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Immunodominance in TCD8+ Responses to Viruses: Cell Biology, Cellular Immunology, and Mathematical Models

Meeting Review

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Summary

Highlights from a Fundación Juan March Interdisciplinary Meeting. $CD8^+$ T cells (T_{CD8+}) play a critical role in immunity to viruses. A central feature of antiviral T_{CD8+} responses is immunodominance: out of thousands of potential target peptides, only a handful generate measurable responses. A recent Fundación Juan March Meeting brought together scientists working on the various steps in antigen presentation and T cell biology that contribute to immunodominance, whose understanding is key to rationally developing vaccines meant to elicit effective antiviral T_{CD8+} responses.

Introduction

Viruses pose a serious threat to living organisms. In response, jawed vertebrates evolved a multilayer immune response that includes one of evolution's great creations, the MHC class I-$CD8^+$ T cell (T_{CD8+}) immunosurveillance system. Nearly all cell types constitutively express class I molecules, which bind oligopeptides generated through the action of proteasomes and other proteases. By displaying oligopeptides on the cell surface, class I molecules enable T_{CD8+} to monitor cells for viral infection. Since a prime source of viral peptides are defective ribosomal products (DRiPs), rapidly degraded forms of newly synthesized proteins, T_{CD8+} can detect viruses shortly after they initiate infection and exert their antiviral effects before progeny viruses are released.

The past decade has witnessed an explosion in our capacity to probe and understand antiviral T_{CD8+} responses. It is now possible to define viral peptide determinants recognized by T_{CD8+}, accurately quantitate T_{CD8+} responding to defined determinants, and measure functional capacities of T_{CD8+} on a determinant-by-determinant basis. While T_{CD8+} cannot generally provide sterilizing immunity to viruses, they make a major contribution to controlling viral infections. In the case of human immunodeficiency viruses (HIV), T_{CD8+} may be the best hope for developing a preventive vaccine. Therapeutic T_{CD8+} vaccines may be of benefit for other infectious diseases and tumors, as well.

A major hurdle to developing vaccines that generate effective T_{CD8+} responses is immunodominance (ID) (Yewdell and Bennink, 1999). Despite the presence of thousands to tens of thousands (depending on the coding capacity of virus) of potentially immunogenic peptides, T_{CD8+} responses are predominantly directed to a few peptides (sometimes a single peptide), termed "immunodominant" determinants. Other "subdominant" determinants elicit fewer responding T_{CD8+}. Narrow responses favor the emergence of viral escape mutants. For unknown reasons T_{CD8+} responses to some immunodominant determinants exert poor antiviral activity and compromise the effectiveness of the T_{CD8+} response.

ID in antiviral responses results from a complex combination of factors that encompass all aspects of T_{CD8+} biology, including: (1) generation of the T_{CD8+} repertoire, (2) interaction of T_{CD8+} with professional antigen-presenting cells (pAPC) in lymphoid organs, (3) generation of class I peptide complexes from viral antigens synthesized by pAPCs ("direct priming") or acquired by pAPCs from virus-infected cells ("crosspriming"), and (4) competition between T_{CD8+} clones for activation. The complex problem of ID is poised for a quantum leap in understanding due to the introduction of new technologies, many of which were on display at a meeting entitled: "Immunodominance: The Key to Understanding and Manipulating $CD8^+$ T Cell Responses to Viruses," sponsored by the Fundación Juan March in Madrid, Spain on June 7–9, 2004.

ID in Mouse, Monkey, and Human

Much of what we know about ID has been gleaned from infecting B6 or BALB/c mice with model viruses, particularly influenza A virus (IAV). Responses to approximately ten well-defined IAV determinants in each inbred strain form a hierarchy that evolves during the transition form the primary to the memory phase of the response and that is highly reproducible between individuals of a given strain. But how diverse is the total antiviral response? Jonathan Yewdell (NIAID, Bethesda, Maryland) showed HPLC fractionation of peptides eluted from IAV-infected cells that suggests that mice mount low-frequency responses to a large number of undefined peptides. These may be completely distinct determinants from the defined sets or they may represent extended, shortened, or posttranslationally modified versions of defined determinants. Margarita Del Val (Instituto de Salud Carlos III, Madrid, Spain) showed that in addition to the "optimally" sized peptide used to gauge T_{CD8+} responses to an HIV protein, cells present two extended versions as well as a shortened version. The truth is that precious little is known about the viral "immunopeptidome"; i.e., the diversity and abundance of viral peptides presented to the immune system.

