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PARTICIPATING IN THE EVOLUTION OF TRANSFUSION MEDICINE FROM A DISPENSARY INTO A DISCIPLINE

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Abstract

Collecting, processing and dispensing blood for hemotherapy has evolved into Transfusion Medicine (TM), a newly recognized discipline. Joining my efforts to those of collaborators all over the world during this period of transformation, my scientific career spanned from the investigation of the immunogenetics of Bombay (OhOh) blood to establishing the academic TM program at the University of California, San Francisco (UCSF). The twin discoveries of class-specific antibodies against immunoglobulin A (IgA) causing anaphylactic transfusion reactions, and of anti-IgA of limited specificity defining *A2m(1)* as the first genetic marker of IgA led to the award of the Julliard Prize. My precocious appointment as the head of the Bombay Municipal Blood Center in India launched my academic career in 1969 as the Chief of the Blood Bank at UCSF Medical Center. Viral hepatitis, then the principal risk of transfusion, engaged me in the molecular analyses of purified hepatitis B virus (HBV) and its surface antigen (HBsAg). Consequently the first HBV vaccine, derived from infected plasma (superseded by cloned HBV-envelope protein), and hepatitis B immune globulin (HBIG) were developed for clinical trials that led to FDA-licensed biological products for prophylaxis and therapy. The advent of HIV/AIDS in the early 1980s raised renewed concern about transfusion safety and led me to push for anti-HBc blood screening for improved transfusion safety. The triennial International Symposia on Viral Hepatitis and Liver Disease (ISVHLD), which I started in 1972, continue to be the foremost forum for the contemporary assessment of hepatitis prevention and treatment. Besides viral hepatitis, I undertook multiplexed flow cytometric analyses for markers of infection by blood-borne viruses and their PCR-amplified gene products, kinetics of HIV replication in peripheral blood lymphocytes, leukocyte depletion for safer transfusion, and removal/inactivation of blood-borne viruses. The TM training and research programs I initiated at UCSF in the 1980s with NIH support enabled me to recruit new faculty members who continue to foster the worldwide advancement of transfusion safety.

Keywords

Transfusion reactions; Anti-IgA; Blood; borne infections; Viral hepatitis; Transfusion safety; Passive-active immunization

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PERSONAL BACKGROUND AND EDUCATION IN INDIA

Born in 1933, I was raised in Bombay where I received my entire education in English. After graduating from the Fellowship School, I went to St. Xavier's College and graduated in 1954 as a Medical Microbiology major. With the intention to pursue postgraduate training in virology, I joined the laboratory of D.D. Banker in the Department of Pathology at Seth Gordhandas Sunderdas Medical College and King Edward the VII Memorial (K.E.M.) Hospital. Instead of virology, I was assigned to work on blood groups and other genetic traits among endogamous castes for my Master of Science degree (1956). Then I was trained for a year in Hematology and studied Thalassemia, prevalent in the Lohana caste. L.D. Sanghavi and J.B.S. Haldane connected me to Rev. Fr. G. Fuchs, who was conducting anthropometric measurements among six aboriginal tribes in Western India. With antisera provided by Philip Levine I tested their ABO and Rh blood groups. In addition, I discovered the suppression of *ABH* genes in certain families with the Bombay (*OhOh*) phenotype.

Having trained in blood group serology and genetic hematology, I turned to transfusion-related clinical investigations in 1959 as a Ph.D. student. I worked at the newly formed Bombay Municipal Blood Center (BMBC), where I pursued doctoral work using ^{51}Cr -labelled red blood cells (RBCs) to study blood volume and RBC survival rates in health and disease, and earned my Ph.D. degree in 1964. I was engaged in 1961 to a lovely graduate student, Devi Trivedi, who was studying for her Master's degree at a college 300 miles away from Bombay. She left her program and we were married in 1962. During my doctoral thesis work I worked late in the evenings and week-ends, so she joined the BMBC as a social worker for voluntary donor recruitment so that we could at least have lunch together.

With my cumulative experience in blood groups, genetics, hematology, RBC survival studies, ambulatory transfusion service, exchange transfusion service, and my routine evening investigations of reactions in transfusion recipients at the K.E.M. Hospital, I was appointed as the Officer-in-charge to head the BMBC in 1964. This job entailed donor recruitment, public relations, and laboratory management--unlikely tasks for a Ph. D. scientist. During his visit to BMBC, Arthur Steinberg from the Western Reserve University in Cleveland, Ohio offered me a two year postdoctoral research fellowship beginning in February 1965.

