Evento de Inmunología Mucosal. Reykjavick 28-30 de Julio del 2001

Julio C Aguilar

Departamento de Hepatitis B, División de Vacunas. Centro de Ingeniería Genética y Biotecnología. Ave 31, e/ 158 y 190, Playa, Ciudad de La Habana, Cuba. AP 6162, CP 10600.
E-mail: julio.aguilar@cigb.edu.cu

Hace sólo 5 años, el destacado investigador de la universidad noruega de Oslo, Per Brandtzaeg escribiría en su artículo *Historia de la tolerancia Oral y la Inmunidad de Mucosas:* "Frente a la perspectiva histórica de la instrucción genética que guía a nuestro sistema immunitario, una revisión histórica de la inmunidad mucosal y la tolerancia oral basada en la información científica disponible constituye menos de la punta de un iceberg" [1].

Con el objetivo de promover esta importante rama de las ciencias biológicas, se organizó la Sociedad Internacional de Inmunidad Mucosal (SMI). En ella se reunen los principales investigadores relacionados con la temática, y entre sus principales tareas está la promoción del conocimiento teórico y la celebración de eventos internacionales [2].

En el mes de Julio del año 2001 se celebró el Congreso Mundial de Inmunología en Suecia. Por la importancia creciente de la temática, tuvo lugar en fecha posterior, un evento satélite dedicado especialmente a la Inmunidad de Mucosas, en la ciudad de Reykjavik, Islandia.

Las temáticas que centraron la atención de los debates fueron: la vacunación mucosal, la tolerancia, la adhesión y tráfico linfocitario y, por último, la inflamación mucosal e inmunogenética. Durante la reunión se celebraron sesiones particulares para cada uno de estos temas, que fueron introducidos por conferencias sobre el estado del arte, ofrecidas por investigadores líderes en cada temática. Seguidamente se sucedieron las presentaciones orales de trabajos seleccionados entre aquellos que se presentaron. Al finalizar se dedicó espacio a la exposición de carteles.

La vacunación mucosal ha ganado en atención rápidamente por cuatro razones fundamentales. Primeramente, la mayoría de las infecciones ocurre en las mucosas, o tienen en ellas una puerta de entrada. Se ha visto además que la células inmunocompetentes estimuladas a nivel de vías respiratorias o tracto gastrointestinal se pueden diseminar a otras mucosas interviniendo en la resolución de un amplio espectro de infecciones mucosales. Además, no obstante a que a través de las rutas mucosales se generan importantes respuestas a este nivel, es posible inducir fuertes respuestas de anticuerpos en suero, y en muchos casos respuestas proliferativas y citotóxicas. Por último, y de modo práctico, existe la potencialidad de una fabricación y control de calidad más sencillos y de una administración potencialmente más segura y barata. Por todas estas razones, fueron los trabajos relacionados con la inmunización y tolerogenización mucosal los más numerosos.

La bacteria *Helicobacter pylori* es agente causal de gastritis crónica y úlcera duodenal y un factor de riesgo para cáncer gástrico por lo que el desarrollo de una intervención terapéutica segura es un objetivo importante y actual.

Entre los trabajos seleccionados en la temática de vacunas mucosales se presentaron los resultados preliminares del uso en humanos de *Salmonella typhy* Ty21a como portadora de epítopos de la ureasa de *Helicobacter pylori* (reporte: "Pilot study of a *Salmonella typhi* (Ty21a) vaccine expressing *Helicobacter pilori* Urease"). De manera interesante, estos resultados apuntan a que la inmunidad preexistente hacia el portador es capaz de favorecer la respuesta hacia el antígeno heterólogo.

Estudios en el modelo de infección con *H. pylori* en ratón, utilizando como inmunógenos a lisados del Helicobacter con la toxina del cólera administrada como adyuvante o sola, evidenció la capacidad inmunomoduladora de la toxina del cólera al proteger por si sola contra la reinfección en ausencia de gastritis postinmunización; fenómeno que sí ocurre cuando la toxina del cólera es utilizada junto a la inmunización específica (reporte: "Effects of specific vaccination and/or immunomodulation by cholera toxin on experimental *H. pylori* infection and reinfection").

Una gran variedad de presentaciones recogieron el interés actual en el desarrollo de adyuvantes y estrategias de inmunopotenciación por rutas mucosales. Este es el caso de la administración nasal del antígeno de superficie del virus de la hepatitis B en conjunto con el inmunopotenciador acemanano (reporte: "Nasal monovalent vaccine formulation of HBsAg against hepatitis B virus infection"), así como la formulación que contiene a antígenos de superficie y de nucleocápsida del mismo virus (reporte: Combined formulation of hepatitis B virus antigens as a nasal vaccine candidate). Ambas formulaciones favorecieron un incremento en los niveles de anticuerpos séricos hasta niveles similares a los obtenidos con formulaciones para administración parenteral. En lavados mucosales, los títulos también fueron significativamente superiores a los obtenidos con la preparación parenteral y la variante nasal del antígeno en tampón fosfato. Estos candidatos pueden aplicarse como vacunas preventivas, y en el caso de la mezcla de antígenos virales se discutieron sus potencialidades terapéuticas.