Inbred mice express two or three different class I allomorphs, while humans can express up to six distinct allomorphs. How does the increased complexity of the displayed viral immunopeptidomes affect the ID hierarchy? There are no data for direct comparison between mouse and human responses to similar pathogens acting under similar conditions (e.g., initial encounter). Most data in humans have been obtained with human immunodeficiency virus (HIV), which like many clinically signif-

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icant viruses, does not infect mice. Richard Koup (NIAID, Bethesda, Maryland) summarized current knowledge. On average, chronically infected patients on anti-HIV chemotherapy respond to approximately four determinants. In some individuals, T_{CD8+} to single determinants account for >90% of responses. Response magnitude is not strictly related to clinical status. HLA-B57-restricted T_{CD8+} are strongly associated with decreased pathogenesis. Understanding this observation could provide critical insight into effective vaccine strategies for HIV. The road to an effective HIV vaccine probably passes through the SIV-macaque system. David Watkins (University Wisconsin) reported that though responses to individual determinants frequently predominate during chronic infection, animals may respond to up to 70 determinants during acute infection. T_{CD8+} responding to different determinants exhibit gross differences in their capacity to reduce viral replication in vitro. Koup and Watkins concurred that effective T_{CD8+}-based HIV vaccines may require the induction of T_{CD8+} responses to peptides that first, are from regions of structurally constrained parts of proteins (to prevent selection of escape mutants), and second, for reasons that need explanation, represent effective targets.

Herpesviruses and poxviruses provide a great challenge for immunodominologists, since their genomes encode more than ten-fold the number of potential determinants as IAV or HIV. Louis Picker (Oregon Health Science University, Portland, Oregon) discussed human T cell responses to cytomegalovirus (CMV), a herpesvirus that chronically infects most humans. Picker used a panel of 13,684 (!) synthetic peptides corresponding to a complete set of overlapping (by 10 residues) 15-mers from all 213 in silico-predicted CMV open reading frames (ORFs) to measure T_{CD8+} (and T_{CD4+}) responses in 33 chronically infected and 9 uninfected individuals. On average, ~10% of T_{CD4+} or T_{CD8+} in peripheral blood were CMV specific, despite the fact that CMV expresses multiple "VIPRs": viral gene products that interfere with antigen presentation. Individuals detectably responded to determinants encoded by an average of 12 (T_{CD4+}) and 7 (T_{CD8+}) ORFs. Although representing just ~4% of the CMV genome, CMV proteins synthesized initially in the infectious cycle elicited 28% of the responding T_{CD8+}. Non-CMV infected individuals demonstrated only seven T_{CD8+} responses and no T_{CD4+} responses to the entire peptide panel. This led Picker to propose that memory T cell crossreactivity between CMV and commonly encountered non-CMV agents is unusual, and thus, such crossreactivity may not have the dominant influence on the memory repertoire of humans as has been demonstrated in mouse systems.

Alex Sette (La Jolla Institute of Immunology, San Diego, California) described a similarly ambitious project to define human T_{CD8+} responses to vaccinia virus, information that will be important for benchmarking responses induced by safer alternatives to standard smallpox vaccination. Using a panel of >7,000 peptides encompassing all determinants predicted in silico to bind to class I allomorphs from each of the nine human "supertypes," Sette examined T_{CD8+} responses in transgenic mice expressing HLA-A2, A11, or B7. In each transgenic strain, ~25 peptides were recognized, with 25%–75% of the response focused on one to three determinants. In contrast to CMV, T_{CD8+} prefer gene products predicted to be expressed late in the vaccinia infectious cycle. This underscores the reality that virus systems exhibit unique features based on the precise nature of the virus-host interaction. Variola virus (the cause of smallpox) is highly specific for humans and cannot be studied in mice. Luis Sigal (Fox Chase Cancer Center, Philadelphia, Pennsylvania) described the ectromelia model in mice, which is similar in many respects to human smallpox.

