Private Lives: Reflections and Challenges in Understanding the Cell Biology of the Immune System

Ira Mellman, *et al.*

Science **317**, 625 (2007);
DOI: 10.1126/science.1142955

21. Z. Garcia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 4553 (2007).
22. J. Hataye, J. J. Moon, A. Khoruts, C. Reilly, M. K. Jenkins, *Science* **312**, 114 (2006).
23. R. L. Reinhardt, S. Hong, S. J. Kang, Z. E. Wang, R. M. Locksley, *J. Immunol.* **177**, 1618 (2006).
24. S.L.R. is grateful for support from the NIH and the Abramson Family. A.L. and F.S. are grateful for support from the Swiss National Science Foundation and the Helmut Horten Foundation.

10.1126/science.1143775

PERSPECTIVES

Private Lives: Reflections and Challenges in Understanding the Cell Biology of the Immune System

Ira Mellman

The immune system comprises a variety of cell types whose activities must be carefully regulated to act as a coherent unit for the purpose of host defense. To perform their autonomous functions, immune cells must rely on the same basic organizational features that apply to all cells, although immune cells often exhibit remarkable degrees of specialization and adaptation. The study of these specializations has lagged behind advances in understanding the immune response and cell biology individually. As a result, there are great opportunities, but also great challenges, for new conceptual discoveries by taking a more cell-biological approach to probing the function of the immune system.

Immunology and cell biology share at least one profound common origin. Around the turn of the 19th century, Elie Metchnikoff discovered "innate immunity" by demonstrating the ability of phagocytes to detect, engulf, and kill invading microbes. His definition of this fundamental principle of immunology was wholly enabled by paying close attention to the cell biology of how phagocytes worked. Metchnikoff's attentiveness also provided some of the first fundamental principles in cell biology, including the discovery of endocytosis, the function of lysosomes, and the ability of cells to produce cytotoxic compounds. Despite this auspicious beginning, immunology and cell biology gradually drifted apart. Perhaps because of the emerging complexity of each field, immunologists became less interested in how the cells they study actually work, whereas cell biologists (at least molecular cell biologists) avoided problems involving more than one or two cells. Yet today, like Amanda and Elyot, the divorced couple in Noel Coward's play *Private Lives*, immunology and cell biology now find themselves with new spouses in adjacent hotel rooms, realizing that there had been something wonderful in their previous relationship.

In all complex problems, understanding the mechanism provides the key to understanding the problem itself, even if this relationship is hidden by a preoccupation with the problem. In immunology, this key was long ago demonstrated by the application of molecular biology to unravel how immune cells generate the diversity required for antigen recognition by antibodies and T cells. Attention to cell-biological mechanisms has similarly produced basic insights, particularly in the areas of leukocyte diapedesis, apoptosis, and transcription. What we have learned from studying cytotoxic T lymphocyte (CTL) function has been particularly noteworthy in this regard. We have learned that cytotoxic granules are in fact modified lysosomes whose distribution and secretion are polarized to the site of target-cell recognition, increasing the directionality and thereby the selectivity of the cytotoxic payload. CTL granules polarize by interacting with microtubule-dependent motor proteins after an induced reorientation of microtubule organizing centers and thus the cell's entire microtubule network after antigen recognition (*1*). Both granule biogenesis and polarity were first described with classical cell-biological approaches and then confirmed by the analysis of mutant cells isolated from individuals with various inherited immunodeficiency syndromes (*2*). These studies demonstrated that the features of granule dynamics defined for CTLs apply to other secretory cell types (such as melanocytes). Other surprises await in these systems, such as the posttranscriptional regulation of cytokine production in secretory cells [such as natural killer cells (*3*)].

A variety of other critical problems in the immune response could also be understood at a similar level of cell-biological resolution, revealing basic new information about both immunology and cell biology. We know remarkably little about the mechanisms of cytokine secretion (especially cytosolic cytokines such as interleukin-1β), how cytoplasmic scaffolds control T cell receptor signaling, how Toll-like receptor signaling is controlled in different intracellular compartments, what the immunological synapse actually does and how it works, and the mechanisms by which alterations in cell adhesion cause cellular activation or deactivation. Another central problem in immunology that has created a natural interface with cell biology, the one that we have engaged, is antigen processing and presentation. None of these are new problems. However, they have failed to capture the imagination of more than a handful of cell biologists, leaving them to immunologists, many of whom must learn the methods and criteria of cell biology on the job. The relative lack of interaction between the two communities has created a number of disconnects over the years that perhaps have made progress more difficult to achieve than it already is.