Teniendo como precedente el riesgo potencial de acumulación en el sistema nervioso central de las enterotoxinas del cólera y lábil de *Escherichia coli* por su unión al receptor de gangliósidos GM1, el grupo sueco dirigido por el investigador Nils Lycke presentó un candidato capaz de dirigir las vacunas a células B y que no se une a este receptor (reporte: "CTA1-DD is a potent adjuvant for intranasal vaccines with minimal risk of accumulation in the CNS"). Este candidato a adyuvante es potente y seguro; experimentos presen-

1. Brandtzaeg P. History of oral tolerance and mucosal immunity. Ann NY Acad Sci 1996;778:1n-dosh-27.

2. Brandtzaeg P. The SMI - an international society for mucosal immunology. Immunologist 1995; 3:67n-dosh-9.

tados en paralelo descartaron el posible involucramiento de endotoxinas contaminantes en su actividad.

Otro adyuvante descrito en una formulación para uso nasal fue el dipéptido de adamantilamida que se utilizó en una preparación con la proteína P6 purificada de *Haemophilus influenzae* no tipificable. Este adyuvante tuvo resultados significativos en cuanto potenciación de los niveles de anticuerpos anti P6 en suero y lavados mucosales (reporte: "Antigenicity of a native and recombinant outer membrane protein (P6) of typeable and non-typeable *Haemophilus influenzae* intranasally administered using AdDP as adjuvant").

Una de las estrategias de más interés en este campo lo constituye la inmunización utilizando plantas transgénicas que expresan antígenos vacunales. En un estudio presentado en el evento utilizando la toxina lábil de *E. coli* o proteínas de fusión de la misma, se evidenció que esta estrategia permite generar respuestas sistémicas y locales en animales no inmunizados previamente, aunque la respuesta resultante fue superior en el caso de los que previamente fueron sensibilizados por ruta parenteral y en aquellos en que había intubación intragástrica, respecto a los que se alimentaban por vía oral. Esto sugiere la necesidad de continuar optimizando este sistema (reporte: "Oral immunization of naïve and primed animals with transgenic potato tubers expressing LT-B and a viral glycoprotein or a fusion protein thereof").

El desarrollo de bacterias atenuadas mantiene la atención de muchos investigadores. Un trabajo que presentó una variante atenuada de una *E. coli* enteropatogénica, fue expuesto por investigadores franceses, quienes desarrollaron su trabajo inmunohistológico en conejos. En este trabajo se generó una cepa doble mutante capaz de inmunizar a los conejos sin causar daño histológico detectable, (reporte: "Vaccination of rabbits with a Tir/EspB null mutant of enteropathogenic *E. coli* strain").

Recientemente se ha atribuido a la subunidad B de la toxina del cólera (CTB) la propiedad de potenciadora de la tolerancia oral. El uso potencial es diverso, fundamentalmente para enfermedades autoinmunes e inflamatorias que requieran la inactivación de respuestas patológicas en curso. Varios trabajos trataron la temática. En el trabajo del grupo de Czerkinsky se pudo evidenciar la diferente actividad moduladora de la subunidad B y la holotoxina sobre la presentación antigénica por las células dendríticas, como posible explicación del mecanismo que gobierna la inducción o eliminación de la toleracia oral por la CTB y la CT respectivamente (reporte: "Cholera toxin and its B subunit differentially modulate antigen presentation by mucosal dendritic cell subsets").

Una de las teorías que explican la aparición del fenómeno de la tolerancia es la relacionada con la formación del tolerosoma. Esta es una partícula de aproximadamente 50 nm, que se puede obtener del suero de ratones a los que previamente se les administró proteínas solubles por vía oral y desarrollaron tolerancia a la misma. Se plantea que está compuesta fundamentalmente por moléculas por el complejo principal de histocompatibilidad de clase II *(MHC,* del inglés *major hystocompatibility complex)* portando los péptidos de la proteína administrada. Además, en estos tolerosomas no está presente el componente coestimulatorio. Luego de la purificación y transferencia a otros ratones no inmunizados de estas partículas, se ha podido transmitir la capacidad de generar tolerancia a la proteína con la que se administraron los ratones donantes de dichas partículas. Estas partículas han podido obtenerse a partir de células dendríticas. También han sido obtenidas *in vitro* a partir de células epiteliales intestinales, evidenciándose una funcionalidad mucho mayor a la que tradicionalmente se consideró para estas células. En un primer reporte se demostró que partículas de látex, semejantes en tamaño a los tolerosomas, son captadas y presentadas directamente por células dendríticas hepáticas portadoras del MHC de clase II, situadas adyacentes al lumen sinusoidal. Este resultado indica un posible destino de los tolerosomas, que serían presentados directamente por células dendríticas hepáticas (reporte: "Uptake of latex particles by cells in livers from untreated and gadolinium-treated rats").

