PASPCR Commentary – September 2007

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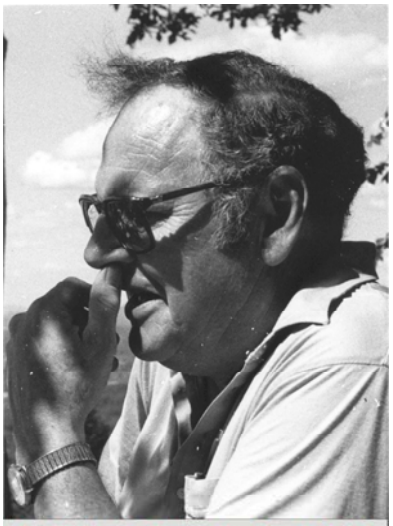
Serendipity can govern the course of ones career, providing you are prepared for it. As a young undergraduate Zoology major at the University of Massachusetts I had never imagined the scientific voyage I would embark upon. Now that I reflect back on it for this commentary, I am amazed and thankful for the colleagues and opportunities I have had to help paved the path to reach this point.

Undecided as a senior with an interest in cell biology what I should do after graduation, . my poultry genetics professor unexpectedly called me into his office. Realizing my indecision, Dr. J. Robert Smyth began his story about a specific chicken in his flock of 2000 that he used to analyze inheritance of genes on micro-chromosome with plumage color patterns as readouts. This Brown Leghorn chicken began to develop white feathers shortly after reaching the age of sexual maturity and laying eggs and within months of that became blind. With foresight, Dr. Smyth mated this chicken back to her father resulting in several offspring that "turned white and went blind". Pose to me was the opportunity to analyze the cellular events underlying this depigmentation and blindness for a graduate thesis while Dr. Smyth himself concurrently analyzed the genetics. This was a wondrous time where I learned hypothesis driven experimental exploration and critical thinking assessment of data. Research was captivating and enticing, characteristics I had not fully or passionately gleaned from course work as an undergraduate. Using light and electron microscopy of biopsied feather and ocular tissues, we were able to piece together a pathologic scenario where melanocyte dysfunction and concurrent inflammation resulted in removal of the melanocyte population from these tissues [1]. Subsequent limb bud transplantation studies, immune response evaluation and analysis of bursectiomized neonates (spearheaded by Dr. Sue Lamont) suggested that there was both an innate defect in the melanocytes and a hightened immune response in the delayed amelanotic chicken [2, 3], or DAM chicken as it was fondly called in Smyth's lab. But what came first, the melanocyte defect or the immune response, remained a mystery. But what did become apparent was that the DAM chicken was an animal model for Vitiligo!! We were notified of this by Dr. Aaron Lerner,


J. Robert Smyth, Jr., Ph.D.

Chair of Dermatology at Yale University School of Medicine, who had heard about the DAM chicken while on a transatlantic flight sitting next to a Massachusetts Eye & Ear Institute scientist who was a colleague of a UMass Psychology professor who was mentoring me on the assessment of visual acuity in the DAM chicken!!

So I had completed my graduate research and was getting ready to sit down and compose my dissertation with thoughts of arduously searching for a post-doctoral fellowship being delegated to the back of my mind when a telephone call arrived. Dr. James Nordlund invited me down to Yale to train with him and Aaron Lerner. What an opportunity that was!! This was the center for pigment cell research at the time with the likes of John Pawelek, Ruth Halaban, Gisela Moellmann, Lynn Lamoreux, Drew Lambert, Janos Varga, Joe McGuire, Jean Bolognia, and Seth Orlow buzzing around the department. In addition, Aaron and Jim had just begun the first program project on Vitiligo that also included Harvard University, the University of Pennsylvania, the University of Massachusetts, and Howard University. This was an incredible place to train and continue my small project on the DAM chicken, renamed the Smyth chicken. Gisela (morphologist extraordinaire) and I pursued electron microscopic histochemical evaluation to demonstrate the induction of hydrolytic enzymes that preceded the melanocyte dysfunction, predating the knowledge of caspases. Ruth and I established cultures of melanocytes from embryonic neural tubes to demonstrate that Smyth melanocytes could undergo premature cell death isolated from the immune system, predating the knowledge of apoptosis. Biochemical experiments demonstrated that elevated levels of melanogenesis correlated with the induction of melanocyte death in the Smyth line, predating the damaging effects of oxidative stress. So began formulation of the hypothesis that alterations in pigment synthesis could lead to melanocyte apoptosis and may also elicit an autoimmune response resulting in Vitiligo [4, 5]. And more collaborations ensued while in New Haven. Lynn had identified a potential mouse model for Vitiligo, tentatively called the Vitiligo mouse. She and I eventually demonstrated that postnatal melanocyte death could occur independent of an immune response in this model as observed in the histological evaluation of tissue, mice treated with cyclosporine, and melanocytes isolated in culture [6, 7]. However, this mouse has turned out to be a mutant allele at the Microphthalmia loci and hence not related to Vitiligo but appropriate for analysis of mechanisms for melanocyte specific apoptosis.

