The discovery of T–B cell lineages: An interview with Professor Max D Cooper

Chen Dong[1,2,*], Max D Cooper[3,*]

[1]Shanghai Immune Therapy Institute, Shanghai Jiao Tong University, Shanghai, China
[2]Institute for Immunology, Tsinghua University, Beijing, China
[3]School of Medicine, Emory University, Georgia, USA
*Correspondence: chendong@tsinghua.edu.cn (C.D.); mdcoope@emory.edu (M.D.C.)
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In the early 1960s, despite a general understanding of antibodies and immunoglobulins, their source and their relationship with anti-infection immunity remained largely enigmatic. However, this changed with the groundbreaking discoveries of Dr. Max D Cooper, professor at Emory University School of Medicine. In the 1960s, Dr. Max D Cooper discovered the existence of two different lymphoid lineages, B and T cells, which are now widely recognized as the cornerstone components of the adaptive immune system. B and T cells are responsible for humoral and cellular immunity, respectively. This discovery served as the foundation of modern immunology. Here, Dr. Max D Cooper shares his inspiring journey, from career choice to research experiences, as well as offering sage advice to aspiring immunologists.

Chen Dong

Max D Cooper

Chen Dong: As my PhD mentor, Dr. Max Cooper provided invaluable mentorship and guidance all along the years of my study. Dr. Max Cooper grew up in Mississippi; he then pursued his medical education with a specific interest in the field of immunology. He honed his skills through medical training in London and clinical allergy experience in San Francisco. I must tell you, Max, that clinical allergy is still in its early stages in China. There is a considerable scarcity of hospitals with dedicated clinical allergy departments, and it is an area I am working to develop in China. After that, you decided to become a researcher and went to Minnesota. So let me first ask you what the field of immunology was like when you arrived there and why you decided to take on this research-focused fellowship?

Max Cooper: I was encouraged to pursue an academic career, which I initially thought would take some courage at that time. I soon realized that there were only two routes to advancing up the academic ladder: a heavy teaching workload or a novel research endeavor. As such, I had to start over again to learn how to conduct proper research. I applied to several famous immunologists, including Macfarlane Burnet in Melbourne, Jonathan Uhr at New York City, and Bob Good in Minnesota. All of them accepted me except Bob Good. He requested that I wait a year, as his lab was quite full, I was advised by my friends to convince him to accept me immediately. I was required to secure my own funding through grants in order to be accepted.

Chen Dong: You opted for an academic career and started enhancing your research skills. When Bob gave you chickens to study, were you surprised? After all, you were originally trained as a pediatrician. And if I recall correctly, you were born in the year of the rooster. Was that a coincidence, or was there any particular significance to it?

Max Cooper: Well, I was drawn to the field of pediatric immunology, especially children with inherited susceptibility to infections because I wanted to gain a deeper understanding of how the immune system develops and functions, with the aim of improving treatments for immunodeficiency diseases.

Chen Dong: What did we know about immunology or lymphocytes at that time?

Max Cooper: Well, we knew about antibodies and immunoglobulins, as well as the basic structure of IgG; we knew that plasma cells produced antibodies. Additionally, a finding was reported in *Poultry Science* by a graduate student from Ohio State, Bruce

Text edited by Hao Cheng (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China), Qi Xing (Institute for Immunology and School of Medicine, Tsinghua University, Beijing, China)

Glick, who thought that the bursa of Fabricius, a cloacal organ in chickens, might have something to do with their sexual maturation. He found that its removal from young chickens had no effect on their sexual maturation. A colleague later requested some of Glick's chickens to demonstrate antibody production after immunization. When the colleague returned to reported that only some of the chickens produced antibodies, they realized that Glick's removal of the bursa of Fabricius was the reason. Accordingly, they redid the experiments and found that the failed antibody responses correlated with the early removal of bursa of Fabricius. They sent their reports to *Nature*, but although the results were clear, it wasn't considered a topic of general interest at that time. As such, the research was published in *Poultry Science*. Later, Jacques Miller was studying the development of a lymphoid malignancy and found that it seemed to depend on the thymus. To understand how this occurred, he removed the thymus from newly born mice and noticed that they were not developing well and were susceptible to infections, leading him to realize the importance of the thymus in the development of the immune system. Studies in Robert Good's lab in Minnesota, where I was working, had similar findings, coming from other sets of clues. The prevailing theory then was that the thymus gave rise to lymphocytes, which seeded to the periphery. By labeling DNA in lymphocytes, James Gowans had shown that some of them became plasma cells that produced antibodies. So, the prevailing idea was that the thymus assisted in the development of lymphocytes, which could differentiate into plasma cells and produce antibodies, forming a single lineage.

