

Portrait

Coincidences, convergences and opportunities

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Born in 1944, I grew up in a world in which polio was both a gripping fear and real threat. Then in a matter of a few years—polio was eradicated by a vaccine developed by Jonas Salk. Later I learned that Salk's efforts were built on pioneering work of many others, including John Enders, Thomas Weller and Frederick Robbins (Nobelists, 1954), and David Bodian, who pioneered studies of polio pathogenesis and immunity. Bodian became my teacher in medical school, and Robbins became a colleague. Later, Salk, Robbins and I shared a platform at an infectious diseases symposium, and I was privileged to speak at Robbins' retirement. But that gets ahead of my story. In January 1956, at age 12, I received my first of dose of the "Salk" vaccine. Other kids had pictures of athletes in their rooms; I had a picture of Jonas Salk.

As formative as this moment was, I could not foresee that I would choose a career in infectious diseases; nor that I would have incredible opportunities to work on vaccines that would help control two infectious diseases. Instead, a series of coincidences, convergences and opportunities led me to rediscover my passion for the power and promise of vaccines.

In 1963, at age 19, I arrived at Johns Hopkins University School of Medicine in Baltimore, Maryland. My first mentor, John Money, was a brilliant medical psychologist but a controversial figure. Money pioneered research on sex and gender identity long before terms like transgender were part of our lexicon. He taught me how to write a scientific paper, and the

importance of checking and rechecking data. I vividly remember taking a manuscript to his office around 7 p.m., and leaving at midnight without a single sentence untouched. The result was my first publication, in 1965, on Turner's syndrome.¹

While working with Money, I met Ernesto Pollitt, a post-doc from Peru, recently retired as University Professor of Nutrition at the University of California, at Davis. I went to Lima with Pollitt to investigate cognitive development of infants who had suffered from severe malnutrition. Our results revealed major impairments of language and motor development.² The following year, I approached Evelyn Howard, a Professor in the Department of Physiology at Johns Hopkins, who was investigating changes in DNA, RNA and cholesterol in the brains of developing mice. Jointly we studied the effects of malnutrition on mouse brain development. She introduced me to brain chemistry, animal and behavioral research, and reinforced for me many lessons on scientific paper writing learned from Money.

By the time I left medical school, I had a number of publications on the cognitive effects of malnutrition on brain development in both human and animal studies.^{1–6} Until my post-medical school internship and residency in pediatrics— I believed nutritional research was my true calling.

In 1964–65, the United States experienced one of the largest recorded rubella epidemics with more than 20,000 congenital rubella syndrome cases. In 1968, when I began my pediatric internship at Philadelphia Children's Hospital, the

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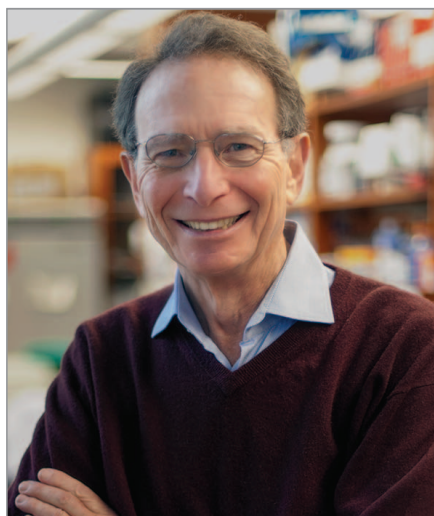
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wards were full of congenitally rubella infected infants with heart disease, blindness, and severe neurologic disabilities. The need for a vaccine was urgent. My attending physician was Stanley Plotkin, who pioneered a rubella vaccine attenuated in human diploid cells that eventually became the vaccine of choice.⁷ A superb clinician, an inspiring teacher, and a gifted researcher, Plotkin reinforced an appreciation of the power of vaccines, and was a role model for me to consider a career as a physician-scientist. In 2010 I felt especially honored to be selected by the Pediatric Infectious Diseases Society to give the Stanley A. Plotkin Lecture in Vaccinology.

