E. E. Just Award Lecture

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ABSTRACT
What started as a game of discovery for a child with a chemistry set has resulted in a lifetime of fulfillment and dedication to science. It is deeply rewarding to continuously let my curiosity ask questions for which there are no known answers. Equally rewarding has been the opportunity to mentor young students and postdoctorate graduates through their formative years in science.

When I was a child growing up, my parents had an orchard. In the orchard, they cultivated oranges. But my father expanded his one-man operation to other crops including corn, peppers, and tomatoes. At our house, we also had several fruit trees. At nights in the summer, I would be awakened by hard thumps on the tin roof of the building where we parked vehicles and farm equipment. These sounds indicated that either a mango or an avocado had fallen from a tree. Early in the morning, I would climb up on the roof and collect as many of them as I could. Then I would set up a small stall by the door to our house and sell my goods to passersby. When the corn was in and had dried, it would take hours to shell by hand, but it brought a great price. So what did I do with all the money I made? I bought the most amazing chemistry set! It was like magic to me. That was it for me. I was hooked. Even though neither of my parents had a formal education beyond secondary school, and no one in my family at that time had gone to a university, I wanted to be a scientist. My father worked three jobs to take care of my six siblings, my mother, and me. It was a great sacrifice to send me to college. When I first arrived there, I did not do so well. It took me a while to academically catch up with my peers, but eventually I did. The most important lesson I learned in college was to have a good work/study ethic. The second was chemistry. While I was in college, my dad died in a tragic automobile accident that killed two men and left 14 children without a father. My mother took over raising and financially supporting my siblings and me, and she set for us a very high example of success. Even though she did not have a formal high school or college education, she gave us all an education and then helped all of us to obtain postgraduate education. After completing this monumental task, and 10 grandchildren later, she ran and was elected to Congress. Did I say she has been a hard act to follow? She is my source of inspiration.

As an aspiring student of science, I was very fortunate to have outstanding mentors. By the time I arrived at graduate school, I was well prepared for the rigors of the lab. I earned a PhD in chemistry with a focus on nucleic acid/protein interactions in a tremendously rigorous program that provided me with the type of fundamental quantitative skills that have served me well the rest of my academic life. Even courses like molecular dynamics have provided me with the type of broad scientific base that is needed to work in a highly competitive discipline.

If there is one piece of advice I would give to anyone interested in a career in science, or in any other field for that matter, it would be: be flexible. Let everything around you guide you in whatever is your quest. Don’t think the answer you thought up is the correct one. It might be. Yet it might not always be the only one or the complete story. Many years ago, I heard someone say, “the truth is somewhere in the middle.” How can that be in science? Science is about precision. Absolutes. Certainty. Proof. Then I was told that I was “more interested in the middle than the truth.” In other words, many
so-called truths have changed, been proven wrong, developed further, or amended. All of these new discoveries were made because someone did not accept something as an incontrovertible truth. The development of the humanized bone marrow/liver/thymus (BLT) mouse model is a prime example of this (Melkus et al., 2006).

Our laboratory had been developing gene transfer vectors based on the human immunodeficiency virus (HIV) (now called “lenti” vectors; Douglas et al., 1999). We had great success in using these vectors to transduce human hematopoietic stem cells in vitro but did not have a way to establish whether or not this could be translated into future clinical applications, as we lacked suitable in vivo animal models. We set up a human hematopoietic stem cell transplantation model in which we could demonstrate the capacity of these vectors to efficiently transduce these cells, resulting in multilineage reconstitution with human immune cells. Even though this approach was highly successful, and we were able to demonstrate the presence of transduced B-cells, monocytes, macrophages, dendritic cells, and so on, we were not able to detect any human T-cells in these animals (Gatlin et al., 2001). This was a problem because we wanted to develop gene therapy for AIDS, and T-cells are a key cell type during the course of HIV infection. We were aware of another in vivo model in which human thymic/liver tissue implanted in a different type of immunodeficient mouse developed into a well-defined human thymus–like organ. These thymic “organoids” produced human thymocytes and could be infected with HIV. Unfortunately, these thymocytes did not exit the organoid, and there was no peripheral reconstitution with human T-cells, which severely limited the use of this model. Interestingly, in contrast with the transplant model described above, in this tissue implant model there were no other type of human immune cells present, only thymocytes. We decided to test whether these two systems could be combined into one that would have a complete human hematopoietic system. When the student who was carrying out these experiments discussed his project with the “experts,” he was told that his approach had been extensively tested and that it had failed in all its previous interactions. He was utterly distressed about all the negativity we encountered from colleagues and grant reviewers. However, I reminded him that we had one ace in the hole. What we had noted was the fact that, whereas all tissue implants were done with one specific strain of mice, all hematopoietic stem cell transplants were performed in a different strain of mice. The strain of mice used for tissue implants was not used for transplants, because of resulting low engraftment levels. The strain of mice used for transplants was not used for implants, because those mice had a shorter life span. When we implanted one small piece of human liver tissue sandwiched between two small pieces of thymic tissue into the mice that could be efficiently transplanted with autologous human hematopoietic stem cells, the results of this combination approach were dramatic. In essence, the animals developed a bona fide human thymic organoid, and, in addition to a full complement of T-cells, they also developed virtually all other bone marrow–derived human hematopoietic cells (Melkus et al., 2006). In short, we had created a chimeric human/mouse model that had a functioning systemic human immune system. These animals were designated bone marrow/liver/thymus mice, because they are the results of a bone marrow transplant with autologous CD34+ hematopoietic stem cells of animals coimplanted with liver and thymic tissue. Since then, the name has been shortened to BLT.

