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Molecular T cell biology – basic and translational challenges in the twenty-first century

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THE BEGINNING OF MOLECULAR T CELL BIOLOGY

The field of immunology has been grounded in basic biology since its inception, with myriad applications to human disease. Development of both preventive and therapeutic vaccines as treatments for human infectious diseases dates to the time of Pasteur's efforts in the nineteenth century (Debre and Forster, 1998). Since then, harnessing immunity through precise knowledge of molecular and cellular mechanisms was perceived as important in medical science.

Adaptive immunity is the most sophisticated and effective system to combat and rid infectious pathogens (Murphy et al., 2007). Adaptive immunity endows jawed vertebrates, including mammals, with precursors of T (thymus-derived), and B (bone marrow-derived) lymphocytes able to generate a repertoire of clonotypic antigen receptors (TCR and BCR) of immense diversity from somatic rearrangements of variable gene segments (VDJ recombination). Spatio-temporally controlled differentiation and selection processes of those cells shape two complementary "arms" of the immune system, offering protection with exquisite specificity, sensitivity, and long-term memory.

Key discoveries during the last quarter of the twentieth century began to unravel the cellular and molecular nature of adaptive immunity. In the 1960s, T and B lymphocytes were identified and their interactions shown to be essential for antibody production. The basic paradigm of immunoglobulin (Ig) gene rearrangements that generate antibody diversity was revealed in 1976 (Tonegawa, 1993). The "dual" specificity of T cells for foreign peptide and self-MHC inferred by functional studies was discovered and clearly noted to be distinct from the "single" specificity of antibody recognition of foreign proteins (Zinkernagel, 1997). This realization then led to an intense effort to understand the molecular puzzle represented by the self versus non-self recognition and the receptor and ancillary molecules on T cells responsible for this unusual recognition.

Initial studies suggesting the existence of an "I-J-specific" suppressor factor secreted by T cells and TCR specificity achieved through Ig genes were refuted. Rather, the discovery of how to expand T cells *in vitro*, via IL-2 dependent T cell cloning (Baker et al., 1979), in conjunction with monoclonal antibody (Milstein, 1993), and flow cytometry screening (Julius et al., 1972) technologies together with *in vitro* functional analyses were decisive in molecular identification for the long sought-after TCR. The key breakthroughs came in the early 1980s with the identification in human of a clonotypic disulfide-linked heterodimer, the αβ Ti, which together with CD3 molecules, were essential for antigen/MHC recognition and cellular activation (Reinherz et al., 1982; Acuto et al., 1983a; Meuer et al., 1983a,b). Biochemical evidence showed that, similar to Ig molecules, both Ti α and β chains possessed variable and constant regions (Acuto et al., 1983a,b). A comparable αβ Ti was soon identified also in the mouse in 1983, with similar cognate immune recognition features (Haskins et al., 1983; Kappler et al., 1983). Those murine studies supported an earlier suggestion that a tumor-specific marker on mouse T-lymphoma cells might be TCR-related (Allison et al., 1982). Within 2 years, cDNAs for TCR αβ subunits were obtained by several groups including Davis and Mak with the bona-fide identification established by the Ti αβ protein sequence (Acuto et al., 1984; Hedrick et al., 1984a,b; Yanagi et al., 1984). Collectively, these results confirmed the clonotypic nature of the Ti αβ first identified biochemically. These studies showed that TCR combinatorial diversity was generated by the same type of site-specific gene recombination mechanisms as with Ig genes, but without somatic hypermutation and led to identification of a second type of TCR, the γδ TCR (reviewed in Tonegawa, 1993).

CD4 and CD8 co-receptors identified during the same period, were soon recognized as ancillary structures that optimize TCR recognition and T cell activation via interaction with monomorphic segments of MHC class II and I molecules, respectively (Meuer et al., 1982). A few years later, the "dual recognition" puzzle was solved when it was shown that MHC class I and class II proteins bound foreign and self-peptides derived from degradation of intracellular or exogenous proteins and that such complexes could be recognized by the TCR (reviewed in Unanue, 2006). Structures of peptides complexed with MHC molecules then followed (Bjorkman et al., 1987; Jardetzky et al., 1994).

T CELL ACTIVATION AND REGULATION

TCR signaling evokes T cell lineage commitment and repertoire selection during development, maintains the peripheral T cell pool, and further differentiates naïve T cells into effector or memory cell populations upon immune stimulation. Through many studies, we know that the TCR is a multimeric transmembrane complex composed of an antigen binding clonotypic heterodimer (αβ or γδ) in non-covalent association with the signal-transducing CD3 subunits (CD3εγ, CD3εδ, and CD3ζζ; Clevers et al., 1988). Structural analyses of TCR or CD4 and CD8 co-receptors alone or in complex with pMHC using X-ray crystallography and NMR in conjunction