Pathways of Antigen Processing: MHCII

With the realization more than 20 years ago that major histocompatibility complex class II (MHCII) molecules bound peptides derived largely from extracellular antigens, there has been much interest in understanding the pathways and organelles involved. It was appreciated early on that an invariant chain directed newly synthesized MHCII αβ dimers to be diverted from the secretory pathway into endocytic organelles where they encountered internalized antigens (*4*, *5*). Proteases clearly degraded invariant-chain and protein antigens, with peptide loading facilitated by chaperones such as HLA-DM, and the resulting peptide-MHCII complex then proceeded from the loading site to the plasma membrane. However, the identity of the intracellular compartment(s) in which these events transpired (as well as the order of events) remained uncertain.

Initially, the issue was, in effect, ignored by collectively referring to any endocytic organelle containing MHCII as the MHCII compartment (MIIC) (*6*). This raised a problem because the endocytic pathway comprises several distinct organelles that have decidedly different functions. Worse, the term MIIC came to imply the existence of a unique compartment specific to antigen-presenting cells (APCs). This situation obscured an underlying complexity of functional importance and substituted it with a complexity (i.e., a novel organelle) that probably did not exist (*7*, *8*). MIICs were generally assumed to have the properties of late-endocytic compartments, namely because the cells most commonly used in these studies localized most of their MHCII to late endosomes and lysosomes. Yet, not all APCs accumulate MHCII in late compartments, not all antigens are processed in late compartments, and not all APCs maintain a characteristic distribution of MHCII under all conditions. This serves to illustrate the importance of understanding organelles of immune cells with

Department of Research Oncology, Genentech, 1 DNA Way, Mail Stop 212, South San Francisco, CA 94080–4990, USA; and Departments of Cell Biology and Immunobiology, Ludwig Institute for Cancer Research, Yale University School of Medicine, New Haven, CT 06520–8002, USA. E-mail: mellman.ira@gene.com

the use of the terms and criteria established to describe organelles in general; only in this way will any unusual, immune cell–specific specializations be revealed and effectively communicated to those outside the field of immunology.

The problem is best illustrated by myeloid dendritic cells (DCs), which concentrate MHCII in late endosomes and lysosomes while immature, but after maturation translocate the MHCII to the plasma membrane (*9*). Yet, it is not clear that the depletion of MHCII from late compartments eliminates the ability of a given antigen to be processed and presented. Many primary B cells and B cell lines can act as efficient APCs without localizing much MHCII to late-endocytic compartments (*10*). Although DCs can form peptide-MHCII complexes in lysosomes for delivery to the surface upon maturation, this may reflect the particular biology of DCs and not represent a general phenomenon. To solve these issues, we need better and more sensitive imaging and biochemical tools to identify when and where peptide-MHCII complexes form, combined with the use of genetics or conditional switches to disrupt predicted individual steps. Such tools must be applied not only to solve the problem in culture but also to investigate APCs in vivo.

Understanding the order of events remains a problem as well: Textbooks often imply that internalized antigens are converted by proteolysis to peptides, which bind at equilibrium to MHCII molecules. Yet, a variety of considerations suggest that intact antigens can bind to MHCII before degradation, with peptides being generated after exoproteolysis from both the N- and C-terminal ends [for review, see (*11*)]. Such a mechanism would be consistent with the observations that protein degradation in lysosomes does not produce even a transient accumulation of peptide intermediates and that professional APCs, such as DCs and B cells, exhibit greatly attenuated levels of lysosomal and endosomal proteases, conditions that would facilitate the sculpting of denatured antigens bound to MHCII. How peptides are loaded onto MHCII molecules may be the most fundamental issue in the field, but it still awaits direct proof by kinetic analysis and biochemical reconstitution.