Un segundo trabajo demuestra el involucramiento de las células epiteliales intestinales en el proceso de formación del tolerosoma. Se conoce que la administración intraperitoneal de IFN gamma a ratones SCID, los capacita para producir MHC clase II en sus células epiteliales intestinales, lo que no ocurre normalmente. A partir de este hecho se obtuvieron tolerosomas en los ratones administrados con IFN gamma y posteriormente se demostró su funcionalidad por transferencia de los mismos a receptores no tratados en los que se generó tolerancia (reporte: "Production of a tolerogenic serum factor in OVA fed IFN-gamma treated SCID mice").

Un punto que se discutió en varias sesiones fue el tema del envejecimiento y la inmunidad de mucosas. Aunque se reconoció que este tema está poco estudiado, es evidente a partir de los resultados que se presentaron por Owen y colaboradores que algunos aspectos del sistema inmunitario intestinal se deprimen con la edad y otros no pierden toda su capacidad (reporte: "How aging compromises intestinal mucosal immunity").

De la sesión destinada a la adhesión celular y el tráfico linfocitario, se destacan dos trabajos. Un primer estudio dedicado al análisis de la expresión endotelial en mucosa gastrointestinal de la molécula de adhesión celular MAdCAM-1, de la proteína de adhesión vascular VCAM-1 y de la selectina E en biopsias gástricas y duodenales luego de voluntarios humanos inmunizados por distintas rutas mucosales. Se pudo evidenciar una sobre-regulación de los niveles de MAdCAM-1 en la mucosa duodenal luego de inmunización local, a diferencia de la inmunización a distancia, fenómeno que no ocurre para las restantes proteínas. Lo cual sugiere fuertemente que la molécula MAdCAM-1 está involucrada en el desplazamiento de los linfocitos a sus sitios originales de activación (reporte: "Endothelial MAdCAM-1 expression is increased in the human gastrointestinal tract after mucosal vaccination"). Un segundo estudio de patogénesis y tráfico celular evidenció que la *Bordetella pertussis* es capaz de evadir la inmunidad adaptativa mediante la disrupción del tráfico de células dendríticas en el tracto respiratorio. Este proceso puede ocurrir a partir de la subversión de los elementos de la vía inductiva por productos derivados del patógeno como la toxina, la adenilato ciclasa y otros factores de viru-

lencia, quedando afectada la actividad sentinela de las células dendríticas en el tracto respiratorio por diferentes mecanismos. Este hallazgo evidencia que la red de células dendríticas de los conductos aéreos es crítica para una inducción normal de la respuesta inmune (reporte: *"Bordetella pertussis* evades adaptive immunity by disrupting dendritic cell trafficking in the respiratory tract*"*).

*P*ilot Study of a *Salmonella typhi* (Ty21a) Vaccine Expressing *Helicobacter pylori Urease*

Metzger WG,[1] Bumann D,[1]Mansouri E,[2] Palme O,[1]Wendland M,[1]Hurwitz R,[1]Haas G,[1] Aebischer T,[1] von Specht B-U,[1]Meyer TF[1]

Salmonella enterica serovar Typhi Ty21a is a common live attenuated typhoid fever vaccine. We stably expressed two heterologous proteins, *Helicobacter pylori* urease subunits A and B, in this strain, yielding Ty21a(pDB1). Nine volunteers received three doses of 6-9 x 10^9 cfu Ty21a(pDB 1) and a control group of three volunteers received three doses of 6-9 x 10^9 cfu Ty21a. No serious adverse effects were observed in any of the volunteers. Both groups of volunteers developed similar humoral immune and cellular immune responses to the *Salmonella* carrier indicating that the heterologous expression of *Helicobacter* antigens did not impair the immunogenicity of Ty21a. Three of the volunteers that had received Ty21a(pDB1) showed a clear T cell response to urease and three additional volunteers showed weak responsos of borderline significance while no volunteer had detestable humoral responses to urease. Interestingly, two of the responders to urease had previous contact to *S. enterica* serovar Typhi suggesting that pre-existing immune responsos to the *Salmonella* carrier might enhance responses to heterologous antigens.