At the time I was examining children with Wiskott–Aldrich syndrome, an inherited-disease complex was characterized by X-linked immunodeficiency and susceptibility to infections, particularly bacterial infections. As they aged, if these boys contracted the herpes simplex virus, instead of being controlled, the infection would progress to become fatal. When I examined tissues from some of the patients who did not survive the herpes infection, I found that their thymus was atrophic, lacked sufficient lymphocytes, and their blood was also deficient in small lymphocytes, but these patients possessed lymphocytes in their lymph nodes and an abundance of plasma cells as well as high levels of gamma globulins. The single-lineage theory just did not fit well with these findings.

In the meantime, other researchers had been studying the impact of thymus and bursa removal in chickens with inconsistent results. One group in Australia noticed that treating chickens with testosterone, which inhibited bursa development, resulted in reduced antibody production, and some of their chickens had an underdeveloped thymus. They interpreted their results to indicate that the bursa controls antibody production and delayed-type hypersensitivity, the bone marrow somehow controls graft-versus-host disease, whereas the thymus seemed to control graft rejection. However, this picture did not fit well at all with the contemporaneous findings in mice. One of the problems was that all of us conducted our studies on chickens using different strains, and different strains of chickens have different levels of maturation. Given the evidence against single-lineage differentiation in our Wiskott–Aldrich syndrome patients, I decided to go back to the chicken model and use whole-body irradiation in a near-lethal dose to wipe out immune system components that develop prior to hatching, and then immediately after hatching, surgically remove either the thymus, bursa of Fabricius, both, or neither. I then waited several weeks for their immune system to recover from the irradiation, before testing the experimental subjects. The results were absolutely clear-cut. Bursectomy after near-lethal irradiation eliminated germinal center development, plasma cells, gamma globulin and antibody production, whereas thymectomy and irradiation depleted the small lymphocyte population and reduced graft-versus-host capability and delayed type hypersensitivity. Suddenly, it was clear that these were two separate lineages of lymphocytes with different functions.

Chen Dong: Were you excited at that time? Did you think about the implications for a long time at that moment?

Max Cooper: I did not sleep for a week; I was so excited. My mentor, Bob Good, was traveling, and I called him as the new finding unfolded. At that moment, I knew that I had mapped out the next decade of my research. Lymphoid lineages could be divided into T cells or B cells according to their origin in the thymus or the bursa. The implications were far-reaching, such as the realization that T cells are incapable of producing antibodies, the need to identify the equivalent of the bursa in mammals, and the potential to use this information to develop a roadmap for the study of stem-cell-based development of immunity. Using contemporary information provided by others, we could begin to classify different immunodeficiency diseases based on thymus/T cell deficiency, bursa/antibody deficiency. We also needed to know where the B cells were generated in mammals in order to begin studying the early aspects of their B cell development.

Chen Dong: Your findings served as the foundation of current lymphocyte biology by demonstrating the existence of B and T lineage cells, responsible for humoral and cellular immunity, respectively. That is a remarkable contribution.

Max Cooper: Immediately, the discovery was well received by physicians as it aligned with what they had observed in their patients. However, basic immunologists took more time to come around the idea.

Chen Dong: Subsequently, you moved to Birmingham, and you started your own career. You studied evolution in chickens and humans. How would you describe your experience in Birmingham? Also, how did you combine both clinical and fundamental research in your work?

Max Cooper: You accurately described what we were doing. During that period, I worked closely with a graduate student, Paul Kincade, to study early B cell development in chickens. First, we mapped out the developmental pattern of immunoglobulin gene expression, starting with IgM and moving on to IgG and IgA, with a focus on class switching. Our experiments involved removing the bursa at different times to assess the effect on the expression of these immunoglobulins. We found that removing the bursa after birth resulted in IgA deficiency but elevated IgM and IgG levels, whereas removing it earlier in development, through embryonic bursectomy, caused a substantial elevation in IgM levels but no IgG or IgA expression. If we intervened even earlier, we could prevent the expression of all three classic immunoglobulins. Later, we also showed that ligating the

cell surface IgM on B cells during early bursal differentiation resulted in apoptotic cell death. We did not know the underlying mechanism at that time, but we did observe cell death in the anti-immune-injected chicken embryos as one of the things we learned. Then we started applying our knowledge to classify immunodeficiency diseases and lymphopoiesis. We spent a considerable amount of time working back and forth between our patients and the lab, with many researchers joining our team at various stages.

