



**Commentary:  
Vitiligo – Lessons learned from the Smyth line chicken**

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For the past 18 years, the Smyth line chicken model for vitiligo has been part of my scientific career. I consider myself very fortunate to have had the opportunity to work with this animal model and to have found a career I feel so passionate about.

I grew up in Wiesensteig, a small town in South-Western Germany, very close to Ulm [Ulm was the venue for the 9<sup>th</sup> meeting of the European Society for Pigment Cell Research in 2000]. I did well in school and dreamt of becoming a veterinarian some day. However, during my last year of high school, I worked with a veterinarian and soon realized that this was not the career for me. After high school graduation, my parents allowed me to spend a summer in New Brunswick, Canada, with friends of the family. There, I enrolled for one year at the University of New Brunswick in Fredericton. From then on, my educational path led me to an Associate Degree from the Nova Scotia Agricultural College, in Truro, Nova Scotia; a B.S. in Animal Science and then a M.S. degree in Reproductive Endocrinology, both from the University of Guelph, Guelph, Ontario; and lastly a Ph.D. degree in Immunology from Cornell University, Ithaca, New York. Other than loving science, having the desire to keep learning, and being open to interesting opportunities that presented themselves at the right times, there was not much planning involved in this educational path. During my graduate studies, I discovered my love for research and teaching. My M.S. research was on the role of steroid and pituitary hormones in the ovulation cycle of the hen and my dissertation research on thyroid-immune interactions in chickens. My enthusiasm for teaching was instilled by excellent teachers in my classes and by the Cornell faculty members in charge of the introductory biology laboratory course I taught as part of my graduate teaching assistantship. By the end of my graduate studies, I knew that I wanted a career in teaching and research and that immunology would continue to be my major area of interest.

When I graduated in January of 1988, I bypassed a post-doc position to become an Assistant Professor in the Department of Biological Sciences at Smith College, a liberal arts college for women in Northampton, Massachusetts. Suddenly, within days of completing my dissertation, my sole focus was preparing and teaching lecture and lab courses in immunology, cell biology, human reproductive endocrinology, and later, comparative animal physiology. Although research had to take a back-seat to teaching, I started a project on immune system development and function in the congenitally hypothyroid (hyt/hyt) mouse. With the help of immunologists at the University of Massachusetts and Amherst College, I learned to work with mice and to write grant applications, but with the exception of a small NSF grant for Women in Science, I was only able to get internal funding for this project. The funding allowed me to provide undergraduate students with research opportunities, but the future of developing an independent research program was not very promising. I was struggling and, in retrospect, didn't think it was such a great idea to have skipped the post-doc experience.



J. Robert Smyth, Jr.

At this point (late 1989), Dr. J. Robert (Bob) Smyth, Jr. and the Smyth line chicken came to my rescue. As many of you know, Dr. Smyth, a member of PASPCR, was a poultry geneticist at the University of Massachusetts in Amherst. In the early 1970's, he discovered one white female in a flock of Brown line chickens. A true geneticist, he was immediately interested in this mutation. He back-crossed the amelanotic hen into the Brown line and through selection of amelanotic offspring, he developed the DAM (delayed amelanosis) line of chicken – later known as the Smyth line chicken. This line of chicken exhibits a spontaneous, vitiligo-like, post-hatch loss of melanocytes in feather and choroidal tissue. Vitiligo occurs in approximately 70% to 95% of hatch-mates, with about 70% of those affected expressing complete

depigmentation in adulthood (>20 weeks of age). The development of the Smyth line chicken model and research conducted by Dr. Smyth's research group is comprehensively described in Smyth (1989) and I'll only highlight some of the observations that formed the foundation of my work with this animal model.

At the time Dr. Smyth and I made contact, it had been well established that the post-hatch, vitiligo-like loss of pigmentation in the Smyth line chicken was due in part to an inherent melanocyte-defect, manifested in irregularly-shaped melanosomes with pigmented membrane extension and the formation of melanosome-containing autophagocytic compartments. Histological examination of the feather and eye tissue revealed that the loss of pigmentation was associated with mononuclear leukocyte infiltration, suggesting involvement of the immune system in the loss of melanocytes. Through immunosuppression studies it was clearly shown that the melanocyte defect alone was not responsible for the development of Smyth line vitiligo and that the pigment cell loss in this animal model required an active immune system. Moreover, these studies pointed towards a more important role of cell-mediated than humoral immunity in melanocyte destruction. Other indications of an autoimmune component in the pigment loss in Smyth line vitiligo were the presence of melanocyte-specific autoantibodies, a genetic association with the major histocompatibility gene complex (MHC), and the prevalence of other autoimmune disorders like hypothyroidism (4 % to 5 %) and an alopecia areata-like feathering defect (2 % to 3 %) as well as uveitis (20-40%), frequently resulting in blindness.



