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Defining Yourself: Tolerance Development in the Immune System

Paul M. Allen

It was 25 years ago that I first came to Boston, just having completed my graduate work in John Niederhuber’s laboratory at the University of Michigan. In 1981, I started my postdoctoral fellowship in Emil Unanue’s laboratory at Harvard Medical School. My research interests, then and now, involve how the MHC is implicated in the recognition of Ag by T cells and how self-tolerance is achieved. In this presentation, I will focus on two model Ag systems I developed, hen egg-white lysozyme (HEL) and hemoglobin (Hb), and review the discoveries made using these models. Over the years, I have had the privilege of working with a group of talented and dedicated students, postdoctoral fellows, and technicians, and I would like to thank them all for their effort and making this all possible.

Science in 1981 was different than it is today, as was America. The winner of the Academy Awards Best Picture of the year was “Chariots of Fire.” A popular student car was the remarkably ugly AMC Pacer. In sports, the Curse of the Bambino was still continuing, with the Yankees dominating the Red Sox. Also, 1981 was a watershed year for the music industry with the introduction of the Walkman, ushering in the era of personal music listening. The top 10 songs that year were remarkably forgettable, with hits such as “Bette Davis Eyes” by Kim Carnes and “Jessie’s Girl” by Rick Springfield. Many aspects of science in 1981 are remarkably similar to today, including 1) pipettors, 2) SDS-PAGE gels, 3) tissue culture hoods and supplies, and 4) ELISA and mAbs. However, there are many aspects that are completely different, including 1) PCR, which revolutionized molecular biology, 2) personal computers, with the original IBM-PC being released that year, 3) clean mice, with spleens from 1981 mice having $>200 \times 10^6$ cells, while a clean mouse in 2006 has $70 \times 10^6$ cells, and 4) kits, with ones available for nearly every protocol.

Thus, arriving in Boston in 1981, I started my research on investigating how T cells recognize Ag and how self-tolerance is achieved, with an emphasis on the involvement of self-peptides. The 1981 model of how a T cell recognizes Ag was prominently displayed on the cover of the popular immunology textbook at the time (1). It was known that this recognition event was MHC restricted and was Ag specific. Therefore, it was proposed that a T cell had two distinct receptors, one for MHC and one for Ag.

As a postdoc, I developed the HEL model Ag system to facilitate the investigation of how T cells recognize Ag. This system involved generating a panel of HEL-specific T cells in H-2$k$ mice, including the extensively studied 3A9 T cell, and then identifying the I-A$k$-restricted immunodominant epitope recognized by these T cells to be contained in a tryptic fragment of

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3 Abbreviations used in this paper: HEL, hen egg-white lysozyme; Hb, hemoglobin; APL, altered peptide ligand.
HEL, residues 46–61 (2–4). The HEL (46–61) peptide was then used in studies, published in 1985 (5), with Bruce Babbitt and Emil Unanue, in which we were the first to demonstrate that a peptide could directly bind to an MHC molecule. In these studies, we labeled HEL (46–61) with a fluorescent tag, NBD, and then performed equilibrium dialysis with it and purified I-Ak molecules. The HEL (46–61) peptide bound to I-Ak in a saturable manner, and Scatchard analysis revealed a single binding site with micromolar affinity. The binding of HEL (46–61) to I-Ak was then shown to be peptide sequence specific and MHC allele specific in that HEL (46–61) did not bind to I-A^d. These findings were a seminal contribution to the field of immunology in that they directly showed how an MHC molecule was involved in the recognition of Ag by T cells and how immune response genes could be operating. Thus, by 1985, our view of T cell recognition had significantly changed from the 1981 version. Our studies had shown that peptides directly bound to MHC molecules, thus defining the ligand for the TCR. Mark Davis, Tak Mak, and others (6–9) had cloned and identified the TCR in 1984. Therefore, by 1985, it was established that a single TCR recognized a pMHC complex on the surface of an APC. This was the year that I started my own laboratory at Washington University in St. Louis.