Upon completing my pediatric residency at the Johns Hopkins Hospital, I accepted a post-doctoral fellowship in infectious diseases at Cleveland Metropolitan General Hospital (part of Case Western Reserve University School of Medicine). As a mentor, I chose Eli Gold, an investigator of the prenatal effects of cytomegalovirus (CMV) infection on neurologic development of children. When I arrived in 1973, the hospital's closed polio unit with iron lungs stood eerily quiet, was a testament to the success of polio vaccination. I discovered that Gold was taking a sabbatical year, from which he would never return.

While I learned tissue culture methods and viral isolation from George Nankervis, Co-Director of the Lab, I became impatient with virology and the CMV project and searched for a new project. During my first clinical rotation I consulted on two newborns with septic arthritis from *Hemophilus influenzae*. In one patient, I found complement-mediated bactericidal activity in maternal serum, which should have conferred protection but didn't.⁸ Thus, in the middle of a virus laboratory, I began my studies of immunity to *Hemophilus influenzae* type b (Hib). What began with a seemingly simple question of how Hib caused bacteremia despite serum bactericidal activity, progressed to investigations of an infant rat model of Hib bacteremia, and immune responses to clinical infection.

After completing my post-doctoral fellowship, I accepted a position at the Valley Medical Center of Fresno California.



About Dr. Granoff:

Dan M. Granoff, MD holds the Clorox Endowed Chair and is Director of the Center for Immunobiology and Vaccine Development at the Children's Hospital Oakland Research Institute (CHORI), Oakland California. Before joining CHORI, he was Vice President of Scientific Affairs at Chiron Corporation, and Professor of Pediatrics and Associate Professor of Molecular Microbiology at Washington University School of Medicine, St. Louis, Missouri and Director of Pediatric Infectious Diseases at St. Louis Children's Hospital. After earning his bachelor of arts and medical degrees from Johns Hopkins University in Baltimore, Maryland. Dr. Granoff completed his pediatric internship at the Children's Hospital of Philadelphia, pediatric residency at the Johns Hopkins Hospital, and post-doctoral fellowship in pediatric infectious diseases at Case Western Reserve University School of Medicine and Cleveland Metropolitan General Hospital in Ohio. Author or co-author of more than 200 peer-reviewed research articles in areas of microbiology and vaccine research, Dr. Granoff's current research focuses on identification of new vaccine antigens and approaches for prevention of meningococcal disease, including strains causing epidemics in Africa.

Located in the Central Valley, the hospital provided care to poor people, including migrant farm workers. Although it was not a major research center, I initiated population-based surveillance of invasive *Hemophilus* disease,⁹ which led to studies of the spread of Hib among household contacts of cases, and children in day care centers.⁹⁻¹² (At the time, Hib was not thought to cause secondary cases of disease). In these studies I was fortunate to have a gifted pediatric resident, Janet Gilsdorf, as a coworker. Later she became Professor and Head of Pediatric Infectious Diseases at the University of Michigan where she maintained a highly productive research focus on *Hemophilus pili* and molecular epidemiology of non-typeable *Hemophilus* infections. In Fresno, I also obtained my first research grant from the National Institutes of Health (NIH) on Hib outer membrane proteins as vaccine candidates. David Smith and Porter Anderson at Boston Children's Hospital, and John Robbins and Rachel Schneerson at NIH, were already conducting vaccine

research on the Hib polysaccharide. I knew as an inexperienced researcher I couldn't compete with these labs, and would need to find a different approach. Hence, I decided to focus on outer membrane proteins.

In 1980, I unexpectedly got a call from Philip Dodge, Chair of the Department of Pediatrics at Washington University School of Medicine in St. Louis Missouri, recruiting me to head their pediatric infectious diseases division. I had gotten to know two UCLA professors, Bascom Anthony and Joseph St. Geme, during monthly visits to Los Angeles to teach and see patients. They recommended me to Dodge for the division head position. In addition a few years earlier Dodge spent a sabbatical year writing a book on nutrition and brain development. Not only had he read my papers from medical school, he included them in his book.

An unprecedented rubella outbreak during medical school; a virology fellowship missing its mentor; an encounter with rare cases of *Hemophilus influenzae* disease

in neonates, and a Pediatric Chair hiring in infectious diseases who was familiar with my nutrition and brain research: this series of coincidences converged and formed an incredible opportunity at age 36 to become Professor and Head of an infectious disease division at a distinguished medical school.

