Neisseria meningitidis Group A Bactericidal Activity Assays using Pooled Human Complement

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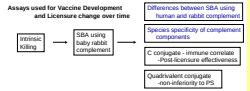
Background

Neisseria meningitidis

- Obligate human pathogen
  - No reservoir outside of the human host.
  - Aerobic, non-spore-forming Gram-negative coccobacilli
  - Well adapted to inhabit human mucous membranes
  - Require moist warm environment
- Polysaccharide encapsulated organism
  - Serogroups A, B, C, Y, W-135 associated with invasive disease
  - A,C, Y, W-135 capsular polysaccharides (PS) are immunogenic
  - Polysaccharide and and PS-conjugate vaccines are effective for disease prevention
  - Therefore – disease caused by these organisms are potentially vaccine preventable

Immune responses to Meningococcal Polysaccharide Antigens

- Anti-capsular antibody – ELISA
- Complement mediated bacterial killing – SBA
- Opsonophagocytosis
- Animal protection models

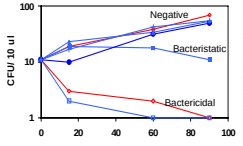


Research Outline
Problem, Hypothesis, Plan

- **Immune correlates of protection are a likely mechanism for licensure of new meningococcal vaccines…..*but***
  - Anti-polysaccharide antibody (ELISA) ≠Serum Bactericidal Activity (SBA)
  - SBA determined with rabbit complement (rSBA) is not always predictive of human complement SBA (hSBA)
  - Human complement (hC') may be difficult to identify and qualify
  - Different sources give different titers – **what source is relevant?**
- **Hypothesis**
  - Intrinsically negative sera can be identified or created - Adsorption may be necessary
  - Pooled hC' may provide
    - Less variation between sources
    - A "representative" hC'
- **Study Design**
  - Obtain sera from 100 adult blood donors
  - Assign consecutively to one of three groups
  - Characterize sera
  - Develop and test criteria for suitability as complement source
  - Pool eligible sera within groups → 3 lots of hC'
  - Test a variety of sera using hC' lots and compare hSBA titers
- **Healthy adult blood donors**
  - Age: 18-77 Average = 41, Median = 42
  - 68 Male/ 32 Female
  - Race: 74 C; 21 B; 4 H; 1 NA
- **All samples tested for:**
  - Intrinsic Killing of strain F8238
  - rSBA
  - Anti-A PS IgG, IgGAM
- **Subsets tested for:**
  - CH100, IgA, IgM, anti-class 4 Ab

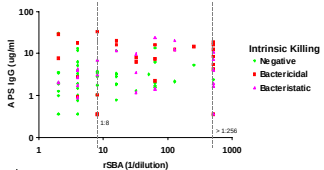
Results – Screening Sera

Group A Intrinsic Bactericidal Assay
Bactericidal, Bacteristatic and Negative Samples



Representative intrinsic killing assay
*HI = heat inactivated

Bactericidal Titer (rSBA), Anti-A PS IgG and Intrinsic Killing in NIH Blood Donor Sera



- No correlation between intrinsic killing, rSBA, or anti-PS A IgG concentration
- Many sera potentially suitable as C'

Test Lots of Pooled Complement

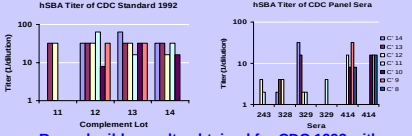
- Preliminary test lots of complement pools using donors with high or low anti-A PS IgGAM in hSBA assay were tested
  - Confirmed complement activity by hemolytic assay
  - Confirmed activity in hSBA assay
  - Investigated various criteria for pooling
- Created three independent complement pools using intrinsic, rSBA and IgGAM criteria
- Role of anti-PS antibody titer in screening unclear so 4th C' pool created with sera that had no bactericidal activity but had IgGAM concentrations > 30 ug/ml