José A. López de Castro (Centro de Biología Molecular, Madrid, Spain) described the careful comparison of over 1000 natural ligands of HLA-B27 expressed in mouse or human cells. The peptide repertoire of this human class I allomorph diverged 15%–25% between the two species. More than half of the divergence could not be ascribed to species-specific differences in protein sequences but was rather accounted for by differential tapasin and proteasome-mediated effects.

Ann Hill (Oregon Health Science University, Portland, Oregon) used a genomic library to identify 18 mouse CMV (mCMV) determinants recognized during the acute phase of the infection in B6 mice. Importantly, the establishment of latent infection with no detectable infectious virus is accompanied by alterations in the ID hierarchy. As described in acute virus systems previously, there is no simple relationship between the ID rank of a determinant and the functional avidity of responding T_{CD8+}. The dominance hierarchy was clearly affected by genes outside of the MHC and NK-resistance loci. This is an important finding since virtually nothing is known about the influence of background genes on ID, which may explain some of the variability in human responses noted above. mCMV is known to express at least three "VIPRs." Surprisingly, although deleting these genes increases in vitro presentation of all five defined determinants tested, VIPR-less mCMV establishes a chronic infection, and induces an indistinguishable response from wild-type virus in both acute and chronic phases.

Matthias Reddehase (University of Mainz, Mainz, Germany) analyzed the ID hierarchy of seven determinants defined in mCMV-infected BALB/c mice and found that contrary to B6 mice, this does not change upon establishment of latency. The ID hierarchy in BALB/c mice also remains unaffected by deleting the three VIPRs. Importantly, T_{CD8+} specific for any of the seven defined determinants were able to protect against lethal mCMV infections. This raises hope for the efficacy of vaccines that induce T_{CD8+} responses to subdominant determinants that have otherwise preferred properties such as conservation due to structural constraints to their variation. By contrast, T_{CD8+} cells specific for the immunodominant determinant in B6 mice were completely nonprotective. This is explained by the negative effect of VIPRs on presentation in productively infected cells, since the same T_{CD8+} protect against VIPR-less mCMV. Thus, T_{CD8+} may be activated by crosspriming rather than by direct priming (discussed in detail below). This neatly makes the point that the effects of VIPRs can be highly specific for individual determinants, and that ID as defined by the magnitude of a determinant-specific response does not always match "ID as defined by the generation of protective immunity.

Peter Doherty (University of Melbourne, Melbourne,

Australia) and Weisan Chen (Ludwig Institute, Melbourne, Australia) described the swapping between T_{CD8+} responding to dominant ($PA_{224-232}$) and subdominant ($NP_{366-374}$) determinants in primary and memory response to IAV. The phenomenon is complex and can be reversed by a number of manipulations that reduce activation of the now dominant memory $NP_{366-374}$-specific T_{CD8+} or by increasing presentation of the $PA_{224-232}$ in secondary immunization. A critical question posed by the studies is the extent to which memory T_{CD8+} can be activated by non-pAPCs, pointing to the dearth of information regarding the requirements for costimulation of memory T_{CD8+} and how this might differ between T_{CD8+} located in lymphoid versus peripheral organs.

Routes of Antigen Presentation In Vivo

The contribution of crosspriming versus direct priming to induction of antiviral T_{CD8+} responses is a contentious topic. Jonathan Yewdell showed that crosspriming with IAV-infected cells results in a robust response exhibiting similar ID hierarchy as immunization with IAV itself. This implies that IAV-responses are either normally induced by crosspriming or that crosspriming and direct priming induce a highly similar response. The latter would be predicted if crosspriming is based on the transfer of processed peptides from donor cells to pAPCs. Yewdell discussed recent findings that instead suggest that crosspriming is based on transfer of proteasome substrates and not proteasome products. Sebastian Joyce (Vanderbilt University, Nashville, Tennessee) emphasized that whatever the physical nature of transferred material, it represents a finite source of antigen. Therefore, the half-life of cell surface peptide-MHC complexes displayed should play a more important role in crosspriming than in direct priming, where biosynthesis constantly supplies peptides.