Chen Dong: Furthermore, you discovered pre-B cells and defined early steps of B cell differentiation.

Max Cooper: During my first sabbatical, after six years in Birmingham, I had the opportunity to work with a renowned developmental biologist and immunologist, John Owen, in Av Mitchison's lab at University College London. John and his colleagues thought that B cells were probably generated in hematopoietic tissues like fetal liver. At that time, I believed that they were coming from gut-associated lymphoid tissues, like Peyer's patches and the appendix, and many of my experiments coincided with that idea. When I was on sabbatical, however, I also worked with Geoffrey Dawes and his Oxford University colleagues, who were fetal physiologists. We made antibodies to sheep immunoglobulins and used these to time the onset of B cell development, which occurs around 70 days into the 150-day gestational period of lambs. With Geoffrey's young surgical colleagues, we conducted an experiment involving twin sheep. We removed the hematopoietic tissues from one twin embryo at 65 days of gestation to verify that there were no B cells present and then removed the entire intestine from the other twin. A few weeks later, we observed that B cell development was completely unaffected by early removal of the gut.

John Owen and Martin Raff developed a method to grow mouse fetal liver pieces in culture, and after a few days of growth, we observed the in vitro generation of B cells. These results revised a widely held view that had persisted for over a decade by showing that B cells are generated in mouse hematopoietic tissues.

Chen Dong: You have always been interested in evolutional immunology. I recall during my time in the laboratory, Robin was studying frogs, and Brent Passer was studying catfish; I still remember his hands stained with catfish blood. But why did you eventually decide to focus on sea lamprey?

Max Cooper: Well, I started those experiments with Jan Klein, who was then at the Max Planck Institute in Tubingen, Germany. We were both fascinated by how lymphocytes developed. Jan had a particular interest in the evolution of the major histocompatibility complex gene and had made several important contributions to this area. One of my interests was determining whether T cells or B cells came first in evolution, a question that I was frequently asked. At that time, we knew that research from multiple labs had shown that the most basal-jawed vertebrates possessed immune systems similar to our own. Along with thymus and hematopoietic tissues where T and B cells were generated; the cartilaginous fishes also used many of the same genes to generate antibody diversity and T-cell-receptor diversity. However, we were unable to examine species antecedent to jawed vertebrates as we knew about them only as fossil remains. We decided to study lampreys and hagfish, two jawless vertebrates that are even more ancestral and still exist, in order to shed light on this question.

Using *in situ* hybridization, Jan Klein's lab identified small round cells in hematopoietic tissues of lamprey larvae that expressed Spi-B, a PU.1 gene family member that is pertinent for B lymphopoiesis specifically and hematopoiesis generally. This led to the question of whether lampreys have lymphocytes and prompted us to collaborate. We collected cells from hematopoietic tissues with light scatter and morphological features of lymphocytes. At the time, we could get sequencing of cDNA libraries done by companies because human genome had been provisionally completed. We obtained a couple of thousand cDNA sequences, but our annotation of the sequences using the National Bioinformatics Institute database did not reveal any of the cardinal genes of the mammalian immune system, including TCR, BCR, MHC, *Rag1*, and *Rag2* genes. So we went back to get another couple of thousand gene sequences, but still did not find any obvious immune system genes. Still, we knew that previous studies conducted by Bob Good and others had suggested that lampreys were capable of producing antigen-specific agglutinins in response to immunization with human red blood cells. Zev Pancer, a developmental and molecular biologist, then joined us in experiments to see if we could detect cells in the act of responding.

We injected 3- to 4-year-old lamprey larvae with live *Escherichia coli* bacteria, sheep erythrocytes, phytohemagglutinin, and pokeweed mitogen and then analyzed a population of cells that looked like our lymphoblasts. We found numerous leucine-rich repeats (LRRs) in the selected cDNA library, which was initially very disappointing as LRRs are used by basically every living creature on our planet. They are very old gene blocks that are used for many different things. However, a summer student wished to learn molecular biology techniques, so we assigned her the sequencing of the LRRs as a learning project. At the end of the summer, when we found that each of her first 21 expressed sequence tags (EST) were different, the realization dawned on us that this kind of diversity could underly the diversity of agglutinin responses observed earlier. We then examined hundreds of LRR sequences made by stimulated and unstimulated lampreys to find that almost all of them had a different sequence, with the very rare exception of duplicates occurring in immuno-stimulated lampreys. We named them as variable lymphocyte receptors (VLRs). The VLRs are expressed on the cell surface, are highly variable, and provided a recognition system. Furthermore, we were able to show that these VLR-expressing B-like cells gave rise to plasma cells that secrete their multivalent variable lymphocyte receptors.