During my 13 years at Washington University, with so many resources and talented students and faculty, I could tackle Hib research from several different angles. My first faculty recruit, Robert Munson, Jr., introduced me to protein chemistry and molecular biology. Munson would become internationally recognized for his work on bacterial pathogenesis. I collaborated with him on investigations of the Hib vaccine-potential of protein antigens,^{13,14} and the use of SDS PAGE of outer membrane proteins as an epidemiologic tool to investigate spread of Hib infection.¹⁵ We also performed the first clinical trial in humans of a polysaccharide-protein Hib conjugate vaccine.¹⁶ With another colleague, Penelope Shackelford, I investigated functional activity of human Hib anticapsular antibodies and showed that IgG1 predominated the IgG subclass response.^{17,18} This result was unexpected – the dogma (from mouse studies) had been that antibody responses to polysaccharides were restricted to IgM and IgG2. I also showed that an Hib conjugate vaccine prepared by Merck, in which the polysaccharide was coupled to meningococcal outer membrane proteins, elicited serum anti-capsular antibody responses after a single injection in two month-old infants.¹⁹ This result also was unexpected; most scientists had believed two-month-olds did not have mature B cells capable of responding to polysaccharide antigens. Our findings proved that B cells, with the appropriate rearranged genes, were in fact present at age two months, but not activated by unconjugated Hib polysaccharide, or Hib polysaccharide coupled to carrier proteins such as diphtheria or tetanus toxoids.

While at Washington University I established productive collaborations with Trudy Murphy, a pediatric infectious disease specialist at the University of Texas Southwestern School of Medicine, Dallas, and Michael Osterholm, the Minnesota Department of Health State

Epidemiologist. Over a ten-year period we used population-based surveillance of Hib disease in Dallas County, Texas and Minnesota, linked with molecular strain characterization and serologic studies, to define risk factors for Hib disease just before and after introducing Hib vaccines.^{20,21} Our studies showed that the unconjugated Hib polysaccharide vaccine was not effective in Minnesota.²² Although the data were not what the public health community had hoped, the results ultimately hastened introduction of a highly efficacious next generation Hib conjugate vaccine in the US. Subsequently we showed that the Hib conjugate vaccine was highly efficacious in preventing disease in Minnesota,²³ also prevented asymptomatic Hib colonization,²⁴ and induced protection in children < 18 mo, an unvaccinated age group (herd immunity).²³

During this period I also collaborated with Alexander Lucas, then at Scripps Research Institute in La Jolla, California, using idiotypic analysis to investigate variable region gene diversity of human antibodies specific for Hib polysaccharide. We found a highly penetrant and predominant cross-reactive idio type expressed in immunized infants and adults, which underscored the limited variable region diversity of Hib anticapsular antibodies.^{25,26} We also found dramatic changes in light chain variable region gene utilization as a function of age,²⁷ and vaccine²⁸; and that light chain usage affected anti-capsular antibody avidity and functional activity.²⁸ Collectively these studies contributed to making the structure and function of Hib antibodies one of the best understood human antibody systems at a molecular level.

In 1986, Harvey Colten, a highly accomplished immunologist from Harvard Medical School and Boston Children's Hospital, became Department Head of Pediatrics at Washington University. Under Colten's leadership, the department flourished. He was always available for consultation on a patient, advice on an experiment, or even to provide critical comments on a grant application or manuscript. Long after we both departed from Washington University, I continued to seek his advice. His untimely

death in 2007 was a personal loss. Even now when faced with a scientific question or administrative issue, I ask myself, what would Harvey have done?

By 1992, Hib disease was disappearing. I decided to take a sabbatical to focus on future plans. My scientific collaborator and friend, Alex Lucas, had moved to Children's Hospital Oakland Research Institute (CHORI), in Oakland, California, where he is presently Executive Director. I spent my sabbatical year in Lucas' lab redirecting my research interests to *Neisseria meningitidis*. I also met Bill Rutter, former Chairman of Biochemistry at UC San Francisco School of Medicine, one of the founders of the Chiron Corporation. Rutter wanted a person with strong scientific and clinical vaccine experience to work on clinical vaccine development at the company headquarters in Emeryville, California —10 min away from CHORI. Rutter and his colleague, Rino Rappuoli, at Chiron in Siena Italy, were very persuasive and, in 1993 I became Chiron's Executive Director of Clinical Vaccine Research. I also had a joint appointment as a Scientist at CHORI, where I transferred my NIH grants from Washington University to continue my investigations of Hib and new studies of meningococcal immunity.