Kenneth Rock (University of Massachusetts Medical School, Worcester, Massachusetts) presented data demonstrating that crosspriming occurs via TAP-dependent and -independent pathways, with the former accounting for perhaps 80% of responses. Intriguingly, TAP-independent crosspriming is impaired in cathepsin S $(CatS)^{-/-}$ mice but not $CatL^{-/-}$ or $CatB^{-/-}$ mice. CatS appears to be involved in endosomal peptide generation. Most importantly, IAV-infected $CatS^{-/-}$ mice demonstrate reduced responses to two defined IAV determinants, suggesting that TAP-independent crosspriming participates in generating anti-IAV T_{CD8+} responses. It will be important in future studies to determine the overlap between peptidomes generated by secretory/endosomal versus cytosolic/ER proteases and class I loading mechanisms: surely the sets cannot overlap completely, and T_{CD8+} may be generated against endosomally processed peptides that are unable to recognize virus-infected cells (and vice versa).

A critical issue in crosspriming and direct priming is the identities of the pAPC that are active in the process. William Heath (Walter and Eliza Hall Institute, Melbourne, Australia) described the six defined mouse DC subsets. Despite constituting ~10% of all DCs, $CD8\alpha^+$ $CD205^+$ DC appear to be responsible for nearly all priming (potentially crosspriming) to viruses and crosspriming to soluble proteins and parasites. This is not simply due to expression of the proper costimulatory molecules or ability to interact with T_{CD8+} in lymphoid organs since all of the DC subsets are capable of priming if the DCs are exposed to synthetic peptide antigens. Thus, $CD8\alpha^+$ $CD205^+$ DC seems to have a special ability to acquire or process protein antigens. This does not necessarily occur at the site of viral infections, but may occur in the lymph node by antigen transfer from tissue DC.

Jacques Nefjees (Netherlands Cancer Institute, Amsterdam, Netherlands) showed that DC can obtain peptides through gap junctions they form with surrounding cells. In principle, this remarkable mechanism enables DCs to monitor the peptides generated by surrounding cells in tissues and lymphoid organs.

José Villadangos (The Walter and Eliza Hall Institute, Melbourne, Australia) reported that $CD8\alpha^+$ $CD205^+$ DC can only crosspresent antigen captured by actin-dependent mechanisms (i.e., phagocytosis or macropinocytosis). Intriguingly, maturation of DC by LPS or CpG-rich oligonucleotides inhibited macropinocytosis and crosspriming in $CD8\alpha^+$ $CD205^+$ DC in vitro. LPS or CpG treatment of mice resulted in the complete loss of T_{CD8+} responses against subsequent antigenic challenge 9 hr later by crosspriming or viruses. Importantly, these findings imply that antigen presentation can only occur within a limited time frame in a local inflammation due to maturation of immature DC. Maria Montoya (Edward Jenner Institute, Compton, United Kingdom) discussed how splenic $CD11^+$ DCs enter apoptosis within 2 days of infection of mice with lymphocytic choriomeningitis virus.

Vincenzo Cerundolo (University of Oxford, Oxford, UK) showed that in vivo activation of NKT cells via oral or parenteral administration of α-galactosylceramide induced DC maturation and enhanced primary and memory T_{CD8+} responses to soluble protein and peptide antigens. NKT cell activation represents, therefore, another target for T_{CD8+} vaccine adjuvants.

Antigen Processing Filters Influencing ID

The most important factor in shaping the immunopeptidome is the individual's set of class I allomorphs. But class I molecules can only present peptides that are provided to them by the antigen processing machinery. Jacques Neefjes presented evidence that contrary to prevailing models of antigen processing, cellular proteasomes most frequently generate peptides greater than 17 residues that must be trimmed by tripeptidyl peptidase II (TPPII).