Results – Pooled Complement Lots

Summary: NIH Donor Sera and Final Complement Selection Criteria

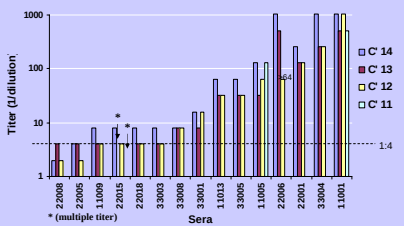
- **Healthy Adult Blood Donors**
  - No intrinsic killing in 52%
  - Intrinsic killing not predicted by rSBA or IgG
  - 31% were both intrinsically negative and had a rSBA liter 1:8 or less
- **Anti-A PS antibody**
  - Importance unclear
  - Arbitrary cut off at 30 ug/ml IgGAM
  - High antibody sera that met the intrinsic and rSBA criteria combined in a fourth lot
- **Complement Pools**
  - Active C' by hemolytic assay
  - Intrinsically negative

Human Complement Pools Tested with CDC Panel Sera



- Reproducible results obtained for CDC 1992 with four different pooled human complement lots
- Many CDC panel sera had negative or low titers by the hSBA assay

Human Complement Pools Tested with Adult Post Immunization Sera
hSBA Titer of MVP I Sera by Complement Lot



Sera from the Meningitis Vaccine Project (MVP) Phase I study in Indian Adults were used to compare complement lots

Titers obtained for the same sera with different complement lots were within the expected variability of the assay

- Similar results were obtained across the range of the SBA using different lots of pooled human complement

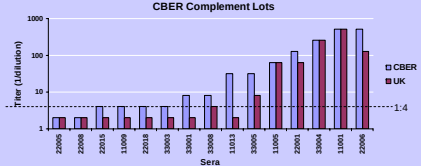
Human Complement Pools Tested at Manchester UK Laboratory

MVP sera were tested for hSBA by the Manchester laboratory using complement Lots 11, 12, and 13

GMT based on all readable results for each sera within each laboratory were compared

More sera were below the limit of detection in the Manchester assay

- Similar hSBA titers were identified at two laboratories for 14 of 15 sera

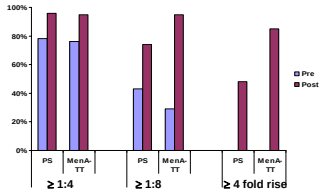


Results - hSBA Titers Pre- and Post Immunization With Polysaccharide or Conjugate Vaccines

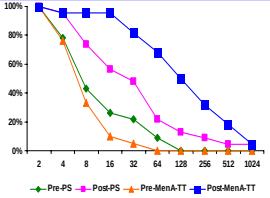
Meningitis Vaccine Project Phase I Study of PsA-TT

	Sera Tested	SBA-H Results	≥1:4	≥1:8	4-fold rise
Total	94	88	76	53	25/41
Pre MenA-TT	21	20	76%	29%	
Post MenA-TT	24	22	95%	**95%**	
Pre PS	24	23	78%	43%	
Post PS	25	23	96%	**74%**	
Pre/Post pairs					
Men-A-TT	23	20			**85%**
PS	24	21			**48%**

Proportion at or above hSBA threshold Titers and 4 Fold Rise in hSBA Titer by Vaccine Group



Reverse Cumulative Distribution of hSBA Titers Pre and Post Immunization by Vaccine Group



Summary

- Suitable donors were identified among normal healthy U.S. adults
- Screening to exclude sera with intrinsic killing and or rSBA titers >1:8 was sufficient to identify acceptable sera
- Pooling 6 to 9 sera resulted in independent complement sources that gave reproducible hSBA titers for a range of sera
- The hSBA titer of 88 sera from a phase I meningococcal group A conjugate vaccine trial were determined. Six sera (6%) had unreadable results (multiple titers)
- Differences between the immune response to conjugate vaccine vs. polysaccharide vaccine were detected by hSBA, even in an adult population

Acknowledgements: Research was supported in part by the Meningitis Vaccine Project. PsA-TT study 001 was conducted at Seth G.S. Medical College & KEM Hospital, The Nizam's Institute of Medical Sciences, and the Topiwala National Medical College & BWI, India. Kshirsagar N, Mur N, Thatte U, et al; Vaccine 258 (2007)A101-A107