Presentation of Endogenous Versus Cross-Presentation of Exogenous Antigens on MHCI

When work on the MHCII system was beginning, it was appreciated that the concentration of antigen required to elicit a CD4 T cell response was small, relative to the amount required to track its behavior by biochemical or immunocytochemical techniques. It was thus formally possible that the observed pathway was not the physiologically important one. Yet, the "obvious" mechanism turned out to be correct: antigen endocytosis and intracellular formation of peptide-MHCII complexes, followed by their delivery to the plasma membrane. On the other hand, understanding antigen processing and presentation via MHC class I (MHCI) molecules presented—and continues to present—a daunting conceptual challenge. It was clear early on that the peptides recognized by $CD8^+$ T cells were often derived from cytosolic viral proteins, proteins that never reached the endoplasmic reticulum (ER) lumen and therefore should never come in contact with the peptide-binding groove of MHCI (*12*). Although the violation of compartment barriers is anathema to cell biologists, the problem was solved in a convincing fashion by the genetic identification of the ER-localized transporter associated with antigen processing (TAP) peptide translocator and the in vitro reconstitution of its activity (*13*). Elucidating how exogenous antigens are "cross-presented" on MHCI after endocytosis has eluded a similarly elegant solution.

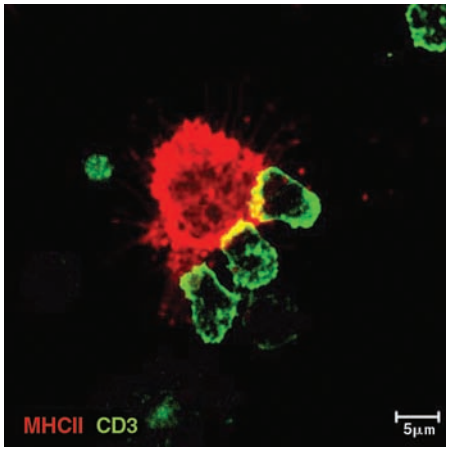

Fig. 1. Mature DCs express large quantities of self- and foreign peptides bound to MHCI and MHCII (red) molecules and can present peptide-MHC complexes to multiple T cells simultaneously (CD3, green). It is assumed, but not entirely clear, that different peptide-MHC complexes can be presented at the same time. [Photograph courtesy of Ona Bloom, Yale University School of Medicine]

Bevan and colleagues were the first to show that an antigen internalized by endocytosis could be loaded onto MHCI molecules, although (using fibroblasts) they had to resort to a "trick" thought to chemically disrupt endosomes after antigen uptake (*14*). More recent work has established that DCs are by far most efficient at cross-presenting peptides derived from internalized antigens (*9*). Although some peptides can be loaded within the confines of endocytic organelles (*15*), there is excellent evidence that most incoming antigens must gain access to the cytosol in order to be cleaved by the proteasome before MHCI loading (*16*). This creates two compartment barrier violations: (i) the escape of antigens or peptides across the endocytic organelle membrane to the cytosol and (ii) reimportation across (presumably) the ER membrane to bind to MHCI. Although it is clear that TAP plays a role in translocating antigen-derived peptides in the ER (*11*, *17*), how antigens egress from endocytic organelles remains a mystery. Among the suggested mechanisms are the stochastic or induced rupture of endosomes, regulated endosomal pores, and the presence of ER-derived channels responsible for retrotranslocation of misfolded proteins. The latter has received much attention after initial excitement over the possibility that forming phagosomes physically fused with the ER, a suggestion based on qualitative static electron microscopy images but not confirmed by subsequent work (*18*).

It nevertheless remains possible that ER components are present in endocytic compartments, although it has been difficult to demonstrate this point by conventional or well-accepted approaches to immunolocalization. In other words, if they are present they represent trace components, a situation quite unlike the ER, where TAP, subunits of the translocon (such as Sec61 and derlins), and the various chaperones and glycosyltransferases associated with translocation are easy to detect (*18*). There is certainly earlier evidence that some ER components can reside at least transiently in the Golgi, endosomes, or even the plasma membrane (*11*), but using elegant and sensitive assays to demonstrate their accessibility to endocytic probes (*19*) cannot be taken as definitive evidence that they perform a physiologically relevant function outside the ER. For example, small amounts of antigen may reach the ER itself by retrograde transport, as occurs for a number of viruses and bacterial toxins (*20*): A small escaped fraction of an antigen may create the signal, rather than a small endosome-localized fraction of ER components. As described above, one must use biochemical reconstitution and genetics to clinch an idea created by localization and functional studies. A problem as vexing and important as the mechanism of cross-presentation provides fertile common ground that will continue to challenge immunologists and cell biologists alike.