Peter Van Endert (Necker Institute, Paris, France) described the contribution of TAP to shaping the human immunopeptidome based on selectivity for the three NH_2-terminal residues and the COOH terminus of transported peptides. COOH-terminal filtering has a great impact on the immunopeptidome since ER is essentially devoid of carboxypeptidase activities. NH_2-terminal filtering has a less direct effect, since peptides are trimmed by ER aminopeptidases. Van Endert described two ER peptidases with distinct specificities that participate in antigen processing, human ERAP1 and ERAP2. Although each is functional when studied separately in vitro, ERAP1 forms a heterodimer with ERAP2 that may be required for efficient trimming of longer and

more complex NH_2-terminal extensions. There are many puzzling features that remain to be explored, including highly disparate expression of the two enzymes in different tissues, possible association with the class I loading complex, and the absence of an ERAP2 homolog in mice.

Focusing on T_{CD8+}

One of the least understood factors in ID is the T cell repertoire. T_{CD8+} precursor frequency plays an important role in establishing the ID hierarchy in minor histocompatibility systems (Sebastian Joyce). Todd Schell (Pennsylvania State University College of Medicine, Hershey, Pennsylvania) showed that tolerance can greatly shape the ID hierarchy to foreign antigens by deleting T_{CD8+} specific for immunodominant determinants and sparing only the lowest affinity T_{CD8+} specific for the least immunogenic determinants.

Peter Doherty reported that systematic sequencing of TCR genes from single cell sorts of T_{CD8+} specific for dominant and subdominant IAV determinants reveals considerable differences in diversity and CDR3 length in TCRs responding to the two determinants. More such studies will be needed to arrive at useful generalizations relating these factors to ID.

Benedita Rocha (Necker Institute, Paris, France) used single cell PCR to measure levels of mRNA encoding 20 distinct genes in studying the dynamics of naive and memory T cell activation. Surprisingly, expression of genes encoding cytokines, their receptors, and lytic machinery occurs at random and in widely varying amounts in individual cells during the early (day 4) primary response. Three days later, concerted expression of these genes is finally achieved in activated T_{CD8+}. Disappearance of gene activity in the contraction phase is again random at the single cell level. Memory T_{CD8+} respond to antigen within hours with a concerted and efficient expression of effector gene products. Characterization of T_{CD8+} by RNA and protein profiling may provide the key to understanding determinant specific differences in anti-viral activity that plague development of T_{CD8+}-based vaccines for HIV and other "difficult" pathogens.

Philippa Marrack (HHMI, Denver, Colorado) studied the mechanism of immunodomination, which is a major contributor to ID in many viral systems. T_{CD8+} recognizing antigen on a pAPC inhibit proliferation of other T_{CD8+} engaged with the same pAPC. As only dividing cells mature into memory T_{CD8+}, this ought to have a large effect on the numbers of memory cells. Paradoxically, discrepancies in ID hierarchies seem to narrow in secondary responses. The mechanism for immunodomination is uncertain. T_{CD8+} can clearly be observed to nonspecifically deplete peptide class I complexes from APCs in vitro but the in vivo relevance of this phenomenon is uncertain. Marrack suggested that dominating T_{CD8+} may physically sequester APCs, or monopolize costimulatory signals or cytokines provided by pAPCs.

Resolving these possibilities will probably require visualizing the T_{CD8+}-APC interaction. Gillian Griffiths (University of Oxford, Oxford, UK) used confocal microscopy to study the interaction of T_{CD8+} with target cells. Membrane fusion between the two cells frequently can be found in such synapse, which results in the transfer of class I molecules (and other cell surface proteins) observed by Marrack and others. Patients with genetic defects in perforin secretion demonstrate impaired response to infections, and a surprising failure to contract the numbers of activated T_{CD8+} after the primary infection. Intriguingly, this also occurs in certain patients with fully normal secretion of perforin-containing granules. Griffiths suggested that this may be due to a defect in the ability of T_{CD8+} to acquire target membranes from APCs, and that T_{CD8+}, like those from perforin deficient patients, may not be susceptible to fratricidal lysis, which may contribute to the contraction phase.