[1]Max-Planck-Institut für Infektionsbiologie, Abteilung Molekulare Biologie, Schumannstraße 21/22, D-10117 Berlin, Germany.
[2]Chirurgische Universitätsklinik der Albert-Ludwigs-Universität Freiburg, Hugstetter Straße 55, D-79106 Freiburg, Germany.

*E*ffects of Specific Vaccination and/or Immunomodulation by Cholera Toxin on Experimental *H. pylori* Infection and Reinfection

Raghavan S, Svennerholm A-M, Holmgren J

H. pylori is the cause of chronic gastritis and duodenal ulcer and a risk factor for gastric cancer. A mouse model of *H. pylori* infection was used to study the effects of oral immunomodulation with cholera toxin (CT) alone or in combination with specific immunization, with a *H. pylori* total antigen lysate preparation (lysate), against both an initial infection and against reinfection. In this study we describe for the first time that administering CT at the time of initial infection protects significantly against reinfection and that the protection is not associated with post immunization gastritis. Specific immunization together with CT, on the other hand resulted in significant protection against an initial infection as well as against reinfection, but was associated with post immunization gastritis. A 3-fold increase in proliferative response of mononuclear cells to *H. pylori* antigens *in vitro* was seen before reinfection. The proliferative response was comparable in mice treated with CT alone or immunized with *H. pylori* lysate. Clarification of the mechanism by which CT protects against reinfection in the absence of post immunization gastritis should be importanto the development of a safe therapeutic intervention against *H. pylori* infection.

Department of Medical Microbiology and Immunology. Göteborg University. E-mail: sukanya.raghavan@microbio.gu.se

*N*asal Monovalent Vaccine Formulation of HbsAg Against Hepatitis B Virus Infection

Aguilar JC, Lobaina Y, Leal MJ, Muzio V, Pentón E, Urquiza D, Pichardo D, Guillén G

The infection by the hepatitis B virus (HBV) is a serious world health problem. Approximately 5% of the world population are infected with the HBV, responsable of a necroinflammatory disease of variable severity and duration, hepatic cirrhosis and primary hepatocarcinoma. The HBV is considered the second carcinogen after tobacco.

The hepatitis B surface antigen has been largely and successfully used to prevent the HBV infection in commercially available vaccines. The aim of this work was the evaluation of the humoral immunity raised with a monovalent formulation of hepatitis B surface antigen (HBsAg) and the natural polysaccharide acemannan to obtain a nasal vaccine candidate improving the immune response in systemic compartments and mucosal tissues against the HBV.

Groups of 8 balb/c mice 8 to 12 weeks old, were immunized three times, 2 weeks apart by nasal and systemic routes, with equal doses of HBsAg. The concentration of acemannan was detemined by the Antrona colorimetric method. IgG and IgA titers in sera and vaginal washes were measured by ELISA. Titers were compared using the Student's t test.

Serum IgG and secreted vaginal IgA response for the acemannan group was higher than that obtained with HBsAg in saline-phosphate buffer. No difference was observed for the seric IgG titers after i.m and s.c. inoculations using the same dose of antigen in alum after three inoculations. Nasal inoculations of HBsAg-acemannan formulations gave a high length and stronger responses compared to cholera toxin and alum formulations.

It has been convincingly demonstrated in the literature that secretory antibodies are able to limit the absorption of protein antigens through the mucosal membranes and neutralise a broad spectrum of viruses that could use mucosal portals of entry as HBV.

In conclusion, we have demonstrated in mice that using acemannan-HBsAg formulation, the nasal route can be as efficient as systemic routes in the induction of strong and high length anti-HBsAg antibody responses in sera, with the advantage of inducing strong mocosal responses.

Vaccine Division, Biomedical Research. Center for Genetic Engineering and Biotechnology, Havana, Cuba. E-mail: julio.aguilar @cigb.edu.cu

Combined Formulation of Hepatitis B Virus Antigens as a Nasal Vaccine Candidate

Aguilar JC, Lobaina Y, Muzio V, Pentón E, Urquiza D, Pichardo D, Guillén G

The purpose of this study is the evaluation of the humoral immunity raised with a combined formulation of hepatitis B virus antigens. One of the main mechanisms involved in HBV chronicity is the tolerance induction to HBV antigens. It is known the tolerogenic activity of HBeAg in the pathogenesis of HBV chronic disease. The relative importance of oral tolerance in HBV pathogenesis is unclear. A recent epidemiological report evidenced a higher percent of persons infected with HBV secreting HBeAg in saliva, respect to their percent of HBeAg in sera during a large period of time. The mucosal route (oral and nasal) has been used in different vaccine strategies to immunize and also to modulate ongoing pathologic responses.