Antigen Targeting to DCs

With the appreciation that antigen presentation by DCs is responsible for initiating adaptive immune responses has come considerable interest in therapeutic uses of DCs in augmenting or attenuating immune responses. Antigen delivery to DCs is a minimum prerequisite for any therapeutic setting, and it is now clear that the induction of antigen-specific immunity (or tolerance) can be dramatically enhanced by targeting a desired antigen with antibodies against DCs in the presence (or absence) of a DC-maturation signal (*21*). DCs express a plethora of surface receptors, many being C-type lectins that have proved to provide effective portals of entry.

Several cell-biological questions are of interest concerning these receptors: (i) Are they internalized, and where do they deliver bound antigens? (ii) Are some receptors better at programming cross-presentation? (iii) Can individual receptors target functionally distinct DC subpopulations? (iv) Does antibody binding to a given receptor trigger DC maturation, and if so, does receptor activation help to control the balance between immunity and tolerance? In general, we must ask what the relationship is between the mode of antigen endocytosis and the function of antigen presentation to T cells (Fig. 1).

In recent work published in *Science*, Dudziak *et al.* have shown that two lectin receptors on mouse DCs, DEC-205 and DCIR (33D1), are differentially expressed by two different populations of DCs in the spleen: $CD8\alpha^+$ and $CD8\alpha^-$ DCs, respectively (*22*). Targeting antigens via DEC-205 to the $CD8\alpha^+$ subset was found to selectively prime MHCI-restricted responses by cross-presentation, whereas MHCII-restricted responses were more efficiently triggered by DCIR targeting to the $CD8\alpha^-$ subset. This finding is consistent with the idea that cross-presentation is, in general, more the purview of $CD8\alpha^+$ DCs (*23*). Reversing cell-type restriction of receptor expression did not appear to change this conclusion substantially, suggesting that specializations associated with $CD8\alpha^+$ DCs may favor their ability for cross-presentation.

At the same time, in vitro work suggested that another lectin (the mannose receptor) was also quite effective at inducing the formation of peptide-MHCI complexes from exogenous antigen in bone marrow cultures and macrophages, where subpopulation identities are less clear (*24*). The mannose receptor appeared to deliver bound antibody to early endocytic compartments, suggesting that cross-presentation may occur from here as opposed to in late endosomes and lysosomes, which was the case for loading onto MHCII. Although these results suggest that the receptor used and route of antigen entry may also help determine the resulting form of antigen presentation, the data relied only on low-resolution qualitative immunofluorescence to define the intracellular localization of delivered antigens—criteria too limited to support a firm conclusion. Moreover, the data did not account for the fact that DEC-205, which also efficiently mediates MHCI-restricted cross-presentation, has been extensively characterized as delivering its bound antigens to late endosomes and lysosomes as opposed to early compartments (*25*). In any event, these findings highlight a whole new problem set involving the relative contributions of endocytosis and DC subpopulations in determining the nature of the immune response.

Looking Forward by Looking Backward

There are many other problems that would benefit immediately from a more effective and bidirectional relationship between immunologists and cell biologists. For example, the dynamics and function of the immunological synapse remain incompletely understood, in part because these critically important structures have yet to be subjected to the type of rigorous analysis applied to "simpler" problems of cell adhesion. The relationship between autophagy and antigen presentation in viral immunity is also emerging as critical (*26*, *27*). Signaling in immune cells will provide a rich area to mine, and some direct interchange over what lipid rafts can and cannot do would be of great value in itself. Finally, there is the issue that immunologists have always appreciated far better than most molecular cell biologists: the in vivo or systems-level context. Immunology exists to study the way the immune system works as a whole to confer protection against disease. Broad and conceptually profound, it is understandably difficult for the field to devote equivalent attention to the cellular mechanisms involved. However, further progress will require such effort, and the best path forward will be to take steps to make the language, concepts, and culture of immunology more accessible to colleagues in cell biology to attract them in and to outsource what may be too diversionary to learn. One area that is particularly ripe for spectacular advance is in the area of in vivo or "intravital" imaging. Although still in a largely descriptive phase of development, immunologists are nicely demonstrating to cell biologists the conceptual value of this platform. When this area is combined with emerging technologies to permit interventional experiments using actuation switches and quantitative molecular reporters, we will have entered a new age of "systems cell biology," combining the best of both worlds.