Then came another big surprise for me. I thought that B cells must have evolved before T cells, but that turned out to be wrong. Subsequent studies revealed the presence of other VLR genes, including VLRA and VLRC, which were used by cells with gene expression patterns consistent with our $\alpha\beta$ T cells and $\gamma\delta$ T cells, respectively. This led us to conclude that two T-like

cell lineages and one B-like cell lineage originated from a common ancestor of both jawed and jawless vertebrates over 500 million years ago. Moreover, we think it is most likely that the two types of highly diverse antigen receptors, LRR-based vs Ig-based, are yet another example of convergent evolution. The primordial availability of these Lego-like building blocks to generate a diversified repertoire of receptors allowed for the specific recognition of antigens.

Chen Dong: So, you also discovered that the earliest lymphocytes possess both humoral and cellular components, specifically B and T cells, which you did not anticipate at the time. Now, when you think back, would it be reasonable to assume that these early life forms had both B and T cells?

Max Cooper: I think that the genetic programs for different lymphocyte pathways must have evolved near the dawn of vertebrate evolution, but what they used for recognition receptors is a complete mystery. The two different kinds of receptors must have evolved much like the divergent evolution of vision in primitive invertebrates that developed eyes or at least the ability to perceive light, using lenses with completely different chemical and protein structures. Any visual organ that could allow an organism to see what it might eat or what might be coming to eat it would be selected as a fitness value.

Chen Dong: Max, I still remember when I read your paper. I was in Yongjun Liu's lab; at that time, I had just joined MD Anderson from Seattle. I killed my evening time talking with Yu-hsi and other Chinese postdocs. I came across your paper, which was wonderful. I was so delighted. Then I read your article in the annual review, which was talking about your career. I wrote an email to you, saying: "what a life, what a career, because you finished a circle." You initially discovered the presence of both B and T cells in chickens, then went on to study these cells in humans before finally identifying the earliest known lymphocytes possessing both T and B components in sea lamprey. It was remarkable to see that the beginning and the end of your scientific career converged on the same conclusion. The same cell types, B and T cells in chickens and mammals, and B- and T-like cells in sea lamprey. I was so happy to see that paper and read your article.

Max Cooper: Thank you. But you never finish, that's the fun. There are always new questions.

Chen Dong: Prior to the discovery of lymphocytes in sea lampreys, do you believe there were already lymphocytes that possessed natural killer (NK)-or innate lymphoid cell (ILC)-like cells?

Max Cooper: One possibility is the presence of receptors akin to those found in natural killer cells. We found cells that utilize the same or very similar genetic program as human NK cells and differentiate into cells that you cannot distinguish from our own NK cells. They exhibit all the hallmark features of NK cells, including the presence of NK lineage granules. We also discovered cells with a genetic program similar to that of ILC2, but not type 1 or type 3.

Chen Dong: Given that sea lampreys do not produce IFNg, it is unlikely they possess ILC1. However, do they produce IL-4, IL-13, and IL-5?

Max Cooper: Our findings, published in the *Journal of Immunology*, indicate the presence of an IL-4-like receptor in sea lampreys. The studies were primarily conducted by Jean-Louis Boulay and were a collaboration between Boulay, Louis Du Pasquier, and Bill Paul.

Chen Dong: The sea lamprey also has a high number of *Il17* genes, though some were lost during evolution. Are these lymphocytes or innate lymphocytes responsible for producing IL-17?

Max Cooper: Yes, T-like cells are responsible for producing IL-17, and their receptors are expressed on B-like lymphocytes. Our studies have shown that IL-17 can affect the activation of B-like cells.

Chen Dong: As we progress from sea lampreys to human beings, what do you think governs the evolution of our immune system? Environmental factors, self-driven diversification, or natural selection?

Max Cooper: That's a deeper philosophical question. I believe it is the selection of survival. Our genome has undergone duplications at least twice, from a single gene to two and then to four, which has generated diversity and new functions through gene amplification. That would be selected on the basis of the functional advantages as described by the theory of evolution. The immune system is also subject to these rules.

Chen Dong: Recently, I found myself reflecting on the organization of the immune system and the interplay between its various components. Because as scientists, we often employ a reductionist approach, studying individual cells and dividing them based on their developmental stages and phenotypes, focusing on a special program. However, when confronted with pathogens, it becomes apparent that both B and T cells must work together to provide comprehensive protection to the host. This integration of humoral and cellular immunity has likely been a crucial aspect of our immune system throughout evolution. While we recognize B and T cells as key players, there are many other cells within the immune system, such as $\gamma\delta$ T cells. Is there a special job or responsibility for $\gamma\delta$ T cells in mammals? I still do not quite get why we need them. Can you shed light on their significance, and would we be able to cope without them?