TRAINING LEADING TO THE UCSF PROGRAM IN TRANSFUSION MEDICINE

In the Steinberg laboratory I was trained in Gm allotypes, immunochemistry, genetics, purification of plasma proteins, and audited immunology courses. My training in Cleveland formed the foundation for my academic career in the U.S.A. In 1965 my wife joined the Western Reserve University for graduate studies in speech and hearing. Once again she had to leave graduate school because our two sons were consecutively born in summer of 1965 and 1966. With the most generous support of Albert and Marguerite Kronheim in Cleveland we managed to raise our two sons, Jay and Shrikrishna (Kris), and comfortably cope with the transition to the American way of life. The Kronheims called our sons Cleveland Indians, who are now finally the champion team in baseball. Cleveland has been very good to us. My family, including the Kronheims, continues to play a pivotal role in my career.

At the 1966 AABB meeting in Los Angeles I was recruited by Hugh Fudenberg from the University of California, San Francisco (UCSF). During my 30 months in the Fudenberg laboratory, I published 10 papers and received the Julliard Prize for my work on anti-IgA at the 1969 ISBT Congress in Moscow. This accomplishment, combined with my blood banking background in India, led George Brecher to appoint me as Assistant Professor and Director of Blood Bank at the UCSF Medical Center in 1969.

At UCSF I trained 28 medical/graduate students and 45 M.D./Ph.D. postdoctoral research fellows, whose collective contributions are reflected in many of my publications. From a publication record of 220 papers and 12 books/monographs edited by me, I have selected in Table 1 topics highlighting my contributions to TM-related virology and immunology. The 15 topics cover three broad areas: antibodies to immunoglobulin A (IgA) and anaphylactic transfusion reactions; molecular immunology of hepatitis B virus (HBV) antigens and antibodies applied in passive/active immunization; and risk reduction in transfusion-transmitted HIV/AIDS.

ANTI-IgA CAUSING ANAPHYLACTIC TRANSFUSION REACTIONS

Unlike anti-Rh coated RBCs used for Gm typing, I developed a passive hemagglutination assay using a panel of purified IgA1 and IgA2 myeloma proteins, coupled with O Rh-negative RBCs by using the CrCl₃ method, for detecting antibodies against human IgA [1]. Similar assays using viral proteins (HBV/rhinoviruses) subsequently catapulted me into studying viral hepatitis.

Class-specific anti-IgA, reacting with both IgA1 and IgA2 proteins, are high-titer (>1024) IgG antibodies occurring in persons with absent IgA (aIgA), who suffer complement-mediated anaphylactic reactions to small transfusions of IgA-containing blood or blood products. In contrast, low-titer antibodies (<1:256) of limited specificity, reacting with only some IgA1/IgA2 proteins, are found in the serum of patients with normal IgA levels who occasionally suffer an anaphylactoid/allergic transfusion reactions [2]. The laboratory diagnosis of anti-IgA continues to guide the clinical management of patients with anaphylactic or anaphylactoid transfusion reactions caused by anti-IgA [3]. Other investigators, notably Gerald Sandler, have extended this seminal work.

Selective deficiency of IgA (dIgA), characterized by serum IgA less than 1% of normal level (284 mg/dL), is the most common immunodeficiency disorder in humans. A majority of dIgA individuals are normal and unaware of their immune deficiency, excepting rare patients with recurrent sino-pulmonary infections resistant to most antibiotics. The dIgA phenotype is a heterogeneous one occurring with varying worldwide frequencies (1:600 to 1:3000). To manage the rare anaphylactic transfusion reactions, Herbert Perkins and I established in 1975 the first registry of dIgA donors. We screened 73,569 blood donations in San Francisco and found 113 donors with dIgA and among them 13 with high-titered class-specific anti-IgA, which characterizes the homogeneous aIgA phenotype [4]. No anti-IgA was detected among the remaining 100 dIgA donors. Notably, patients who received blood products with passive transfusion of high-titer anti-IgA from the 13 aIgA donors reported no reactions. Since 1975 several dIgA registries have been established worldwide, including the latest one in Quebec in 2006. Why some persons produce class-specific anti-IgA without exposure to blood products is explained in part by isoimmunization to IgA occurring in intrauterine life [5]. Investigating the pedigrees of aIgA phenotype from the dIgA registries, using human genome data and comparative genomic hybridization, may reveal the pathogenesis of class-specific anti-IgA.

Routine anti-IgA screening of patients appears unwarranted for detecting those at risk of rare anaphylactic reactions because only one reaction occurred in 1980 among an estimated 160,000 patients transfused at UCSF Medical Center during last 40 years. Such uncommon occurrences are optimally managed by providing blood products devoid of IgA, i.e. washed RBCs or blood products from dIgA donors from regional registries. Patients with primary immune deficiency are now treated with gamma globulins devoid of IgA or dIgA plasma when anti-IgA becomes a clinical problem.