We have immunized groups of 8 to 10 female Balb/c mice with different formulations containing HBsAg and HBcAg through the nasal route in a total volume of 50 mL per mice. The IgA, IgG, IgG1 and 2a titers in sera and vaginal washes were determined by ELISA. The interaction between HBsAg and HBcAg on HBsAg immunogenicity was carried out using as control adjuvants cholera toxin and acemannan.

Initially we demonstrated a high immunogenicity of recombinant HBcAg after nasal inoculation. Then we evaluated the effect of antigen combination on HbsAg immunogenicity co-administering both antigens nasally. We observed a strong adjuvant effect of HBcAg on HBsAg immunogenicity in serum and vaginal secretions, similar intensity to CT and acemannan. We also achieved a higher and significant increase in the rate IgG2a/IgG1 against HBsAg for the group immunized with HBsAg, following the Th1-like response also observed for HBbcAg subclass pattern.

The higher coverage of nasal immunizations, the resistance of mucosal immunity to concomitant systemic depressions and the fact that it has been considered that any strategy favoring Th1 responses could abrogate oral tolerance and the contrary. Along with the results about the better cellular resposes against HBsAg when inoculated in PBS compared to alum, support this formulations as an attractive candidate for therapeutic vaccination against HBV.

We concluded that the inoculation of the soluble formulations of HBsAg and HBcAg enhanced the immunogenicity of HBsAg, in serum and mucosal secretions and modulated the IgG subclass pattern to the Th1-like pattern.

Vaccine Division, Biomedical Research. Center for Genetic Engineering and Biotechnology, Havana, Cuba. E-mail: julio.aguilar@cigb.edu.cu

CTA1-DD is a Potent Adjuvant for Intranasal Vaccines with Minimal Risk of Accumulation in the CNS

Eriksson A, Schön K, Lycke N

Recent studies clearly indicate a potencial risk of accumulation in the nervous system of the enterotoxins cholera toxin (CT) and *Escherichia coli* heat labile toxin (LT), when used as intranasal adjuvants. This is due to their promiscuous binding to all nucleated cells via the GM_1-ganglioside receptor. Our newly developed gene fusion protein CTAI-DD, is a non-toxic, B cell-targeted vaccine adjuvant that cannot bind to this receptor.

Experiments were undertaken to determine the efficiency of CTA1-DD as an intranasal adjuvant, and evaluate the potential contribution of contaminating endotoxin in the vaccine preparation. Endotoxin has in itself potent adjuvant functions, and could contribute to the adjuvant effect of CTA1-DD. CTA1-DD exhibited comparable adjuvant function when given intranasally as when given intraperitonealy. Dose-response experiments revealed that the concentration of contamining endotoxin had a negligible effect on the adjuvant function. Also, the enzymatically inactive CTA1-E112K-DD mutant, contaminated with endotoxin to a similar degree, exerted no adjuvant function.

Our results suggest that CTA1-DD is a safe and attractive adjuvant candidate to be included in intranasal vaccines with minimal risk accumulation in the CNS.

Dept. of Clinical Immunology, Göteborg University, Guldhedsgatan 10, 413 46 Göteborg, Sweden. E-mail: anna.eriksson @microbio.gu.se

Antigenicity of a Native and Recombinant Outer Membrane Protein(P6) of Typeable and Non-typeable *Haemophilus influenzae* Intranasally Administered Using AdDP as Adjuvant

Bertot G,[1] Becker P,[1] Guzman C,[2] Grinstein S[1]

The introduction of an effective vaccine against *Haemophilus influenzae* type b (Hib) has dramatically reduced the incidence of Hib disease in children and consequently non-typeable *Haemophilus influenzae* (NTHi) becomes relevant as common cause of otitis media, pneumonia, exacerbation of chronic bronchitis and other invasive and non invasive diseases, both in children and in adults. P6 is a highly conserved peptidoglycan lipoprotein exposed on the outer membrane of both capsulated and non-capsulated strains of *Haemophilus influenzae* (Hi). P6 has been proposed as a candidate for an effective vaccine against NTHi infection. In previous reports we demonstrated the adjuvant effect of adamantylamide dipeptide (AdDP). We intranasally immunized mice with P6 purified from Hi (10 μg) and AdDP (100 μg) as adjuvant on day 0,7 and 14. On day 21 mice were killed and serum, bronchoalveolar lavage (BAL), nasal lavage (NAL) and saliva samples were obtained and P6-specific immune response was tested. P6-specific IgA antibody titer in BAL was significantly elevated after intranasal immunization with P6 and AdDP, meanwhile a weak response was obtained in NAL and saliva. However, P6-specific IgA antibodies was not induced by intranasal immunizatíon with AdDP alone ($p < 0.05$). The levels of IgG antibodies in serum were also higher in P6+AdDP immunized mice than in AdDP immunized mice ($p < 0.002$). These findings suggest that nasal immunization with P6 outer membrane protein using

AdDP as adjuvant is an effective vaccination regimen for the induction of antigen-spocific mucosal immune responses. We have recently constructed and expressed a P6 recombinant protein and we are carrying out the same experiments as with native P6 purified from Hi.