In 1987, a seminal study was published by Bjorkman et al. (10), namely the crystal structure of the class I molecule. In the famous picture of the electron density of this structure, there was the presence of non-MHC-encoded density in the groove of the structure, which we know now were self-peptides. This structure gave us the first direct visualization of the pMHC ligand recognized by T cells and implicated the critical role self-peptides play in the formation and function of MHC molecules. Using the HEL (46–61) system, we made an important observation involving self-peptides (11). When the sequence of the corresponding mouse lysozyme (46–61) sequence was compared with the HEL (46–61), only two amino acid differences were found. The mouse lysozyme peptide did not stimulate any of the HEL-specific T cells or prime any T cells in a mouse, a clear indication of self-tolerance. When we tested the mouse lysozyme 46–61 peptide for its ability to bind to I-Ak, we found that it bound identically as HEL (46–61). Thus, this observation showed that MHC molecules could bind self-peptides in a manner indistinguishable from foreign peptides and implied that the observed self-tolerance had to be occurring at the level of the T cell and not the MHC. These studies with the mouse lysozyme (46–61) molecule showed that a synthetic self-peptide could bind to an MHC molecule; however, there were many unanswered questions regarding how the immune system handled self-proteins: 1) are self-Ags constitutively processed and presented in vivo; 2) do all APCs process and present self-Ags; and 3) are self-peptides involved in T cell development? (n.b. At this point, there was a 3-day hiatus in my presentation at the AAI Annual Meeting, following a dramatic exit from the podium, a trip to the ER, and recovery from viral gastroenteritis.)

To address these questions about the processing and presentation of self-Ags, in my laboratory, Robin Lorenz developed the murine Hb self-Ag model system (12). The basis for this model is that there are allelic forms of the murine β-chain of Hb. There are mouse strains that share H-2^k but express different Hb alleles, Hb^d and Hb^b, which have 12 aa differences. We purified Hb^d from CBA/J mice and immunized CE/J mice, which express the Hb^b. A strong T cell response was produced, from which a series of T cell hybridomas and clones were generated. These T cells were specific for Hb^b and were H-2^k restricted. This panel of T cells was dual in purpose in that they could recognize Hb^b as a self-Ag when APCs from CBA/J mice (Hb^b) were used, or they could recognize Hb^b as a foreign Ag when APCs from CE/J(Hb^b) mice were used. These Hb^b-specific T cells recognized a single determinant composed of residues 64–76 bound to I-E^k. The crystal structure of the Hb/I-E^k complex was solved by Fremont et al. (13) and revealed that there were two dominant MHC anchor residues at P1 and P9 and four TCR contact residues at P2, P3, P5, and P8. We used the Hb/I-E^k-specific T cell hybridomas to probe for the existence of self-peptide/MHC complexes on APCs from CBA/J mice. We found that Hb is constitutively processed and presented by APCs in the spleen, lymph node, and thymus and in isolated APCs such as macrophages and B cells (12, 14). Thus, this was a direct demonstration that MHC molecules do not distinguish between self and foreign Ags and that APCs are constitutively processing self-proteins and displaying self-peptide/MHC complexes on their surface. We then examined thymic APCs for expression of Hb/I-E^k complexes (15, 16). At the time of these studies, one theory for how T cells could be positively selected and not be negatively selected proposed that the thymic cortex was sequestered from most self proteins due to the blood-thymus barrier. We showed that both cortical epithelial cells and bone marrow APCs in the medulla constitutively expressed Hb/I-E^k complexes, thus proving that self-peptide/MHC complexes of a circulating Ag were expressed constitutively in the cortex and medulla, where they could potentially participate in both positive and negative selection of T cells. These studies from our laboratory, and those from several other labs, firmly established how self-proteins were constitutively handled by the immune system and implicated that self-peptides were playing essential roles in T cell development in the thymus.

Using the Hb system as a foreign Ag model allowed us to manipulate the Hb peptide and make the important observation that T cells have an essential flexibility in their recognition of Ag and established the phenomenon of TCR antagonism (reviewed in Refs. 17–19). The initial observation was made by Brian Evavold when he showed that an altered peptide ligand (APL) could stimulate full IL-4 production in the absence of a proliferative response (20). Recognition of an agonist peptide by a TCR, which has an optimal fit, induces full T cell activation and comprises agonists and weak agonists ligands. Recognition of APLs, which have a less than optimal interaction with the TCR, induce some, but not all, T cell functions and have characteristic TCR phosphorylation patterns (21, 22). They include partial agonists and antagonists, and it is interactions such as these that are involved in positive selection. The third category comprises null ligands, which have no recognition and do not induce any T cell activation. One Hb T cell that has been very useful in the study of APLs is 3.L2 (23, 24). For this T cell, we have generated a TCRtg mouse (25), have developed a clonotypic Ab called Cab, and have defined a series of ligands containing single amino acid substitutions at the P5 position, residue 72, of the Hb64–76 peptide (25–27). All of these peptides bind identically to I-E^k but have differing biological activities due to the changes in the TCR contact residues. These peptides cover the full range of activities and include agonists, APLs/antagonists, and null ligands. To study the in vivo effect of each of the
these ligands on T cell development and function, we expressed each of them as chimeric membrane HEL proteins as transgenes controlled by the MHC class II promoter. The chimeric Hb/HEL molecules were highly expressed on all APCs and were efficiently processed and presented on I-Ek molecules. We crossed each of these Hb/HEL mouse lines to the 3.L2tg and studied the effect on T cell development. We found a wide range of effects of the ligands (28). As expected, the wild-type Hb and a strong agonist, T72, induced complete negative selection of the 3.L2 T cells. Similarly, the APLs, I72 and A72, also resulted in negative selection, demonstrating the ability of weak ligands to induce negative selection. The previously characterized null ligand, Q72, turned out to enhance positive selection of the 3.L2 T cells, whereas the other null ligand E72 had no effect. These studies demonstrated the incredible sensitivity of developing T cells in the thymus to interactions with weak ligands, the biochemical basis for which has yet to be established.