Like Elyot and Amanda, immunology and cell biology were once intimate partners; we find ourselves again in close proximity, but this time with the chance to rekindle a beautiful relationship.

References

1. J. C. Stinchcombe, E. Majorovits, G. Bossi, S. Fuller, G. M. Griffiths, *Nature* **443**, 462 (2006).
2. J. Stinchcombe, G. Bossi, G. M. Griffiths, *Science* **305**, 55 (2004).
3. D. B. Stetson *et al.*, *J. Exp. Med.* **198**, 1069 (2003).
4. P. R. Wolf, H. L. Ploegh, *Annu. Rev. Cell Dev. Biol.* **11**, 267 (1995).
5. P. Cresswell, *Annu. Rev. Immunol.* **12**, 259 (1994).
6. P. J. Peters, J. J. Neefjes, V. Oorschot, H. L. Ploegh, H. J. Geuze, *Nature* **349**, 669 (1991).
7. P. Pierre *et al.*, *Immunity* **4**, 229 (1996).
8. M. J. Kleijmeer, S. Morkowski, J. M. Griffith, A. Y. Rudensky, H. J. Geuze, *J. Cell Biol.* **139**, 639 (1997).
9. I. Mellman, R. M. Steinman, *Cell* **106**, 255 (2001).
10. S. Amigorena, J. R. Drake, P. Webster, I. Mellman, *Nature* **369**, 113 (1994).
11. E. S. Trombetta, I. Mellman, *Annu. Rev. Immunol.* **23**, 975 (2005).
12. A. R. Townsend, J. J. Skehel, *J. Exp. Med.* **160**, 552 (1984).
13. M. T. Heemels, H. Ploegh, *Annu. Rev. Biochem.* **64**, 463 (1995).
14. M. W. Moore, F. R. Carbone, M. J. Bevan, *Cell* **54**, 777 (1988).
15. L. Shen, L. J. Sigal, M. Boes, K. L. Rock, *Immunity* **21**, 155 (2004).
16. A. Rodriguez, A. Regnault, M. Kleijmeer, P. Ricciardi-Castagnoli, S. Amigorena, *Nat. Cell Biol.* **1**, 362 (1999).
17. A. Y. C. Huang, A. T. Bruce, D. M. Pardoll, H. I. Levitsky, *Immunity* **4**, 349 (1996).
18. N. Touret *et al.*, *Cell* **123**, 157 (2005).
19. A. L. Ackerman, A. Giodini, P. Cresswell, *Immunity* **25**, 607 (2006).
20. J. M. Lord, L. M. Roberts, *J. Cell Biol.* **140**, 733 (1998).
21. L. Bonifaz *et al.*, *J. Exp. Med.* **196**, 1627 (2002).
22. D. Dudziak *et al.*, *Science* **315**, 107 (2007).
23. P. Schnorrer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10729 (2006).
24. S. Burgdorf, A. Kautz, V. Böhnert, P. A. Knolle, C. Kurts, *Science* **316**, 612 (2007).
25. K. Mahnke *et al.*, *J. Cell Biol.* **151**, 673 (2000).
26. H. K. Lee, J. M. Lund, B. Ramanathan, N. Mizushima, A. Iwasaki, *Science* **315**, 1398 (2007).
27. D. Schmid, M. Pypaert, C. Munz, *Immunity* **26**, 79 (2007).

10.1126/science.1142955

PERSPECTIVE

Emerging Challenges in Regulatory T Cell Function and Biology

Shimon Sakaguchi[1] and Fiona Powrie[2]

Much progress has been made in understanding how the immune system is regulated, with a great deal of recent interest in naturally occurring $CD4^+$ regulatory T cells that actively engage in the maintenance of immunological self-tolerance and immune homeostasis. The challenge ahead for immunologists is the further elucidation of the molecular and cellular processes that govern the development and function of these cells. From this, exciting possibilities are emerging for the manipulation of regulatory T cell pathways in treating immunological diseases and suppressing or augmenting physiological immune responses.

Walter B. Cannon, the originator of the concept of homeostasis, emphasized in his book *The Wisdom of the Body* that "when a factor is known which can shift a homeostatic state in one direction it is reasonable to look for a factor or factors having an opposing