[1]Laboratorio de Virología, Hospital de Niños Ricardo Gutiérrez, Gallo 1330,(1425) Ciudad de Buenos Aires, Argentina. Phone/Fax: 54-11-4963-7569; E-mail:virologia.hnrg@usa.net
[2]Vaccine Research Group, GBF, Germany.

Oral Immunization of Naive and Primed Animals with Transgenic Potato Tubers Expressing LT-B and a Viral Glycoprotein or a Fusion Protein Thereof

Lauterslager TGM, Hilgers LATh

Oral vaccination can lead to protection against infectious agents entering the body via mucosal surfaces. Oral vaccines have the advantage of being safe and easy to administer. At present, few mucosal inununogens are known, amongst them the heat-labile enterotoxin of *Escherichia coli,* LT. Effective oral vaccination requires administration of large doses of antigen. Plants, especially edible plants or parts thereof, are ideal production systems for antigens, since complicated production facilities and purificafion procedures are not necessary. In our study, we evaluated the applicability of edible vaccines by detennining mucosal and systemic immune responses in naive and primed mice. For this purpose, transgenic potato plants were developed expressing B subunits of LT (LT-B) and glycoprotein E2 of Classical Swine Fever Virus separately and these proteins as fusion protein. The recombinant LT-B was able to bind to GM1 and both LT-B and E2 could be detected in plants by Westernblot. Naive and primed mice were fed tubers or were intubated intragastrically with a tuber extract. The vaccines elicited both systemic and local antigen specific IgA responses in naive animals. Antibody responses were significantly higher in parentally primed animals. Lower efficacy of feeding than intubation suggests that processing of antigen in the gastro-intestinal tract is an important factor in the outcome of oral vaccination. Further research is required to optimize our approach and to identify underlying mechanisms.

ID-Lelystad B. V., Institute for Animal Science and Health. PO Box 65, NL-8200 AB Lelystad, The Netherlands.
E-mail: t.g.m.lauterslager@id.wag-ur.nl

Vaccination of Rabbits with a Tir/EspB Null Mutant of Enteropathogenic *Escherichia coli* Strain

Nougayrède J-P, Marches O, Boury M, Oswald E, De Rycke J, Milon A

Enteropathogenic *E. coli* (EPEC) is a well-established cause of serious diarrhea in young children and in different animal species like rabbits (REPEC). EPEC strains induced a specific "attaching-effacing" lesion, caracterized by a destruction of the enterocyte brush border and an intimate bacterial attachment. Bacterial effectors are coded in the locus of enterocyte effacement (LEE). Inactivation of every gene of the LEE leads to a decrease of virulence, although bacteria could still colonize the intestinal tract. The aim of this study was to generate a vaccinal REPEC strain to protect rabbits in breeding units. We inactivated by allelic exchanges two genes of the LEE, coding proteins injected into the host cell by a type III secretion system: the gene coding EspB, a protein forming a pore in the host cell membrane and the gene coding Tir, a protein injected in the host cell surface and representing the receptor of another bacterial protein, Intimin. Tir-intimin interaction allows intimate attachment of bacteria to cells. In the first part of our study we showed that the vaccinal strain, E22?Tir/EspB, was completely safe: it did not induce diarrhea nor any histological intestinal lesions but it was still able to colonize the intestinal tract. We then showed that E22?Tir/EspB was able to protect rabbits against an early (7 days post vaccination) and a late (28 days post vaccination) virulent challenges with the parental strain E22. In addition, the vaccinal strain blocked the shedding of the virulent strain, decreasing the risk of bacterial transmission between rabbits. Abs against LPS O103, the bacterial adhesin AF/R2 and the Intimin were detected as soon as 7 days post E22?Tir/EspB inoculation. Anti-AF/R2 Abs could blocked bacterial adhesion *in vitro.* These results indicated that E22?Tir/EspB is a good vaccine candidate to protect weaned rabbits against REPEC infections in fattening breedings, efficient with a single inoculation dose. A similar vaccinal strategy could be used to protect young children against EPEC infections.