Left to right: Smyth line chick with normal pigmentation at hatch; normally pigmented Brown line female; vitiliginous Smyth line females; feather samples: growing portion of normally pigmented (top) and vitiliginous (bottom) feathers

After talking with Dr. Smyth and learning about vitiligo and the Smyth line chicken, I instantly wanted to be part of this research effort. With Dr. Smyth as my mentor and through the R15 NIH Academic Research Enhancement Program, I was successful in receiving funding to study the role of the immune system in Smyth line autoimmune vitiligo. This was the jump-start I needed to develop a research program that would make research a viable and exciting part of my career and that would allow me to make significant contributions to biomedical research.

As I learned more about vitiligo and attended PASPCR meetings, the strength and value of this animal model and the opportunities it offered became more and more apparent to me. Dr. Smyth had developed three MHC sublines of Smyth line (SL101, SL102, SL103) and parental control Brown line chickens (BL101, BL102, BL103) and one other control line, the Light Brown Leghorn (LBL101). All three Smyth line sublines had a similar incidence of vitiligo but the age of onset of vitiligo and the incidence of associated autoimmune disorders differed between these lines (Smyth and McNeil, 1999). All three of the parental Brown lines had a low incidence of vitiligo (<2%), and the LBL subline had no incidence of vitiligo. This differential susceptibility of MHC-matched SL, BL and LBL lines to develop vitiligo was further substantiated based on studies using 5-azacytidine, a DNA methylation inhibitor. When treated with 5-azacytidine, 71% of the parental BL101 control chickens and 0% of LBL101 control chickens expressed vitiligo (Sreekumar et al., 1999). Hence, the Smyth line chicken model provides opportunities to study MHC-matched individuals that are 1) vitiligo-susceptible and predictably express vitiligo (SL), 2) vitiligo-susceptible but low or not expressing (BL), and 3) vitiligo resistant (LBL). Moreover, when studying the immunopathology involved in Smyth line vitiligo, we became aware of the uniqueness of the feather as a target tissue: 1) the removal of a growing feather is easier and less invasive than taking a blood sample; 2) the feather regenerates; and 3) feathers can be repeatedly collected for down-stream analysis prior to, during,

and after the development of vitiligo. With the predictably high incidence of vitiligo, this animal model provides the unique opportunity to study the evolving lesion in the same individual. Other important advantages of the avian animal model in this context are the ability to establish melanocyte cultures from 72h embryos as well as from growing feathers and to simultaneously and repeatedly obtain embryos/siblings from various families. (e.g., fertile eggs can be collected for two to three weeks and then set in the incubator at the same time).

With the high teaching load at Smith College and undergraduate research assistants who had full schedules themselves, research progress was slow and my life revolved primarily around work. Although my husband was the primary care-taker of our son, it became more and more difficult for me to juggle family and work responsibilities. Therefore, it was time to seek a position with a better balance between research and teaching.



Poultry Science Center

Once again, I was lucky. The University of Arkansas's newly formed Center of Excellence for Poultry Science was seeking an avian immunologist. The job description was completely in line with my experiences and expertise, including teaching immunology, and I was captured by the idea of being part of building a new program. I successfully applied for the position and we relocated to Fayetteville, Arkansas. The transition was easy as far as teaching was concerned, but I had to prove myself in research, making ties with the poultry industry and developing a research program addressing fundamental and applied aspects of poultry immunology. To my surprise, the administration was very supportive of my research on the Smyth line chicken model. To integrate this project into my research program, I needed to establish breeding populations in Arkansas. For this we had to follow stringent import regulations, including hatching and raising the chicks in HEPA-filtered isolation rooms. It took more than two years before the birds kept in isolation passed all the required health tests and could be housed on the University of Arkansas poultry farm. Despite disrupting the continuation of our vitiligo research effort, the quarantine process led to the exciting discovery that the expression of vitiligo in Smyth line chickens is dependent on an environmental trigger. The incidence of vitiligo in the first chicks hatched in Arkansas dropped to 10 % and vitiligo could only be observed in females. Although we first suspected that the clean conditions in the HEPA-filtered isolation rooms were removing the environmental trigger, it turned out that it was the vaccination with herpesvirus of turkey (HVT) which we, due to the isolation, had not administered to the chicks (Erf et al., 2001). HVT is an alphaherpesvirus commonly used in commercial chicken production as a live virus vaccine to protect chickens from Marek's disease caused by serotype 1 Marek's disease viruses (MDV-1). HVT is a non-oncogenic serotype 3 MDV isolated from turkeys that causes only minor inflammatory lesions, but, like other MDV, exhibits strong tropism for feather follicles. With the exception of the Smyth line, this vaccine does not affect pigmentation in chickens. We conducted several studies to examine the association between HVT vaccination and Smyth line vitiligo and found live HVT, but not dead HVT and other live-virus vaccinations, to be a reliable environmental factor for the expression of Smyth line vitiligo.