with biophysical studies have contributed to our understanding of cognate recognition (Wang and Reinherz, 2002; Rudolph et al., 2006). Structures of CD3εγ and CD3εδ ectodomain heterodimers (Sun et al., 2001; Arnett et al., 2004; Kjer-Nielsen et al., 2004; Sun et al., 2004) and of transmembrane CD3ζζ homodimers (Call et al., 2006) have provided clues as to their unique dimerization interfaces and topology relative to the αβ dimer, which should be relevant to understand how the TCR transduces signals. Upon interaction with pMHC, the αβ TCR heterodimer induces phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic tails of the associated CD3 subunits (Reth, 1989; Irving and Weiss, 1991; Letourneur and Klausner, 1992). Recent evidence suggests that TCR complex quaternary changes by means of torque exerted during recognition of pMHC on the opposite face of an antigen presenting cell could be the initial trigger (Kim et al., 2009). However, this signal transduction mechanism appears rather unconventional in that the TCR and the tyrosine kinase Lck required to initiate signaling are not constitutively associated, instead interacting only after TCR engagement. In addition, a relatively large fraction of Lck is constitutively activated (Nika et al., 2010), suggesting that TCR and kinase co-localization is the relevant event to induce rapid and sensitive signal transduction. This scenario poses a challenge requiring a deep understanding of the biophysics of protein dynamics in biological membranes. Likewise, clarification of the basic physical and chemical events that convert a recognition of pMHC by a weakly interacting (~1–100 μM K_d) TCR into an intracellular signal with great specificity, sensitivity, and diverse biological outcomes remains an intense challenge for future investigation.

In vivo, chemokines function as chemoattractants to guide cell migration, including movements of T cells via interaction with the chemokine receptors, G protein-coupled receptors on the surface of leukocytes (Fernandez and Lolis, 2002). Their ligation promotes signal cascades including changes in avidity and cell adhesion via integrins. TCR stimulation also modulates integrin avidity on T cells (Hynes, 2002). It can be anticipated that these areas will continue to be intensely investigated for at least two reasons: (1) understanding *in vivo* activation of the adaptive response; and (2) exploiting this knowledge for modulating these mechanisms as additional tools to fend off autoimmunity and improve vaccination.

FROM "BLACK BOX" TO "PANDORA'S BOX": DYNAMIC SIGNALOSOMES, IMMUNOLOGICAL SYNAPSE, AND COMPUTATIONAL MODELING

Since the mid 1980s, many groups engaged in comprehending the role of TCR, co-receptors, co-stimulatory, and cytokine receptors in T cell activation. T cells were mysterious "black boxes" in which an input (e.g., signal one, signal two, cytokines) may result in diverse outputs (development, effector cell differentiation, lethal hit, cell death), with few clues as to how these processes develop biochemically. Work from the early 1990s until the present has brought to light most of the T cell signaling tool-box elements, now confronting T cell biologists with the opening of "Pandora's box." The interesting surprise has been that highly specific or unique sets of signaling proteins are grafted onto a template of prototypic signaling schemes and components found in virtually all cell types. This feature is discernible from the TCR and ancillary receptors down to the control of gene expression (Weiss, 2009) and likely serves distinctive developmental, survival, and cell activation processes associated with the T cell arm of the immune response. The most recent discovery is of a T cell unique signalosome component important in T cell positive selection (Fu et al., 2009; Johnson et al., 2009; Lesourne et al., 2009) suggested to regulate the Ras pathway (Brockmeyer et al., 2010). Not unexpectedly, such a vital TCR-based signaling machinery is counter-balanced by negative regulators of activation. Both immunologic (CTLA-4/CD80 or 86, PD-1R/PD-1) and more general (Adenosine receptor A2A-R/adenosine) inhibitory checkpoint pathways have been revealed. These immunosuppressive pathways prevent collateral damage from inflammation leading to termination of immune cell activation in intimate associate with regulatory T cells (Rudd et al., 2009; Sitkovsky, 2009). Control mechanisms establishing positive and negative feedback with their tunable thresholds have been brought to light in TCR signaling (Acuto et al., 2008). Combinations of quantitative data and computational modeling of such events are just beginning to provide us with the basic rules that shape the unique biology of the "system" T cell. This should help to explain how transient events of phosphorylation and other protein modification allow T cells to coordinately switch on and off functional pathways by integrating signals in time and space from multiple receptors, thereby controlling gene expression, morphological changes, and mobility. We can anticipate that deep insights in this area will be revealed by determining the dynamics of the global proteome of different T cell subsets, during distinct differentiation stages and following one or more stimuli. The era of this bold challenge has already begun with enormous technological progress complementing sophisticated transcriptome and metabolome approaches (Luber et al., 2009; Mayya et al., 2009; Brockmeyer et al., 2010).

Of great interest has been the discovery that, upon pMHC stimulation, the T cell/APC interface forms micrometer-scale TCR clusters arising from smaller ones creating what is referred to as the Immunological Synapse (Dustin et al., 2010). These molecular platforms, connected to underlying signalosomes through organization of dynamic protein complexes, likely provide signaling stability as well as signaling complex disposal/recycling. Thus, the IS ensures prolonged signaling and orchestrates expression of T cell differentiation programs and consequently cell fates. Increasingly sophisticated technologies of super-resolution optical microscopy and other biophysical approaches in live cells will add further understanding of the spatial organization of the cellular chemistry.