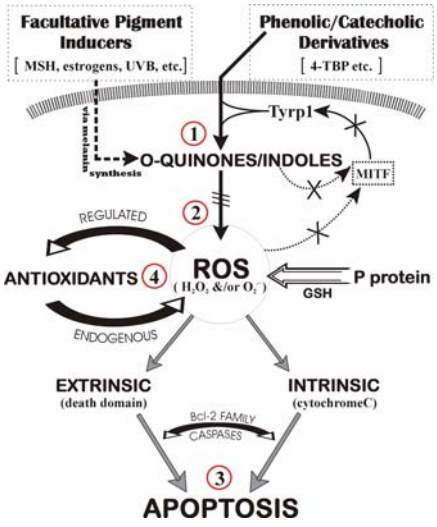

Gisela Moellmann, Ph.D.

At some point I was itching to move on. Jim had left Yale a few years prior to chair the Dermatology Department and develop a research program at the University of Cincinnati College of Medicine, which was undergoing a renaissance towards becoming a research one institute. Jim's design was to develop a multidiscipline group of scientist who all focused on the melanocytes to foster collaboration and training in this discipline. Duly intrigued I committed, initially for a short term but have never had the desire to leave. I have had the great fortune to collaborate with colleagues that are at or have passed through our department, including Zalfa Abdel-Malek, Larry Rheins, Estela Medrano, Jamal Farooqui, Ram Tripathi, Huiquan Zhao, Rangaprasad Sarangarajan, Caroline Le Poole, and Prashiela Manga. Again, the Smyth chickens came with me, via a u-haul on a steamy August trip replete with a flat tire that required an unexpected overnight stay in a hotel with an hasted early departure before the sun rose so …… (opps, but that is a different story and I am digressing). Once my lab was set up, my first graduate student Lisa Austin began to characterize the autoimmune response that developed in the Smyth chicken. To our surprise, the recently cloned tyrosinase related protein (Tyrp-1) proved to be the target of autoantibodies in the Smyth chicken that appeared prior to the development of feather amelanosis and could wane after complete depigmentation was developed [8, 9]. This subsequently left the task of identify how aberrant melanocyte could generate a melanocyte specific autoimmune response to immunologist more talented that I. However, in the research environment at UC we were able to pursue the issue of innate melanocyte defect in vitiligo using the human system quite readily. Estela had perfected a method for culturing melanocytes from adult skin tissue [10]. Under these conditions, melanocytes from the pigmented areas of numerous Vitiligo patients could be readily established and appeared to proliferate normally, albeit exhibiting unique ultrastructural aberrations particularly dilated rough endoplasmic reticulum [11]. However, when stressed by tittering down several growth factors in the culture medium or treating melanocytes with phenolic/catecholic derivatives, premature apoptosis occurred more rapidly in vitiligo-derived melanocytes than controls. Exciting, and to be continued…..

The ability to culture adult human melanocytes and assess them with a plethora of experimental approaches with valued, passionate colleagues afforded me the opportunity to explore the pathophysiology of various other human depigmentary disorders. Oculocutaneous albinism (OCA) had recently been genetically defined as a tyrosinase related (OCA-1) and a P-protein related (OCA-2) form, leaving many albino patients genetically undefined. Paternal twin African-American boys were born at University Hospital, one with and one without albinism. Melanocytes cultured from foreskins demonstrated that the albino had normal expression of tyrosinase and P-protein, but not Tyrp-1, thus defining OCA-3 [12 – please note the cast of characters who helped on this project listed as authors]. Chediak-Higashi Syndrome (CHS) is a rare form of OCA with additional neutrophil dysfunction. Again, melanocytes from a patient with CHS born in Cincinnati's Children's Hospital afforded us the opportunity to demonstrate the mistrafficking of tyrosinase and it aberrant secretion leading to muted pigment synthesis [13], predating the cloning and identification of LYST. And then there is Hermansky-Pudlak Syndrome (HPS) in which the OCA is accompanied by a bleeding diathesis, ceroid storage disease, and pulmonary distress!! A serendipitous collaboration with

William Gahl and then Marjan Huizing at the National Institutes of Health has lead to the culturing of melanocyte from numerous patients representing the extremely heterogenetic syndrome. Ultrastructural and biochemical analysis of HPS melanocytes paved the way for defining the BLOCs they represent and the multiple trafficking pathways that exist in the melanocyte [14]. [Please reread the amazing story compiled by Marjan Huizing in her March 2007 Commentary].

However, I must come back to Vitiligo. We have continued to explore the inherent melanocyte defect in this disease by assess a specific subtype, contact/occupational Vitiligo. Vililigo melanocytes upon exposure to a specific phenolic/catecholic derivative 4-tertiary butyl phenol (4-TBP) undergo premature apoptosis. It appears that 4-TBP is used as a substrate by Tyrp-1 to putative produce related quinones that in turn generate oxidative stress in the melanocyte. The 4-TBP generated premature apoptosis can be ameliorated by antioxidants, specifically catalase, suggesting that Vitiligo melanocytes may have alter defense mechanism and/or apoptotic regulation that confers sensitivity to physiological and/or environmental events that precipitate the disease [15]. Our current working hypothesis for the inherent defects in Vitiligo melanocytes are depicted in the accompanying illustration below and represent the horizon for our future studies.