Since our original description of this model, BLT mice have been extensively used to study numerous aspects of human immunology, hematopoiesis, cell biology, microbiology, and so on. However, the area in which BLT mice have made their most significant contribution is HIV and AIDS research.

According to estimates by the World Health Organization (WHO) and the Joint United Nations Program on HIV/AIDS, 34 million people were living with HIV at the end of 2011. That same year, some 2.5 million people became newly infected, and 1.7 million died of AIDS-related causes, including 230,000 children. BLT mice have opened numerous new lines of investigation that were not possible even a few years ago. In addition, they have contributed to our detailed understanding of the complex interactions between pathogen and host. The first highly significant contribution using the BLT model was to our understanding of mucosal HIV transmission (Sun et al., 2007). Specifically, in developed countries like the United States, the vast majority of transmission events occur during unprotected anal intercourse. We therefore investigated the reconstitution with human lymphoid cells of the gastrointestinal tract of BLT mice. Our result showed that the gastrointestinal tract of BLT mice closely resembles that of humans and, in addition, that these mice (like humans), if exposed rectally, can be infected by HIV. In addition, we were able to demonstrate the critical role of cryptopatches as the essential anlagen for the development of organized immune tissue in the gut (Nochi et al., 2013). Subsequently, we found that the entire female reproductive tract of BLT mice is reconstituted with human immune cells. Vaginal HIV transmission is responsible for the majority of new HIV infections worldwide. This prompted us to determine whether human immune cells were present in the female reproductive tract of BLT mice. Remarkably, the presence of human immune cells in this tissue rendered female BLT mice susceptible to vaginal HIV transmission (Denton et al., 2008). We subsequently leveraged the fact that BLT mice are susceptible to rectal and vaginal HIV transmission to evaluate a variety of different inhibitors for their ability to prevent HIV acquisition. The results demonstrated that several novel HIV inhibitors, as well as several FDA-approved HIV inhibitors, could efficiently block both rectal and vaginal HIV transmission. Subsequently, the FDA has approved two of these agents for use in HIV prevention.

Despite the availability of numerous antivirals, every day, 1000 children are newly infected with HIV. A significant number of these children acquire the infection via breast-feeding. Novel approaches to oral HIV prevention are urgently needed. In this regard, we have recently shown that BLT mice recapitulate key aspects of oral HIV transmission, and this information was used to test novel approaches to prevent infection. One unexpected but nevertheless very interesting outcome of these experiments was the demonstration that human breast milk has a very strong and highly reproducible innate inhibitory activity that prevents both HIV infection and its transmission (Wahl et al., 2012). These experiments addressed a paradigm in the field: If milk has an anti-HIV activity, how is it possible for infants to become infected? For this, one has to keep in mind that children born to HIV-infected mothers receive liters and liters of HIV-infected milk during their first six months of life and yet only ~1/10 become infected. Thus the results obtained in the BLT model strongly support the protective effect of milk in preventing HIV transmission and have opened up new avenues of investigation addressing the nature of the innate viral inhibitors present in human breast milk and have provided support for the WHO’s recommendation to provide breast milk to infants of HIV-infected mothers.

Now our research includes a new ambitious goal: to find a cure for HIV/AIDS. In this regard, BLT mice have proven to be an outstanding tool for discovery. BLT mice have been shown to establish the same type of HIV persistence that is observed in HIV patients treated with antiretroviral therapy (Denton et al., 2012). This provides an opportunity to use this model to evaluate novel...
approaches aimed at eradicating HIV from infected patients. Whether or not the observations made in BLT humanized mice can eventually be translated into human clinical applications has yet to be determined. Nevertheless, to date, the information obtained from experiments using BLT mice has been demonstrated to be highly relevant when making evidence-based decisions concerning which novel approaches have the best chance of success when implemented in humans and should be given priority in development pipelines.

What started as a game of discovery for a child with a chemistry set has resulted in a lifetime of fulfillment and dedication to science. It is deeply rewarding to continuously let my curiosity ask questions for which there are no known answers. Equally rewarding has been the opportunity to mentor young students and postdoctorate graduates through their formative years in science. Their curiosity and dedication inspires me, and I have learned from them as much, if not more, than I have taught. I look toward the future with the hope that together we will continue to contribute to the advancement of science in general and ultimately find a cure for HIV/AIDS. For each one of my students and trainees, I have one wish: that they will surpass anything in science that I have accomplished during my lifetime.

REFERENCES