Ecole Vétérinaire, Migrobiol. Mol. 23 chemin 31076 Toulouse, France.
E-mail: s.boullier@envt.fr

Cholera Toxin and its B Subunit Differentially Modulate Antigen Presentation by Mucosal Dendritic Cell Subsets

Anjuère F, Lebens M, Holmgren J, Ardavin C, Czerkinsky C

Mucosal administration of antigens linked to cholera toxin B subunit (CTB) can induce peripheral T cell tolerance whereas coadministration of cholera toxin (CT) breaks it. The mechanisms that govern induction or abrogation of tolerance in that system remain unknown. We examined the phenotypical and functional properties of DC subsets in the gut-associated lymphoid tissues from mice fed either CT or CTB.

Two CD11c+DC subsets were identified in both Peyer's patches (PP) and mesenteric lymph nodes (MLN), based on expression or not of CD8a. A third and predominant subset with intermediate levels of expression of CD8 was exclusively disclosed in MLN.

Further, a discrete subset of CD11c+but CD8-DCs was identified in the intestinal wall. Feeding mice with CT led to the selective increase of the CD8int DCs in MLN whereas CTB had no apparent effect. Further, CD8int DCs of mice fed CT were shown to be able to prime naive T cells more efficiently than corresponding CD8int DCs isolated from mice fed CTB.

Thus, this MLN DC subset may play a major role in the induction of mucosal immune responses to gut luminal antigens adjuvantized by CT. Adoptive transfer studies are now underway to examine the potential

role of these APCs in the induction or abrogation of oral tolerance (EC Biotech IV).

INSERM U364, Nice, France; Department of Microbiology, Göteborg University. Sweden and Faculty of Biology, University Complutense, Madrid, Spain.

*U*ptake of Latex Particles by Cells in Livers from Untreated and Gadolinium Treated Rats

Larsson M, Telemo E

It has been shown in an earlier study that intravenous injection of carbon particles led to their uptake by dendritic cells (DCs) in the liver and lymph. The diameter of these carbon particles was 50 nm approximately the same size as exosomes/tolerosomes, which we have previously have shown to be involved in processing fed antigens. This suggests that liver DCs clear tolerosomes from the blood. To further investigate this, we injected fluorescent latex beads of different charge, size and colour directly into the hepatic portal vein of rats. Gadolinium chloride is known to inhibit tolerance induction by blocking Kupffer cell phagocytosis. We pretreated some of the rats with gadolinium chloride before injection of the beads under anaesthesia. After 20 minutes to permit sytemic circulation of the beads, the livers were removed and frozen for immunohistochemistry analysis. Preliminary results from this experiment show that smaller beads accumulated in MHC class II positive cells (liver DCs) adjacent to the sinusoidal lumen and in no other cells in the liver, indicating that antigen complexes in tolerosomes may be presented directly by liver DCs. The larger particles accumulated in other cells, MHC class II negative cells (Kupffer cells), throughout the parenchyma.

Department of Clinical Immunology, University of Gothenburg, Sweden. E-mail: maria.larsson@immuno

*P*roduction of a Tolerogenic Serum Factor in OVA Fed IFN-gamma treated SCID Mice

Evertsson S, Taube M, Telerno E

Severe combined immunodeficiency (SCID) mice do not normally express MHC class II molecules in their small intestinal epithelial cells (IEC) and have very few intraepithelial lymphocytes. IFN-gamma treatment leads to a normalisation of MHC class II expression in the IEC. It has previously been reported that SCID mice are unable to produce a tolerogenic serum factor after antigen feeding. We set out to examine if this inability was connected to the lack of MHC class II in the IEC. SCID mice were injected i.p. with an IFN-gamma producing cell line or a control cell line. One week later all mice were tube fed with ovalbumin (OVA) and serum was collected one hour there after and injected into wild type recipients. One week later the recipients were immunised with OVA and a bystander antigen (human serum albumin, HSA) and challenged with the antigen three weeks after that. The DTH response was measured 24 h after challenge. Recipients that received serum from IFN-gamma treated SCID mice showed a statistically significant suppression of DTH response, compared to the control mice ($p < 0.05$). There was also a significant bystander effect against HSA in this group. Expression of MHC class II in the IEC is necessary for the formation of a tolerogenic serum factor after antigen feeding.