In recent years, there has been a re-evaluation of the studies involving TCRtg mice and negative selection due to the possible effects of early expression of the TCR or overexpression of a self-Ag in these mice (29). With the chimeric Hb/HEL mice, the 3.L2 T cells were eliminated at the double-positive stage. We had previously gone back and re-evaluated where the 3.L2 T cells are negatively selected when Hb is naturally expressed. In preliminary studies, we crossed 3.L2tg mice to CBA/J mice (Hbβ3) and followed the development of 3.L2 T cells. In marked contrast to our previous studies, we found that the 3.L2 T cells were negatively selected at the single-positive stage, not the double-positive (our unpublished observation). We had previously shown that Hb/I-Ek complexes were expressed in the thymic cortex and medulla but that negative selection was only occurring in the medulla. We find now that there is incomplete negative selection of the 3.L2 cells in these mice, and preliminary examination reveals that some autoimmune process is occurring. Overall, these studies highlight the difference in negative selection between normal expression of self-Ags and transgenic overexpression and show that to even a ubiquitously expressed self-Ag such as Hb, potentially autoreactive T cells can escape negative selection and be part of the peripheral T cell pool.

From our studies, and from other investigators, it has been clearly established that self-peptide/MHC complexes play a critical role in the development and function of T cells (Fig. 1). They are an essential part of the processes of positive and negative selection of T cells in the thymus, where a self-restricted anticipatory T cell repertoire is developed, from which most of the autoreactive cells have been eliminated. The interactions involved in these selection events remarkably involve relatively weak interactions, as revealed through the study of APLs. These same types of interactions are also required to maintain peripheral memory T cells. Self-peptide/MHC complexes also are critically involved in the function of peripheral T cells, with the recent demonstration of them acting as coagonists in the initial stages of T cell activation (30). Alloreactivity also involves self-peptide/MHC complexes; however, the precise role of peptides has not been established. Taken together, these studies highlight the TCR’s essential flexibility in recognition of Ag, especially with self-peptide/MHC complexes, and how these critical but weak interactions allow T cells to develop, maintain tolerance, function, and survive. Thus, over the past 25 years, we have made incredible progress in elucidating how a T cell recognizes Ag; however, these studies have raised as many new questions as answered old ones. The next 25 years promise to be as exciting and productive as the past as we investigate the intricacies of T cell biology.

Over the years, I have been very fortunate to have worked with a wonderful group of scientists in my laboratory, and I would like to thank all of them for their contributions. They include Robin Lorenz, Bob Evans, Ben Hsu, Brian Evavold, Dave Hagertry, Bob Graziano, Joanne Sloan-Lancaster, Karine Vidal, Tere Yule, Claude Daniel, Matt Holsti, Gil Kersh, Cal Williams, Ellen Kersh, Dev Basu, Arash Grakoui, Holly Hanson, Brian Wipke, Ken Matsui, Leigh O’Mara, Fei Shih, and Eric Hailman. I would also like to thank the current lab members Dave Donermeyer, Laura Mandik-Nayak, Silvia Kang, Henry Kao, Nathan Felix, Scott Weber, Lyse Norian, Steve Horvath, Jen Racz, Celeste Morley, Donna Thompson, Darren Kreamalmeyer, and Jerri Smith. I would like to thank my colleagues in the Washington University immunology community for making it an incredible place to do science. Finally, I would like to thank my two mentors, John Niederhuber and Emil Unanue, for their support and encouragement through the years and for showing how science can be a lifelong passion.

References


