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Possibilities for active and passive vaccination against opportunistic infections.
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MEETING SUMMARY: POSSIBILITIES FOR ACTIVE AND PASSIVE VACCINATION AGAINST OPPORTUNISTIC INFECTIONS*

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INTRODUCTION

Opportunistic pathogens present an ever-growing threat to mankind in spite of numerous medical advances. This event is a consequence of an increase in bacterial resistance to commonly used antibiotics all over the world as well as a greater survival of individuals immunocompromised by chronic and acute diseases or injuries. To meet this challenge, the International Study Group for New Antimicrobial Strategies (ISGNAS) took a fresh look at the possibilities for vaccination against opportunistic infections. Although this approach to control these infections has been a goal for many years, to date there are no licensed products available for this purpose. Based on the results presented at an ISGNAS-sponsored meeting in Herborn, Germany, June 23-25, 2003, there is now reason to hope that this situation could change. This volume of the Old Herborn University Seminar Monographs constitutes the report of that meeting. The meeting consisted of one day of formal reports followed by discussion among the conference faculty. The major points presented during this meeting are summarised below.

WHAT IS AN OPPORTUNISTIC PATHOGEN?

Colonisation of skin and mucosal surfaces with microorganisms begins at birth such that a generally beneficial symbiotic relationship is established that lasts throughout life. The importance of some members of this microbial community is illustrated by research presented from Dr. Cebra's laboratory, which provides insight into the role of intestinal microorganisms in the development and maintenance of the intestinal humoral immune system. Infections referred to collectively as "opportunistic" are those resulting from otherwise commensal organisms, either resident or hospital acquired, when the normal state is disturbed by factors which damage mucosal surfaces or mediate immune defects, such as antibiotic use, intravenous catheters, mucosal breakdown or HIV infection. Dr. Romani suggests another piece to this equation. Her work with *Candida* suggests a model where changes in the microorganism promote an alteration in the response of host dendritic cells (DC), which effectively

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transforms the relationship from a commensal state to a disease state. It is with this in mind that the term "opportunistic pathogen" should not only include the traditional culprits (i.e. *Pseudomonas, Staphylococcus,* etc), but other organisms normally colon ising host surfaces, such as *Helicobacter pylori*. These observations raise important unanswered questions about the regulation of the host immune system with respect to the restoration and maintenance of a commensal state with its microflora.

USE OF PASSIVELY-ADMINISTERED ANTIBODIES

Over 20 years ago the passive infusion of antibodies directed against a conserved region of the lipopolysaccharide (LPS) of Gram-negative bacteria was reported to be effective against sepsis. This approach was not further developed, however, as consistent efficacy was not obtained. At this meeting Dr. Cross reported that a subunit vaccine composed of detoxified J5 LPS complexed to group B meningococcal outer membrane protein provided both active and passive immunity and protection in animal models. A phase I study with this material showed it to be safe and immunogenic. These data suggest that further studies with this approach are now warranted.

Specific prevention of *Klebsiella* infections by passive immunotherapy has also received more attention recently. Dr. Trautmann's report focused on the generation of O serogroup-specific antisera in animals. O antigen specific antibodies were able to opsonise non-encapsulated *Klebsiella* strains, while fully encapsulated bacteria were resistant against O antibody-mediated opsonisation. *In vivo* experiments, however, demonstrated a prophylactic effect on *Klebsiella* bacteraemia in mice. Dr. Trautmann's work suggests that O antigen-specific antibodies may be useful to supplement K antigen-specific hyperimmune globulins for passive immunoprophylaxis of *Klebsiella* infections.

In another approach involving passive immunisation, Dr. Matthews reports that recombinant antibodies can be used synergistically with an antimicrobial agent to control disease. Her work showed that patients with invasive candidiasis, being treated with amphotericin B, showed a close correlation between recovery and antibody to the immunodominant heat shock protein 90. Human recombinant antibody to this protein has synergistic antifungal activity with amphotericin B and is now the subject of a clinical trial. The combination of antimicrobial agents and specific antisera against other opportunistic pathogens merits examination as a strategy to better control infection.

CANDIDATES FOR ACTIVE VACCINATION AGAINST OPPORTUNISTIC PATHOGENS

Progress has also been realised towards the goal of active immunisation against many of the major opportunistic pathogens. This approach is facilitated by the facts that compromised patients respond immunologically to active vaccination and the fact that 65 percent of surgeries are elective, which would indicate that at-risk populations could be identified for vaccination. Further reason

for optimism with this approach is the discovery and application of new antigens and techniques, particularly conjugate vaccine technology.