In contrast to class-specific anti-IgA characterizing the aIgA phenotype, anti-IgA of limited (narrow) specificity is encountered in individuals with normal levels of IgA, e.g. a multiparous

patient without any history of transfusion suffered an anaphylactoid transfusion reaction [6]. Her anti-IgA of limited specificity led us to define the first genetic marker of human IgA, the *A2m(1)* marker. I am very proud of my participation in uncovering the science underlying the discovery of *A2m(1)*, which recently led Erna van Loghem to report allelic *A2m(2)*. For the cumulative work on “Immunobiology of Human Anti-IgA” the Jean Julliard Prize was awarded to me by the International Society of Blood Transfusion (ISBT) at its 1969 Congress in Moscow.

VIRAL HEPATITIS

Viral hepatitis, being the principal risk to transfusion recipients, became my main research focus when I assumed responsibility for the Blood Bank at UCSF Medical Center. Baruch Blumberg discovered the hepatitis B surface antigen (HBsAg, originally termed the Au/Australia antigen) and based on population studies his group suggested that Au was a recessively inherited genetic marker of beta-lipoproteins. Evidence against this genetic hypothesis was established by my group in 1974. This observation was subsequently confirmed by Cladd Stevens and Palmer Beasley.

In 1972 I initiated the first International Symposium on Viral Hepatitis and Liver Disease (ISVHLD), which now convenes at 3-year intervals for the contemporary assessment of advances in virology, immunology, epidemiology, prevention and treatment of hepatitis A-E.

A single chronic carrier of HBsAg was repeatedly plasmapheresed to obtain 65 liters of plasma over a two year period for our research on the structure and function of HBsAg. An excess of virally encoded envelope protein synthesized by infected liver cells circulates as 20 nm particles in the plasma of infected persons. Illustrated in Figure 1 is zonal ultracentrifugation in cesium chloride gradients to obtain purified 20 nm particles (peak A), separated from their polymeric filamentous forms (peak B) and the 40 nm virions (peak C) derived from HBV-infected plasma. During my sabbatical leave at the Walter and Eliza Hall Institute (Melbourne, Australia) I used HBsAg from peak A to experimentally prove that immune response to HBsAg is T-cell-dependent and that HBsAg-binding lymphocytes correlated with antibody response. While I focused on the immunochemical structure of HBsAg and humoral and cell-mediated immune responses to purified 20 nm particles, I gave the concentrated HBV in peak C to Harold Varmus for cloning viral DNA and for the characterization of viral polymerase by Donald Ganem. Darrell Peterson in my laboratory isolated the 25 kD polypeptides and 30 kD glycopeptides from the 20 nm purified particles (peak A), and partially determined their amino acid sequences, which were identical. We therefore concluded that a single virally-encoded 25kD protein was post-translationally glycosylated and self-assembled into 20 nm particles. After attending the 1978 ISVHLD in San Francisco William Rutter at UCSF set out to clone HBV DNA and determine its complete nucleotide sequence. Notably, he found that the N- and C-terminal amino acid sequences of our 25/30 kD proteins perfectly matched the HBV-S gene nucleotide sequence.

Purified HBsAg, which we originally used for hemagglutination assay for antigen and antibodies, proved useful in the development and clinical trials of HBIG as well as the plasma-derived HB-Vaccine manufactured by Merck. The plasma-derived vaccine was subsequently replaced by a safer recombinant HBV-S gene product expressed in the yeast system. In 1980, I recruited Pearl Toy and both of us engaged in the multi-center clinical trial led by Cladd Stevens for preventing perinatal HBV transmission by immunizing newborns with HBIG and plasma-derived or recombinant vaccine.

In collaboration with William Robinson from Stanford University, I established Hepatitis B core antibodies (anti-HBc) as a definitive marker of previous HBV infection. George Grady

from the Massachusetts State Laboratory determined in my laboratory the clinical significance of anti-HBc in HBsAg-negative chronic liver disease (CLD). Because HBV DNA was detectable in some HBsAg-negative/anti-HBc-positive plasma I initiated anti-HBc screening of donated blood to try to enhance transfusion safety.