We propose that exposure of melanocytes to facultative pigment inducers and/or phenolic/catecholic derivatives (e.g. 4-TBP), via the action of Tyrp1, results in the generation of intracellular quinones (1) that in turn produces ROS (2) and induces oxidative stress. Production of ROS stimulates the apoptotic pathway (3) that potentially could lead to cell death and/or interacts with endogenous/regulated antioxidants (4) that could lead to cell survival, the balance of which is genetically determined. Therefore, the melanocyte's ability to survive these exogenous triggers of oxidative stress is dictated by the capacity to tolerate and/or combat ROS and prevent apoptosis; processes that may be genetically impaired in vitiligo melanocytes.

Speaking of horizons, I have recently become president of the National Vitiligo Foundation (www.nvfi.org.) and am committed to moving it through a renaissance. Again, another passionate colleague, Robert Haas, has joined me as Executive Director to help build the foundation. Our goals are to [1] raise money for research focusing on basic science and therapeutic development for the disease, [2] raise awareness of the disease in the general public and health profession, and [3] provide support, tools, and information for patients with the disease. This is most rewarding because it provides me the opportunity to give back to the community of patients whose disease has driven my career and research developments; due and honored justice!!

REFERENCES

1] Boissy RE, JR Smyth Jr and KV Fite. 1983. Progressive cytologic changes during the development of delayed feather amelanosis and associated choroidal defects in the DAM chicken line. A vitiligo model. Am J Pathol 111:197-212.

2] Lamont SJ, RE Boissy and JR Smyth Jr. 1982. Humoral immune response and expression of spontaneous postnatal amelanosis in the DAM line chickens. Immunol Comm 11:121-127.

3] Boissy RE, SJ Lamont and JR Smyth Jr. 1984. Persistence of abnormal melanocytes in immunosuppressed chickens of the autoimmune "DAM" line. Cell Tissue Res 235:663-668.

4] Boissy RE, GE Moellmann and JR Smyth Jr. 1985. Melanogenesis and autophagocytosis of melanin with developing feather melanocytes of delayed-amelanotic (DAM) chickens. In: *Pigment Cell 7*: Proceedings of XIIth International Pigment Cell Conference, Giessen, Germany, 1983. Editors: Bagnara J, Klaus SN, Paule E, Schartl M, University of Tokyo Press, Tokyo, Japan. pp. 731-739.

5] Boissy RE, GE Moellmann, AA Trainer, JR Smyth Jr and AB Lerner. 1986. Delayed-amelanotic (DAM-Smyth) chicken: Melanocyte dysfunction in vivo and in vitro. J Invest Dermatol 86:149-156.

6] Lerner AB, T Shiohara, RE Boissy, KA Jacobson, ML Lamoreux and GE Moellmann. 1986. A possible mouse model for vitiligo. J Invest Dermatol 87:299-304.

7] Boissy RE, GE Moellmann and AB Lerner. 1987. Morphology of melanocytes in hair bulbs and eyes of vitiligo mice. Am J Pathol 127:380-388.

8] Austin LM, RE Boissy, BS Jacobson and JR Smyth Jr. 1992. The detection of melanocyte autoantibodies in the Smyth chicken model for vitiligo. Clin Immunol Immunopath 64:112-120.

9] Austin LM and RE Boissy. 1995. Tyrosinase related protein-1 is recognized by autoantibodies from vitiliginous Smyth chickens. Am J Pathol 146:1529-1541.

10] Medrano EE and JJ Nordlund. 1990. Successful culture of adult human melanocytes obtained from normal and vitiligo donors. J Invest Dermatol 95:441-445.

11] Boissy RE, Y-Y Liu, EE Medrano and JJ Nordlund. 1991. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. J Invest Dermatol 97:395-404.

12] Boissy RE, H Zhao, WS Oetting, LM Austin, SC Wildenberg, YL Boissy, Y Zhao, RA Sturm, VJ Hearing, RA King and JJ Nordlund. 1996. Mutation in and lack of expression of tyrosinase related protein-1 (TRP-1) in melanocytes from an individual with tyrosinase-positive oculocutaneous albinism: A new subtype of albinism classified as OCA3. Am J Hum Genet 58:1145-1156.

13] Zhao H, YL Boissy, Z Abdel-Malek, RA King, JJ Nordlund and RE Boissy. 1994. On the analysis of the pathophysiology of Chediak-Higashi syndrome: Defects expressed by cultured melanocytes. Lab Invest 71:25-34.

14] Richmond B, M Huizing, J Knapp, A Koshoffer, Y Zhao, R Morris, WA Gahl and RE Boissy. 2005. Melanocytes from Hermansky-Pudlak Syndrome types 1-3 express distinct defects in cargo trafficking. J Invest Dermatol 124:420-427.

15] Manga P, D Sheyn, F Yang, R Sarangarajan and RE Boissy. 2006. A Role for Tyrosinase-related protein 1 in 4-tert-butylphenol-induced toxicity in melanocytes. Implications for vitiligo. Am J Path 169:1652-1662.