Pseudomonas aeruginosa is an opportunistic pathogen responsible for often life-threatening complications. Based on an extensive number of approaches that have been studied to vaccinate against this pathogen, reviewed in Dr. Holder's presentation, it is now possible to begin to focus on pseudomonas antigens that seem most promising. Of these, the type III translocation protein (PcrV), LPS-O-polysaccharide, OMP and flagellar antigens are noteworthy. In fact, Dr. von Specht described a recombinant OMP vaccine that was safe and immunogenic in burn patients. New data from others show that O antigen or capsular polysaccharide could offer useful vaccine antigens for *Escherichia coli* and the polysaccharide-based vaccine approach may also be applicable to development of a vaccine against *Cryptococcus*.

Polysaccharide conjugate vaccines also offer a promising approach for vaccines against Gram-positive organisms. Dr. Fattom reported clinical trial results with a capsular polysaccharide vaccine against *Staphylococcus aureus* capsular types 5 and 8, which together comprise over 80% of the clinical isolates worldwide. A trial with this vaccine in haemodialysis patients found that efficacy could be observed at 40 weeks post immunisation as vaccination reduced the number of staphylococcal bacteraemias by 57%. Dr. Pier reported that the genes for biosynthesis of certain capsular polysaccharide adhesins of *S. aureus,* the poly-N-acetyl glucosamine (PNAG) molecules, are present in virtually all strains of this pathogen. Immunisation of mice with PNAG elicited opsonic and protective antibodies against multiple isolates of staphylococcus. High titred opsonic antibody was produced to multiple strains of *S. aureus* and *S. epidermidis* when this antigen was coupled with diphtheria toxin to produce a conjugate vaccine.

Enterococci are one of the most common causes of hospital-acquired infections and many strains have developed resistance to all known antibiotics. Dr. Huebner's group has found that enterococci also possess polysaccharide-containing capsules with features of teichoic acids, which may provide vaccine candidates. One of these polysaccharides is expressed by both *E. faecalis* and *E. faecium* and the antigen is a target for opsonic antibodies. Rabbit antibodies raised against this purified polysaccharide were effective as a therapeutic agent in mice even when the administration of antisera was initiated up to 48 hours after challenge with live bacteria. Dr. Fattom's group has also been looking at the possibility of polysaccharide conjugate vaccines for enterococcus. Their vaccine polysaccharide is conserved on the surface of most enterococcal isolates and antibodies to it mediate in vitro opsonic killing and in vivo protection against *E. faecalis* challenge.

Clostridium difficile is a major cause of hospital-acquired infectious diarrhoea and colitis following antibiotic administration and subsequent loss of the protection afforded by intestinal flora. Dr. Giannasca reported that a toxoid vaccine being evaluated in the clinic is well tolerated and very immunogenic. Anti-toxin A IgG titres were found to far exceed the level associated with protection. The utility of the vaccine to generate a hyper-immune globulin for passive protection in acute care settings remains to be determined.

Its possible that in some cases commensal bacteria with cross-reactive antigens to those of a pathogen may be exploited for immunisation. Dr. Braun showed that both *Neisseria lactamica*

and *Moraxella catarrhalis* isolates bound antibodies to epitopes on the meningococcal LPS (epitopes associated with L3,7,9). His studies provided evidence that blood group like glycoconjugate antigens found on some commensal species might be involved in natural immunity to meningococcal endotoxins during childhood. It should be considered that natural antibody to commensal opportunistic pathogens may benefit the response to specific vaccination. Such a vaccine could in essence be viewed as a booster inoculation. In the future, immunomodulatory techniques could be developed which could also boost natural antibodies of interest.

IMMUNE RESPONSES TO OPPORTUNISTIC PATHOGENS

Evidence obtained with most bacterial opportunistic pathogens shows the importance of circulating antibodies in protection. Although many of the organisms colonise normal mucosal surfaces, it was noted that the problem arises when the host defences are altered such that the organisms get to other sites they do not usually inhabit (i.e. the bloodstream). Further, since it is difficult to dislodge organisms when they exist in a commensal mode on mucosal surfaces (i.e. nasal carriage of *S. aureus*), induction of circulating opsono-phagocytic antibodies rather than local immunity offers the most promising strategy for immunological control of bacteraemia. As indicated above, numerous antigen candidates are now available to induce protective immune responses against opportunistic pathogens. The search for conserved protective antigens is an important element of this vaccination strategy because of the relatively large number of pathogens under consideration and the many serotypes which might be clinically relevant. It is not known at present whether common antigens will provoke sufficient immunity compared to type-specific immunity. Combined vaccines for a number of major opportunistic pathogens, such as those described here, should be sought in the future. Whether immunologic interference will be a problem in vaccine combinations remains to be determined.