AMALGAMATION OF BASIC AND TRANSLATIONAL SCIENCES

Translational endeavors aim to block T cell activation in the setting of organ transplantation or autoimmunity or, conversely, stimulate T cell activation to foster immunity to protect against infectious diseases and cancers. The success of these approaches is predicated on unraveling the complexity of immune recognition and pathways of activation. Nowhere would this knowledge be more prescient than for design of novel T cell-based vaccines (Bambini and Rappuoli, 2009). While the world's currently licensed vaccines against infectious pathogens almost exclusively target generation of antibody production, this strategy is

efficient exclusively against those pathogens that are invariant or change little in amino acid sequence over time (i.e., diphtheria, tetanus, H. influenza B, polio, pneumococcus, meningococcus, etc.) Targeting of vaccine-induced antibodies to influenza A hemagglutinin and neuraminidase which vary on an annual basis is a formidable task. Daily variation in HIV-1 evolution makes conventional vaccine approaches virtually impossible: the humoral immune system directed at highly variable sequences of the virus cannot keep pace with the evolving sequence changes.

Influenza A is a case in point. Influenza A is zoonotic (crosses species) and, especially in avian hosts, often enzootic (replicates without overt host damage). It is widely dispersed in waterfowl. The large reservoir of this highly variable virus and its potential for rapid transmission places a premium on developing vaccination methods that are inherently more stable to antigenic variation. This need has focused attention on T cell cellular immunity necessitating the identification of optimal MHCI-bound peptides expressed on virus-infected human lung epithelial cells and the induction of an effective CTL response mediated by these peptides. By "optimal" we mean the target peptides must be expressed on infected respiratory epithelium where productive viral replication occurs, are conserved among virtually all influenza A viruses and are restricted by HLA molecules that cover a broad range of ethnicities. Advances in bioinformatics and proteomics of HLA display can be used to achieve those key goals (Schirle et al., 2000; Lemmel et al., 2004; Weinzierl et al., 2007; Lin et al., 2008a,b; Reinhold et al., 2010). Elicitation of protective CTLs against critical influenza determinants in the lung offers the potential of creating a universal influenza vaccine to prophylax against both seasonal and pandemic strains of virus. Failure to naturally cross-present these optimal epitopes in the regional lymph nodes may favor expansion of T cells with poor protection, accounting for the perception that T cells are not useful in combating influenza infections. Mechanistic understanding of cognate immunity allows us to create new rules, however. In addition to influenza, such an approach has broad implications for $CD8^+$ T cell vaccines against other infectious agents, including Hepatitis C virus where it is already becoming clear that CD8 T cell immunity contributes in an important way to protection (Yu and Chiang, 2010).

Bioinformatics can provide platforms to address pathogen sequence diversity and human HLA diversity through targeting conserved viral sequences and identification of promiscuous binding peptides (affording population coverage to a large segment of the disease-susceptible population), respectively. As HLA is pivotal for functions of the immune system and determinant in antigen presentation, a systematic approach to T cell-based antigen design is required given that HLA is the most highly polymorphic of known human genes. Currently, there are more than 5,000 HLA alleles expressed by human beings on our planet (http://www.imgt.org). Within the United States alone, there are 3,112 HLA-ABC haplotypes with greater than 9,684,544 combinations. Computational methods that predict peptide binding to a majority of alleles within a supertype dramatically reduce the complexity of peptides required to afford population protection coverage (Reche and Reinherz, 2005; Zhang et al., 2010). These predictive methods will become more rapid and accurate in future years. Detection of the subset of predicted peptides actually displayed on infected cells or tumor cells by evolving mass spectrometry methods (Poisson detection MS^3, MS^E, or MS^N) will allow for precise epitope targeting, obviating cross-presentation of epitopes not displayed on the pathologically dysfunctional cell or irrelevant crossreactivity resulting from reverse immunology methods (Reinhold et al., 2010).

Facile determination of complete HLA typing will assist in prediction of useful responses to vaccines among members of the population. In addition, transcriptome/proteome approaches, multiplex assays, multiparameter phenotyping, and systems biology will assist with the understanding of how the human immune system's steady state is perturbed by infection or vaccination approaches. In short order, it should be possible to ascertain whether an individual response to a vaccine may afford protection or whether during the course of natural infection, the outcome will be favorable or unfavorable.

While details of cognate recognition are critical for vaccine design, this is but one part of the story. Determination of how to elicit high avidity T cells as effectors, memory, and effector/memory populations under the appropriate cytokine milieu followed by mobilization of these cells into the infected organ via relevant chemokines and addressins is necessary to induce the protective response. In addition, augmenting the relevant effector/effector memory population by concurrent blockade of inhibitory pathways (CTLA4, PD-1R, and A2AR) seems logical. That said, the details of pathway function and optimal kinetics of interdiction relative to antigen stimulation require elucidation. Finally, it is important to acknowledge that T cell biology needs to be considered in conjunction with that of B cells, immunologic memory, innate immunity, tolerance, and inflammation, all of which are addressed within Frontiers in Immunology.

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