While working at Chiron I was involved in many different vaccine projects and supervised clinical development of the meningococcal serogroup C conjugate vaccine. Ultimately the vaccine was licensed in Europe, Canada and Australia. During my third year, I became Vice President of Scientific Affairs, a role that involved attending meetings at the NIH, FDA, CDC and WHO. Working at Chiron provided valuable insights of the realities and extraordinary complexities of vaccine development, and I learned a lot of science from Rappuoli, Rutter, Mariagrazia Pizza and others. I recognized, however, that my main interests were more compatible with academic laboratory-based vaccine research. In 1998 I left Chiron for a full time Senior Scientist position at CHORI, which led to what unexpectedly would become my most productive and fulfilling period.

During the 1990s, three vaccine manufacturers, including Chiron, developed

serogroup C meningococcal conjugate vaccines in response to approximately 10,000 cases, including 1000 deaths in the UK in the previous decade. In contrast, no manufacturer was developing a meningococcal vaccine for Sub-Saharan Africa, where, during the same period, more than 1 million cases including more than 100,000 deaths had occurred. The difference? The African epidemics were caused by serogroup A strains that rarely caused disease in industrialized countries. The scientific know-how was available; but the pharmaceutical companies' profit margins to develop a serogroup A vaccine were simply too low.

In November 1998, I contacted Maria Teresa Aguado, Coordinator for Immunization, Vaccines and Biologicals at WHO, and proposed to create a not-for-profit virtual company that would develop a low cost serogroup A conjugate vaccine for Sub-Saharan Africa. I secured a WHO grant to analyze existing intellectual property on conjugation technology and the costs and timelines for developing and producing a low-cost meningococcal conjugate vaccine. These efforts, described in a Lancet publication,²⁹ ultimately led to a 10-y \$70 million grant from the Bill and Melinda Gates Foundation and the formation of a partnership between PATH and WHO, called the Meningitis Vaccine Project. Under Dr. Marc LaForce's leadership, the vaccine was licensed in India and introduced in December 2010 for mass immunization in Sub-Sahara. By the end of 2012, more than 100 million people were immunized. Early studies indicated that the vaccine was highly efficacious both in preventing disease and decreasing colonization, which is critical to establish herd immunity.

Unlike serogroup A and C strains, the capsule of serogroup B meningococcal strains cross-reacts with host antigens, and isn't a suitable vaccine target. In 1995, the first genome sequence was reported for *Hemophilus influenzae*. Under Rappuoli's direction, Chiron contracted with The Institute for Genomic Research (TIGR) to sequence the genome of a serogroup B meningococcal strain, which was used to identify potential new vaccine candidates.³⁰ With Rappuoli's blessing, I

selected three of the Chiron candidates to investigate at CHORI.

While genome studies can identify potential vaccine candidates, they don't necessarily reveal the function of the protein in the bacteria. My colleague, Sanjay Ram, now at the University of Massachusetts School of Medicine, observed that complement protein, factor H (fH), bound to *N. meningitidis*, which was critical for the bacteria to survive in human serum (Christoph Tang's lab at Imperial College in London made the same observation. The two papers were published a month apart in the Journal of Immunology^{31,32}). Collaborating with Ram we also discovered that fH bound to one of the three Chiron vaccine candidates called "Genome-Derived Antigen" (GNA) 1870. At the time, the antigens discovered by genome mining were identified only by their genome number. Because of this important function, we proposed that GNA 1870 be renamed factor H binding protein (fHbp).³²

In subsequent studies with Ram, and Jo Ann Welsch at CHORI, we identified that the binding of fH to fHbp was specific solely for human fH.³³ In non-humans in the absence of binding fH, complement activation on the organism is unregulated and proceeds to bacteriolysis, which helped explain why the meningococcus only causes disease in humans. The observation that fH bound fHbp also raised the question of whether binding of a host protein to a vaccine antigen might decrease immunogenicity. The preclinical fHbp immunogenicity studies were done in animals whose fH didn't bind to the antigen. These models, therefore, might not provide an accurate picture of fHbp vaccine immunogenicity in humans in which fH bound to the vaccine.