As a Fulbright scholar in 1980 I joined Pierre Tiollais at the Pasteur Institute in Paris and collaborated with Christian Brechot to learn the molecular biology of HBV and its application in studying HBV-related CLD. Upon my return from Paris, Hubert Blum from Freiburg joined me at UCSF and together we deciphered the biology of HBV replication in human hepatocytes. By in situ hybridization and immunohistochemical analysis of liver tissue from HBsAg-negative patients with CLD, we revealed the presence of occult HBV infection in CLD. Our data on the high incidence of HBV-HIV co-infection was presented at the 1984 ISVHLD in San Francisco initiated my research in HIV/AIDS.

PREVENTION OF HIV/AIDS THROUGH TRANSFUSION

With acquired immunodeficiency syndrome (AIDS) observed among hemophiliacs and the first case of transfusion-transmitted AIDS reported in a baby transfused at UCSF, concern about transfusion safety reached a peak level. The nascent TM Program at UCSF was boosted by the first award of a Program Project Grant for Transfusion-Transmitted Infections by the National Heart, Lung, and Blood Institute (NHLBI). As the Program Director I collaborated with project investigators Michael Busch, Margaret Chesney, Don Ganem, Marvin Garovoy, and Jay Levy. Further, I received two multi-million dollar contracts for (i) Flow cytometric detection of HIV antigens/antibodies and nucleic acids, and (ii) Assessment of HIV infection with seronegative blood. With a team of postdoctoral scientists we studied HIV replication in co-cultures with human lymphocytes, and applied it to assessing leukocyte filtration as a primary means of enhancing transfusion safety. I recruited Edward Murphy as the epidemiologist to strengthen our TM Program.

The Transfusion Safety Study (TSS) funded by NHLBI was designed by James Mosley, who invited me to direct the UCSF component. I recruited Elizabeth Donegan to follow up the patients transfused with a single bolus of HIV-positive blood. Over 90% developed AIDS in 3–4 years. I gave her published data to Peter Duesberg to stop him from campaigning against HIV as the cause of AIDS.

Over 20 million dollars provided by NHLBI during 1984–94 laid a firm foundation for the UCSF TM Program, which has been taken to unprecedented success by Pearl Toy, Michael Busch, and Edward Murphy. I am currently engaged in developing an inactivated HIV vaccine with genetic diversity to induce broadly neutralizing antibodies against population-prevalent HIV-1. This challenging task I hope will be the final fulfillment of my academic career at the University of California.

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Figure 1.

Two successive ultracentrifuge runs for isopycnic banding in a B-29 zonal rotor yielded semipurified HBsAg (HAA) from infected plasma of donor DD. A final cycle in a B-14 zonal rotor separated the morphologic forms into three peaks by rate sedimentation in CsCl_2 gradient. (reproduced from Vyas, et al, Journal of Immunology, 1972)

Figure 2.
The author, Girish N. Vyas in a photograph taken in July 2007.

Table 1

Key contributions to immunology and virology related to Transfusion Medicine.

Set	Topic
I	Antibodies to IgA causing anaphylactic transfusion reactions and their clinical management with blood products from donors with selective deficiency of IgA.
II	Discovery of the first genetic marker of IgA, <i>A2m (I)</i> ; allo-immune anti-IgA cause anaphylactoid reactions.
III	Hemagglutination assay for viral antigen/antibodies (HBV and rhinoviruses).
IV	Anti-HBs in immune plasma and Hepatitis B immune globulins (HBIG) for passive prophylaxis—multi-center clinical trials, most recently in orthotopic liver transplantation.
V	Biochemical structure, protein conformation, amino acid composition and sequence of virally-encoded 25 kD HBV envelope protein (HBsAg).
VI	HBsAg is a T-cell dependent antigen and cell-mediated immune response in HBsAg-immunized animals.
VII	Evidence against the genetic hypothesis of HBsAg as a recessive trait.
VIII	Purification of plasma-derived 20 nm HBsAg and its use as a vaccine against HBV infection.
IX	Anti-HBc as a marker of current/recent and chronic HBV infection; surrogate marker of non-A,non-B hepatitis and AIDS.
X	HBV DNA probes for dot blot detection of HBV in plasma and characterization of replicative HBV in liver cells of HBsAg-negative/anti-HBc positive donors.
XI	Histomolecular detection and analysis of HIV-infected cells in cultures.
XII	Multiplexed flow cytometric assays for antibodies, antigens, and PCR-amplified nucleic acid sequences of blood-borne viruses, viz. HBV, HCV, and HIV.
XIII	Routine leukocyte depletion for safer blood transfusions.
XIV	HIV in PBMC cocultures and PCR to assess risk of infection from seronegative blood.
XV	Neutralizing antibodies against HIV-1: use of CD4-enriched cell substrate for HIV expansion in a bioreactor for an inactivated HIV vaccine candidate.