Most of our research on the Smyth line chicken was recently summarized in Wick et al. (2006) and Erf (in press). Key findings in recent years include: the demonstration of melanocyte specific cell-mediated immunity in vitiliginous Smyth line chickens; death of melanocytes by apoptosis apparently induced by cytotoxic T cells; the ability of IFN- $\gamma$  to act as a trigger for the expression of vitiligo in non-HVT vaccinated Smyth line females but not males; Th1 polarized immune activity in the target tissue; altered antioxidant levels and oxidative damage in Smyth line feathers and in embryo-derived melanocytes; and heightened immune activity to HVT in Smyth line compared to control chickens. Based on these and other observations, we put forward our current working hypothesis to try to explain the multifactorial nature of Smyth line autoimmune vitiligo:

*The translocation of the HVT infection to the feather and the resulting anti-viral immune activity causes changes in the local melanocyte environment that cause the inherently "fragile" Smyth line melanocyte to express antigens/signals that trigger a melanocyte-specific immune response.*

Current projects include a preliminary study on differences in protein expression in embryo-derived melanocytes from Smyth and control lines using a proteomics approach (Nick Tinsley, M.S.). Through this work, we have discovered that Smyth line autoantibodies also have specificity to heat-shock proteins, a phenomenon now being pursued by one of my Honors students (Regina Finley). Bryan Plumlee, a Ph.D. student, is examining gene-expression using qRT-PCR in feathers throughout the development of Smyth line vitiligo. His focus is primarily on cytokines, chemokines, and melanocyte-specific proteins. [With the sequencing of the chicken genome in 2004, we finally have tools to study gene-expression at the RNA and protein level.] Another M.S. student, Kristin Bateman, is currently examining the HVT connection in Smyth line vitiligo by examining viral load and stage (latent versus productive) in feathers and spleen using a time-course approach. Another Honors student (Collin Trovillion) is examining the involvement of macrophages in the development and progression of Smyth line vitiligo, and a high school student (Ashley Smith) is conducting a science project on the effect of additional vitamin supplementation on the incidence and severity of Smyth line vitiligo. Additionally, we are collaborating with scientists at Uppsala University in Sweden who are conducting quantitative trait analyses on the genetic susceptibility of the avian spontaneous autoimmune disease models, including the Smyth line chickens. [Dr. Susanne Kerje and Dr. Olle Kämpe (Department of Medical Science) and Dr. Leif Andersson (Department of Medical Biochemistry and Microbiology)]. They currently are examining the F2 population of the BL101 and SL101 crosses, and in January 2007 the first, although small, batch of the pure SL101, BL101, and LBL101 populations hatched in Sweden.

After this brief description of my journey with the Smyth line chicken, I hope you will agree that many lessons can be learned from this animal model for autoimmune vitiligo. The Smyth line chicken offers unique opportunities to study the interplay between genetic susceptibility, environmental factors and the immune system, that lead to the development of anti-melanocyte autoimmune activity. The similarities of the clinical manifestations and pathological progression between human and Smyth line vitiligo, together with the easy, non-terminal, repeatable access to the autoimmune lesion, the predictability of the disease, the ability to visually monitor the disease development, and the availability of MHC-matched parental and non-vitiligo-susceptible controls, make the Smyth line chicken an excellent model to study spontaneously developing autoimmune vitiligo.

Lastly, I would like to thank my mentor, friend and collaborator Dr. J. Robert Smyth, Jr. whose excellent scientific insight and tireless work in recognizing, developing and validating this valuable animal model has resulted in many significant contributions to vitiligo research for over 30 years.

With the retirement of Dr. Smyth in 1996, the poultry farm at the University of Massachusetts was closed and many valuable genetic lines of chickens were lost. To date the Arkansas breeding populations of the SL101, BL101 and LBL101 sublines are the only remaining genetic lines of the Smyth line animal model. I am hoping that we will be able to build more research collaborations and will be happy to provide cells, tissue samples, eggs and chicks.

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