To investigate this question, Peter Beernink and I at CHORI, and Ram and Peter Rice at the University of Massachusetts School of Medicine, immunized human fH transgenic mice. Our studies showed that fH binding by the vaccine antigen decreased serum bactericidal antibody responses, possibly by covering epitopes and directing the antibody repertoire toward epitopes outside of the fH binding site.³⁴ Beernink also created a mutant fHbp with one amino acid change

that eliminated fH binding (arginine at position 41 was substituted with serine; designated as R41S). In two studies we found that mutant R41S fHbp vaccines elicited higher serum bactericidal antibody responses in human fH transgenic mice than control vaccines containing wildtype fHbp that bound human fH.^{34,35} These data indicated that the immunogenicity of current fHbp-based vaccines might be improved by introducing a single amino acid substitution to eliminate fH binding.³⁶

On the wall in my office, I have a picture of Jonas Salk immunizing a child. In the same frame, I have a small piece of paper—a prescription written by my doctor for my first polio vaccination. In another frame is Jonas Salk's autograph. As a child, I never imagined that some day I, too, might contribute to vaccine research that helped control two sometimes-fatal infectious diseases. These contributions, however, have been grounded on the dedicated research of many students and colleagues; in the vision of mentors who took the time and effort to invest in me; and in that perfect series of coincidences and convergences that brought me back to the wonders of infectious diseases and the power and promise of vaccines that can overcome them.

References

1. Money J, Granoff D. IQ and the somatic stigmata of Turner's syndrome. *Am J Ment Defic* 1965; 70:69-77; PMID:5830573
2. Pollitt E, Granoff DM. Mental and motor development of Peruvian children treated for severe malnutrition. *Int J Psychol* 1967; 1:93-102
3. Howard E, Granoff DM. Increased voluntary running and decreased motor coordination in mice after neonatal corticosterone implantation. *Exp Neurol* 1968; 22:661-73; PMID:5709812; [http://dx.doi.org/10.1016/0014-4886\(68\)90155-6](http://dx.doi.org/10.1016/0014-4886(68)90155-6)
4. Howard E, Granoff DM. Effect of neonatal food restriction in mice on brain growth, DNA and cholesterol, and on adult delayed response learning. *J Nutr* 1968; 95:111-21; PMID:5653270
5. Granoff DM, Howard E. Malnutrition in infancy and brain development. *J Pediatr* 1969; 75:732-3; PMID:5809852; [http://dx.doi.org/10.1016/S0022-3476\(69\)80480-4](http://dx.doi.org/10.1016/S0022-3476(69)80480-4)
6. Howard E, Granoff DM, Bujnovszky P. DNA, RNA, and cholesterol increases in cerebrum and cerebellum during development of human fetus. *Brain Res* 1969; 14:697-706; PMID:5822439; [http://dx.doi.org/10.1016/0006-8993\(69\)90209-1](http://dx.doi.org/10.1016/0006-8993(69)90209-1)
7. Plotkin SA. The history of rubella and rubella vaccination leading to elimination. *Clin Infect Dis* 2006; 43(Suppl 3):S164-8; PMID:16998777; <http://dx.doi.org/10.1086/505950>
8. Granoff DM, Nankervis GA. Infectious arthritis in the neonate caused by *Haemophilus influenzae*. *Am J Dis Child* 1975; 129:730-3; PMID:1080354