Ulrich von Andrian (Harvard Medical School, Boston, Massachusetts) showed remarkable movies chronicling the interaction of naive T cells with DCs in live mice as visualized by multiphoton microscopy. Mature DCs newly arriving into lymph nodes cluster around venules, exhibiting random movement. T_{CD8+} immigrants exhibit high motility, then slow after a few hours and establish prolonged contacts with mature DCs that are lengthened further if DCs present the appropriate antigen. A day after transfer, T_{CD8+}-DC interactions become briefer and T_{CD8+} begin dividing in earnest. Circulating blood DC enter the bone marrow where they can present antigens to the central memory T_{CD8+} which reside there. T_{CD8+}-DC interaction dynamics were dissimilar from those observed in the peripheral node, possibly due to differences between microenvironments or between memory versus naive T_{CD8+}.

The Future of ID

A clear message from the meeting is that ID, like most immunological phenomena, is the complex net result of interactions between a number of processes, themselves highly complex. A reasonable understanding of ID will only come from the combined efforts of biochemists, cell biologists, cellular immunologists and biomathematicians. Interdisciplinary meetings such as this are essential to create and maintain lines of communication between these groups.

Increasingly, we will have to turn to mathematical modelers for a reality check. Readers might chuckle at "reality check," for they probably share the opinion of most biologists that assumptions used to create mathematical models frequently gloss over reality. This will be less true in the future as our knowledge grows in sophistication and our abilities improve to accurately quantitate nature in all of its fine and glorious details.

Fittingly, the meeting concluded with Rob De Boer (University of Utrecht, Utrecht, Netherlands) presenting a mathematical model for T cell responses to an acute viral infection in mice. He showed that a few simple first order equations could explain the differential expansion, contraction and memory phases that define the life of T cells. A surprising and novel conclusion from this model is that slight differences ($<$15%) in division rates of individual clonotypes or in initial time of activation have a large impact ($>$10-fold) on the magnitude of the response at the peak of activation. As these differences are bound to exist in biological processes, De Boer concluded that ID is nearly an inevitable outcome of nature.

In practical terms, it is clear that ID will provide a hurdle to vaccine development. Immunodomination by

T_{CD8+}-specific for determinants from vector proteins limit responses to inserted genes (shown in humans by Cerundolo for a vaccinia virus vaccine). Funding agencies should devote significant resources to develop improved vectors that provide a minimum of extraneous antigens to the immune system. Immunodomination may also dictate that vaccines meant to elicit responses to multiple targets be given as single antigen vaccines in multiple sites that access anatomically distinct lymph nodes – which is contrary to the current trend in vaccine administration. Obviously, rational vaccine design depends on understanding the relative contribution of crosspriming and direct priming, the cell biological mechanisms underlying these processes, and the identities and properties of pAPCs.

Above all, we need to understand allomorph/determinant-based differences in immune effector functions. Just as humoral vaccines aim at inducing antibodies that effectively neutralize viral infectivity, cellular vaccines should aim at inducing T_{CD8+} that exert effective antiviral activity. This means deepening our understanding of: (1) T_{CD8+} antiviral effector functions, (2) how positive and negative selection processes influence the T_{CD8+} repertoire against dominant versus subdominant antigens, (3) nuances in T_{CD8+} activation that lead to differential acquisition and application of effector activities in the afferent and efferent stages of activation, (4) differential processing and presentation of target antigens by virus-infected cells, including the cell type-dependent effects of VIPRs, and (5) how to minimize or counter selection of virus mutants that escape T_{CD8+} action.

Sadly, Fundación Juan March has decided to terminate this series of meetings. In addition to generous funding, the Fundación provided a professional staff that cheerfully and efficiently attended to every task: the scientific organizers' sole job entailed speaker selection. The Juan March meetings have profoundly impacted Spanish science. Due to their intimate size and the high quality of participants (who can resist the charms of Madrid?), they facilitated international scientific progress in a manner disproportionate to their cost. Perhaps a plea from the scientific community might persuade the Fundación to reconsider its decision: http://www.march.es/contactar/contactar.asp.

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