Department of Clinical Immunology. University of Göteborg. E-mail: sofia@immuno.gu.se

*H*ow Aging Compromises Intestinal Mucosal Immunity

Schmucker DL, Thoreux K, Owen RL

Morbidity and mortality associated with infectious diseases of the intestinal tract are associated with marked deficits in the intestinal mucosal immune responses of old animals and elderly humans. Aging may compromise multiple steps in the generation of intestinal immune response. There are no studies on antigen uptake by M cells as a function of age. Little is known regarding the impact of aging on antigen presentation by dendritic cells and subsequent isotype switching. The third event is the differentiation of IgA plasma cells and their migration from the Peyer's patches to the intestinal lamina propria. Our quantitative immunohistochemical analyses indicate that homing of IgA immunoblasts to intestinal effector sites may be compromised during aging. Homing is facilitated by expression of the integrin ?4?7 on peripheral blood lymphocytes destined for the intestine and the accommodating addressin, MAdCAM-1, on the surface of vascular endothelial cells. Flow cytometric analyses show a 30% decline in the expression of ?4?7 on lymphocytes isolated from senescent rats in comparison to cells from young adult animals. Local antibody production/secretion by mature IgA plasma cells in the intestinal wall constitutes the fourth step. We demonstrated that *in vitro* anti-cholera toxin IgA antibody secretion is equivalent in intestinal lamina propria lymphocytes isolated from young adult and senescent rats. The final event is the transport of IgA antibodies across mucosal epithelial cells and secretion onto mucosal surfaces via receptor-mediated vesicular translocation of IgA. Receptor-binding assays did not detect age-associated declines in receptor number or binding affinity in rodent and non-human primate intestinal epithelial cells as a function of donor age.

Cell Biology & Aging Section, VA Medical Center, Dept of Anat and Liver Center, UC San Francisco. Robert.Owen@med-va.gov

*E*ndothelial MAdCAM-1 Expression is Increased in the Human Gastrointestinal Tract After Mucosal Vaccination

Lindholm C

Mucosal immunization results in homing of antigen specific lymphocytes to the mucosa through the interaction between homing receptors on leukocytes and their ligands, addressins, on endothelial cells. However, it is not known if the recruitment of circulating effector cells after vaccination depends on changes of mucosal endothelial addressin expression. Thus, the impact of different immunization routes on the levels of mucosal cell adhesion molecule-1 (MAdCAM-l), vascular adhesion protein-1 (VCAM- l), and E-selectin in humm gas-

trointestinal mucosa was studied. The proportions of blood vessels in gastric and duodenal biopsies expressing MAdCAM-1, VCAM-1 or E-selectin were determined by immunohistochemistry. The addressin levels before and after local, i.e. peroral, intragastric, or intrajejunal, and distant, i.e. rectal, immunization of human volunteers with an cholera vaccine were compared. The levels of MAdCAM-1 expressing vessels in the duodenum increased significantly after local i.e. per oral ($p < 0.05$) or intrajejunal ($p < 0.0$ l), vaccination. In contrast, rectal immunization did not influence the MAdCAM-1 level in the gastric or duodenal mucosa. The levels of VCAM-1 and E-selectin in the gastroduodenal mucosa were not changed after the different immunization routes. The up-regulated MAdCAM-1 levels in the duodenal mucosa after mucosa after local but not after distant immunization strongly suggests the involvement of MAdCAM-1 in the preferential homing of lymphocites to their original site of activation.

Dept of Medical Microbiology and Inununology, Göteborg University. Guldhedsgatan 10. Telephone: +46 31 342 44 92. 413 46 Göteborg. Sweden.

Bordetella pertussis Evades Adaptive Immunity by Disrupting Dendritic Cell Trafficking in the Respiratory Tract

Cahill S,[1] Cassidy J,[2] Lax A,[3] Ennis D,[1] Mahon BP[l]

The network of dendritic cell (DC) populations in the respiratory tract may be important mediators of the initiation of primary or recall adaptive immune responses to inhaled pathogens or allergens. *B. pertussis* infection in murine models results in delayed induction of adaptive immune responses and has proved useful in determining correlates of human protection. Using this model and *in vitro* studies, we show that the chemokine MIP-3a is expressed in the epithelial layer of the tracheobronchal tree, and confirm this by *in situ* hybridization. We demonstrate *in vitro* that MIP-3a is involved in recruitment of immature bone marrow derived DC expressing the receptor CCR6, whereas the exit of mature DC to lymph nodes draining the conducting airways is most likely governed by the MIP-3b/CCR7 interaction. However, we show that key elements in this inductive pathway are subverted by pathogen derived products, including pertussis toxin, adenylate cyclase and other virulence factors. Furthermore we demonstrate that *B. pertussis* can induce DC apoptosis and impair antigen presentation to antigen specific T cells. Thus *B. pertussis* evades adaptive immunity by interfering with the sentinel function of DC in the respiratory tract by at least three mechanisms involving DC intoxication. Taken together these findings strongly suggest that the DC networks of the conducting airways are critical to normal imune induction in the respiratory tract.

[1]Mucosal Immunology Lab. Institute for Immunology. NUI Maynooth, Co. Kildare, Ireland. E-mail: bpmahon@may.ie
[2]Department of Veterinary Pathology. University College Dublin, Ireland.
[3]Department of Oral Microbiology. Kings College London, SE1 9RT, England, UK.