A better understanding of the relationship between antibody responses to specific pathogens and protection is needed. This information will be required as vaccines move toward licensure. For example, what comprises a surrogate marker for protection? Is a calculated protective antibody level a reasonable surrogate marker for protection and equivalency measure in other populations than the one in which an efficacy trial was run? Perhaps in vitro functional equivalency could be used to make the case of antibody levels equivalency more acceptable as a surrogate marker. *In vivo* protection studies in animals may be able to help interpret the significance of antibody responses. A difficulty here is the need for an animal model which closely mimics the population expected to develop an infection.

H. pylori colonises about half of the human population and, for as yet unknown reasons, in some, is associated with symptomatic gastritis, peptic ulcer disease and an increased risk for gastric adenocarcinoma. This opportunistic pathogen may differ from the others considered in this meeting because cellular immunity seems to be the key to control rather than humoral immunity. Dr. Blanchard proposed a model of *H. pylori* pathogenesis in which the pathogen induces local inflammatory and

immune responses that are limited by a population of regulatory T cells in the stomach. Consequently, immunisation might be better achieved by activation of *H. pylori*-specific T cells in peripheral lymph nodes that are capable of promoting either a qualitatively or quantitatively different inflammatory response when recruited into the stomach.

Dr. Romani suggested, based on her work with opportunistic fungi, that optimally effective immunities may be achieved by targeting specific receptors on dendritic cells *in vivo*. Her studies showed that DCs phagocytose fungal components through distinct recognition receptors which translated into disparate downstream signalling events, ultimately affecting cytokine production and co-stimulation. This was responsible for Th polarisation of patterns of susceptibility or resistance to infection. Her research also found that DCs transfected with fungal RNA restored antifungal resistance in haematopoietic transplantation.

APPLICATION OF EMERGING TECHNOLOGIES TO IMMUNOLOGIC CONTROL OF OPPORTUNISTIC INFECTIONS

Current vaccine candidates in advanced development for opportunistic pathogens are relatively immunogenic, but new developments in immunomodulation deserve consideration for future use. For example, adjuvants such CpG ODN and delivery systems such as poly glycolide poly lactide microspheres or attenuated bacterial vectors such as *Listeria monocytogenes* now on the horizon may be able to reduce the number of doses (i.e. 3 doses to one) of vaccine needed or accelerate or enhance the development of protective titres or other immune responses. These types of techniques could be useful in certain groups of immunocompromised individuals or could help overcome possible interference among multiple components of a vaccine for opportunistic infections. The recognition, for example, of the possible benefit of increased expression of FcγR and the importance of Toll like receptors and dendritic cells in determining immune responses should lead to research which will enable better immune regulation through vaccination. The potential options for better, more directed, immunomodulation may make the current term “adjuvant” obsolete. Already the observation that subcutaneous immunisation is better than the intramuscular route for inducing immune responses may be an example of exploitation of the DC in the skin. Future manipulation involving DCs may involve direct loading of antigens into the cells in vitro or targeting them in vivo. The possibility that non-specific modulators of the innate immune system could be used in combination with vaccination regimens is yet to be explored. Further, antigens themselves may be modified to modulate different immune responses.

THE CHALLENGE OF CLINICAL TRIAL DESIGN

It is possible that paradigms appropriate for paediatric and adult vaccines to be administered to healthy persons may need re-evaluation for immunisation against opportunistic infections. The duration of efficacy may need to be considered carefully in the evaluation of vaccines for people in various compromised states. Further, thought must be given to determination of whether effi-

cacy in one population can serve as proof of concept for the approach toward similar infections in other patient groups at risk, which are immunologically and physiologically equivalent or better than the indicated population. This question may become particularly important for patient populations, which are rare. A question, which may apply to all vaccines, is what clinical studies would be necessary to support the addition of an antigen component to a vaccine after licensure? For example, if a third polysaccharide element is added to a bivalent vaccine to increase coverage by the vaccine, would a second phase III efficacy trial be required? Considerable discussion will be necessary to determine what data are required for licensure of such a second generation vaccine. Another complication of trial design involves the need to maintain antibiotic treatment in all trial groups, yet show a statistically significant in groups given the vaccine also. The meeting faculty observed that improved discussion of preclinical and clinical testing approaches among government, academic and industrial entities would be essential to address practical vaccine development issues such as these.