Max Cooper: I believe that $\gamma\delta$ T cells must play a vital role, but I cannot provide you with specific answers. Our understanding of the infections that lampreys encounter is limited, with only a few examples of viruses and bacteria having been studied in depth. Most of the pathogens that infect lampreys are yet to be well-defined or thoroughly investigated. Nonetheless, it stands to reason that all hematopoietic cells work together in a coordinated manner to fight off infections. However, the exact mechanisms by which these cells interact to enable effective responses to pathogens are still enigmatic.

Chen Dong: Max, let us delve into the implications of B and T cells in vaccine development. What do you think of current vaccines? While there are concerns about the declining titers of the

neutralizing antibodies, we say that memory B cells can still differentiate into plasma blasts, and so on. How do you believe that our vaccines can be improved based on our current understanding of B and T cells?

Max Cooper: Well, in terms of specific antibodies, it is important to identify common epitopes. However, I do not currently have a definitive answer on this matter, do you?

Chen Dong: I don't have one either. I think for RNA viruses that replicate and mutate rapidly, proper antigen boosts are necessary to keep up with the evolving pathogen environment, unless we are willing to accept live infections.

Chen Dong: Max, your career in the field of immunology has been remarkable, with numerous fundamental principles discovered in immunology and during evolution. In your opinion, what are the other areas of interest in current immunology or lymphocyte biology that you would recommend to the next generation of immunologists? With so many breakthroughs having been made by you and other researchers, some may feel that all the mysteries have been solved.

Max Cooper: I am incredibly fortunate, and I attribute much of my success to working with talented individuals like you. However, I do not believe that all the mysteries have been solved. Until we have a complete understanding of immunodeficiencies, the basis for lymphoid malignancies, and how to treat them effectively, we cannot claim to have a full understanding of the immune system and its workings.

Chen Dong: So, you suggest starting with diseases that have not yet been fully deciphered to date?

Max Cooper: Precisely. Also, I believe that a solid hypothesis is crucial. Despite the huge amount of data that can be generated with the new advancements, such as single-cell analysis, data alone are not sufficient for generating meaningful results. A clear hypothesis and set of research questions are necessary to guide the interpretation of the data.

Chen Dong: Indeed, a critical evaluation of the data is essential to uncovering its implications. May I ask for your thoughts on the current state of immunology within the global community? Additionally, for young immunologists, do you have any comments regarding the opportunities and challenges within the field, and any advice for their professional development?

Max Cooper: Technology changes; new technologies come along. So my advice would be to choose a problem that truly interests you, find a successful expert in that field, choose a specific project that you believe can make a meaningful impact, and work hard. The selection of your laboratory and mentor is paramount. Choose someone who is successful—someone with whom you can communicate effectively. It has to be a bidirectional give-and-take relationship. That's the only way it really works. I do not remember if I told you this when you came to my lab, but this has always been my advice: if I have a student, I expect them to teach me as much as I can teach them. If you do not have that interaction, that's a no-go.

Chen Dong: Yeah, you have to know what you are doing, and you have to become your own master in a way. So, Max, it has been lovely talking with you. I still recall the time when I was your student. I stood by you when you corrected my English on papers; we do not do that anymore. That was really a lesson in science and also in English. I vividly remember those moments. And also, I would like to take this opportunity to say what I admire most about you is your persistence in science. You persisted in your work for many years, you trusted yourself, you persisted in your research direction, and then success followed you. When I celebrated my lab's 20-year anniversary, you came, and that was very moving as well. So, at that moment, I also made a commitment to my lab that I would continue for at least 20 more years in science. Hopefully, we will keep each other in good company during the journey.

At the end, I want to say that, your discovery of B and T lymphocytes and the definition of cellular and humoral immunity has laid the foundation for modern immunology, and your work continues to inspire and guide future generations of researchers in this field. We all wish you get everything that you deserve.

Max Cooper: Thank you very much Chen. I have to say I got more credits than I deserve already. The real payoff is the joy you get when you make the discovery.

Chen Dong: That moment when you talked with Bob Good about your finding in Minnesota when you were a thirty-years old young man and discovered there were two separate lineages of lymphocytes. I am sure that has been in your good memory for all your life. I wish we all have those moments. All right, thank you, Max. It has been really lovely talking with you. And thank you so much for talking with us.