9. Granoff DM, Gilsdorf J, Gessert C, Basden M. *Haemophilus influenzae* type B disease in a day care center: eradication of carrier state by rifampin. *Pediatrics* 1979; 63:397-401; PMID:312483
10. Gessert C, Granoff DM, Gilsdorf J. Comparison of rifampin and ampicillin in day care center contacts of *Haemophilus influenzae* type b disease. *Pediatrics* 1980; 66:1-4; PMID:6967580
11. Granoff DM, Gilsdorf J, Gessert CE, Lowe L. *Haemophilus influenzae* type B in a day care center: relationship of nasopharyngeal carriage to development of anticapsular antibody. *Pediatrics* 1980; 65:65-8; PMID:6965531
12. Granoff DM, Basden M. *Haemophilus influenzae* infections in Fresno County, California: a prospective study of the effects of age, race, and contact with a case on incidence of disease. *J Infect Dis* 1980; 141:40-6; PMID:6965975; <http://dx.doi.org/10.1093/infdis/141.1.40>
13. Munson RS Jr., Shenep JL, Barenkamp SJ, Granoff DM. Purification and comparison of outer membrane protein P2 from *Haemophilus influenzae* type b isolates. *J Clin Invest* 1983; 72:677-84; PMID:6603479; <http://dx.doi.org/10.1172/JCI111017>
14. Barenkamp SJ, Munson RS Jr., Granoff DM. Subtyping isolates of *Haemophilus influenzae* type b by outer-membrane protein profiles. *J Infect Dis* 1981; 143:668-76; PMID:6972422; <http://dx.doi.org/10.1093/infdis/143.5.668>
15. Barenkamp SJ, Granoff DM, Munson RS Jr. Outer-membrane protein subtypes of *Haemophilus influenzae* type b and spread of disease in day-care centers. *J Infect Dis* 1981; 144:210-7; PMID:6974205; <http://dx.doi.org/10.1093/infdis/144.3.210>
16. Granoff DM, Boies EG, Munson RS Jr. Immunogenicity of *Haemophilus influenzae* type b polysaccharide-diphtheria toxoid conjugate vaccine in adults. *J Pediatr* 1984; 105:22-7; PMID:6610736; [http://dx.doi.org/10.1016/S0022-3476\(84\)80350-9](http://dx.doi.org/10.1016/S0022-3476(84)80350-9)
17. Weinberg GA, Granoff DM, Nahm MH, Shackelford PG. Functional activity of different IgG subclass antibodies against type b capsular polysaccharide of *Haemophilus influenzae*. *J Immunol* 1986; 136:4232-6; PMID:3486228
18. Shackelford PG, Granoff DM, Nelson SJ, Scott MG, Smith DS, Nahm MH. Subclass distribution of human antibodies to *Haemophilus influenzae* type b capsular polysaccharide. *J Immunol* 1987; 138:587-92; PMID:3491852
19. Einhorn MS, Weinberg GA, Anderson EL, Granoff PD, Granoff DM. Immunogenicity in infants of *Haemophilus influenzae* type B polysaccharide in a conjugate vaccine with *Neisseria meningitidis* outer-membrane protein. *Lancet* 1986; 2:299-302; PMID:2874327; [http://dx.doi.org/10.1016/S0140-6736\(86\)90001-2](http://dx.doi.org/10.1016/S0140-6736(86)90001-2)
20. Murphy TV, Granoff DM, Pierson LM, Pastor P, White KE, Clements JF, et al. Invasive *Haemophilus influenzae* type b disease in children less than 5 years of age in Minnesota and in Dallas County, Texas, 1983-1984. *J Infect Dis* 1992; 165(Suppl 1):S7-10; PMID:1588181; http://dx.doi.org/10.1093/infdis/165-Supplement_1-S7
21. Granoff DM, Shackelford PG, Suarez BK, Nahm MH, Cates KL, Murphy TV, et al. Hemophilus influenzae type B disease in children vaccinated with type B polysaccharide vaccine. *N Engl J Med* 1986; 315:1584-90; PMID:3491315; <http://dx.doi.org/10.1056/NEJM198612183152505>
22. Osterholm MT, Rambeck JH, White KE, Jacobs JL, Pierson LM, Neaton JD, et al. Lack of efficacy of *Haemophilus b* polysaccharide vaccine in Minnesota. *JAMA* 1988; 260:1423-8; PMID:3261350; <http://dx.doi.org/10.1001/jama.1988.03410100113035>
23. Murphy TV, White KE, Pastor P, Gabriel L, Medley F, Granoff DM, et al. Declining incidence of *Haemophilus influenzae* type b disease since introduction of vaccination. *JAMA* 1993; 269:246-8; PMID:8417244; <http://dx.doi.org/10.1001/jama.1993.03500020080036>
24. Murphy TV, Pastor P, Medley F, Osterholm MT, Granoff DM. Decreased *Haemophilus* colonization in children vaccinated with *Haemophilus influenzae* type b conjugate vaccine. *J Pediatr* 1993; 122:517-23; PMID:8463894; [http://dx.doi.org/10.1016/S0022-3476\(05\)83529-2](http://dx.doi.org/10.1016/S0022-3476(05)83529-2)
25. Lucas AH, Langley RJ, Granoff DM, Nahm MH, Kitamura MY, Scott MG. An idiotypic marker associated with a germ-line encoded kappa light chain variable region that predominates the vaccine-induced human antibody response to the *Haemophilus influenzae b* polysaccharide. *J Clin Invest* 1991; 88:1811-8; PMID:1752943; <http://dx.doi.org/10.1172/JCI115502>
26. Granoff DM, Shackelford PG, Holmes SJ, Lucas AH; The Collaborative Vaccine Study Group. Variable region expression in the antibody responses of infants vaccinated with *Haemophilus influenzae* type b polysaccharide-protein conjugates. Description of a new lambda light chain-associated idiotype and the relation between idiotype expression, avidity, and vaccine formulation. *J Clin Invest* 1993; 91:788-96; PMID:8450060; <http://dx.doi.org/10.1172/JCI116298>
27. Lucas AH, Azmi FH, Mink CM, Granoff DM. Age-dependent V region expression in the human antibody response to the *Haemophilus influenzae* type b polysaccharide. *J Immunol* 1993; 150:2056-61; PMID:8436834
28. Lucas AH, Granoff DM. Functional differences in idiotypically defined IgG1 anti-polysaccharide antibodies elicited by vaccination with *Haemophilus influenzae* type B polysaccharide-protein conjugates. *J Immunol* 1995; 154:4195-202; PMID:7706754
29. Jódar L, LaForce FM, Ceccarini C, Aguado T, Granoff DM. Meningococcal conjugate vaccine for Africa: a model for development of new vaccines for the poorest countries. *Lancet* 2003; 361:1902-4; PMID:12788589; [http://dx.doi.org/10.1016/S0140-6736\(03\)13494-0](http://dx.doi.org/10.1016/S0140-6736(03)13494-0)
30. Pizza M, Scarlato V, Masignani V, Giuliani MM, Arico B, Comanducci M, et al. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* 2000; 287:1816-20; PMID:10710308; <http://dx.doi.org/10.1126/science.287.5459.1816>
31. Schneider MC, Exley RM, Chan H, Feavers I, Kang YH, Sim RB, et al. Functional significance of factor H binding to *Neisseria meningitidis*. *J Immunol* 2006; 176:7566-75; PMID:16751403
32. Madico G, Welsch JA, Lewis LA, McNaughton A, Perlman DH, Costello CE, et al. The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance. *J Immunol* 2006; 177:501-10; PMID:16785547
33. Granoff DM, Welsch JA, Ram S. Binding of complement factor H (fH) to *Neisseria meningitidis* is specific for human fH and inhibits complement activation by rat and rabbit sera. *Infect Immun* 2009; 77:764-9; PMID:19047406; <http://dx.doi.org/10.1128/IAI.01191-08>
34. Beernink PT, Shaughnessy J, Braga EM, Liu Q, Rice PA, Ram S, et al. A meningococcal factor H binding protein mutant that eliminates factor H binding enhances protective antibody responses to vaccination. *J Immunol* 2011; 186:3606-14; PMID:21325619; <http://dx.doi.org/10.4049/jimmunol.1003470>
35. Beernink PT, Shaughnessy J, Pajon R, Braga EM, Ram S, Granoff DM. The effect of human factor H on immunogenicity of meningococcal native outer membrane vesicle vaccines with over-expressed factor H binding protein. *PLoS Pathog* 2012; 8:e1002688; PMID:22589720; <http://dx.doi.org/10.1371/journal.ppat.1002688>
36. Granoff DM, Ram S and Beernink PT. Does binding of complement factor H to the meningococcal vaccine antigen, factor H-binding protein, decrease protective serum antibody responses? *Clin Vaccine and Immunol* 2013;20: In press