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Uncovering the Role of Invariant Chain in Controlling MHC Class II Antigen Capture

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It has been known for centuries that humans can become specifically “immune” to particular infectious organisms if they survive a first infection. In the 19th and early 20th centuries, it became clear that much of this specific resistance was the property of soluble factors in blood termed Abs. It was thus surprising when Benacerraf and colleagues discovered in the 1960s that immunity to Ags of limited complexity in guinea pigs was under Mendelian genetic control and could not be ascribed to such serum Abs (1, 2). Soon after, McDevitt determined that the relevant genes mapped to the MHC, and studies from multiple laboratories identified the encoded products of these “Immune Response” or “IR” genes as what are now termed MHC Class II molecules (1–4).

How these MHC-encoded cell surface proteins contributed to control of immune responses was a mystery when IR genes were first described. At that time, the distinction between B and T lymphocytes was unrecognized, although earlier work by Benacerraf showed that while Abs to a protein lost reactivity if a protein Ag was denatured, Ag-specific delayed type hypersensitivity reactions nonetheless were retained (5). As the existence of T cells became recognized and associated with so-called cell-mediated immunity, including delayed type reactions, the importance of IR genes in controlling immune reactivity at the T cell level became evident.

But these advances still begged the question of what the MHC class II genes were doing. By this time, the role of MHC gene products in ‘restricting’ the response of T cells to foreign Ags had become apparent through the work of Zinkernagel and Doherty for MHC class I molecules and CD8⁺ T cells (6, 7) and of Shevach, Paul, and others for MHC class II molecules and ‘helper’ or CD4⁺ T cells (8, 9). This led to a major debate in the field: did such restriction involve “dual recognition” of two molecular entities, the MHC molecules and the Ag, presumably through two distinct receptor systems, or was there somehow combined recognition of the two components by one receptor? Several emerging threads of research lent increasing support to the one-receptor model. In particular, on the Ag side, Unanue and associates, along with other investigators, developed clear evidence that T cell recognition in the context of MHC class II molecules required active endosomal/lysosomal processing of the original protein Ag (10, 11). This evidence for proteolytic processing along with careful studies of antigenic specificity and the role of MHC molecule genetic variation in the control of immunity led to the hypothesis that MHC class II molecules stimulated CD4⁺ T cells by capturing and presenting small fragments of protein Ags, with

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the specificity of such capture dictated by the allelic structure of the highly polymorphic MHC class II proteins (12–14). This accounted for IR gene function, as one needed the right MHC class II allele to present at least one such peptide from a simple Ag to the receptor of T cells. A critical breakthrough that solidified this conceptual model came when Unanue (15) and shortly thereafter, Grey and colleagues (16), provided direct biochemical evidence of binding between antigenic peptides and the relevant MHC class II proteins.

These data helped provide a clear picture of how Ag recognition by CD4⁺ T cells occurred but left a major gap in our understanding of CD8⁺ T cell responses that involved MHC class I molecules. Thinking in this area was dominated by the notion that intact viral Ags on the cell surface were the relevant targets of recognition, but seminal work from Townsend first showed that influenza nucleoprotein was a major Ag for CD8⁺ T cells and then revealed that short peptides from nucleoprotein could sensitize target cells with the correct MHC class I expression for killing by NP-specific CD8⁺ T cells (17). This brought CD8⁺ T cell recognition of Ag in line with that of CD4⁺ T cells as involving peptide presentation by MHC-encoded molecules. In the context of crucial data from Braciale and coworkers (18), these findings led to the proposal that MHC class I and class II molecules were specialized for presentation of peptides derived from distinct protein pools in different intracellular compartments (19).

At this point, substantial attention became focused on the cell biology and biochemistry of MHC-peptide complex formation. On the MHC class II side of things, the question was how proteins that assembled as dimers in the endoplasmic reticulum could capture and selectively present antigenic peptides derived from proteins in the endosomal/lysosomal pathway. Enter the invariant chain (Ii), a non-MHC encoded protein Jones et al. had first reported to co-immunoprecipitate with MHC class II proteins (20). Work in numerous laboratories began to define the nature of MHC class II-Ii association and biosynthetic processing. In 1990, Roche and Cresswell as well as Teyton *et al.* showed that MHC class II molecules would not capture peptides unless Ii was removed by detergent (21, 22). Teyton and colleagues concluded that Ii thus served to distinguish the two pathways for MHC molecule Ag presentation: the exogenous pathway that involved MHC class II molecules and endocytically acquired proteins and the endogenous pathway involving MHC class I proteins and their associated peptides that by then were known to bind MHC class I in the endoplasmic reticulum (ER) (22).

However, these data alone did not provide a fully satisfying picture of how Ag capture by MHC class II was regulated. How did MHC class II molecules that formed binding sites in the ER capture substrates that resided in a distinct intracellular compartment? The evidence that Ii controlled binding site accessibility suggested Ii might play a critical regulatory role, but didn't provide a biochemical explanation for this process. In their seminal 1991 paper in PNAS, Roche and Cresswell answered this nagging question by demonstrating that partial proteolysis of Ii by an acidic peptidase (cathepsin B) revealed a functional MHC class II peptide binding site (23). Cathepsin B was known to be active in the same endosomal compartments as those in which Ags were degraded, providing a logical site of convergence of the two cell biological processes, namely Ii removal from MHC class II molecules and the capture of proteolytically degraded Ags. Based on these data, they proposed, in accord with the implications of the preceding Teyton work, that Ii protects the MHC class II binding site and “inhibits immunogenic peptide binding to $\alpha\beta$ I early during intracellular transport.”

The set of findings refocused the field on the details of this molecular interaction, the precise site(s) of Ii proteolysis, and the location of Ag capture. Work from several groups over the next few years revealed that Ii actively targeted MHC class II molecules into the endocytic

pathway (24), where Ii was removed in a step-wise fashion (25), with an interior peptide called CLIP occupying the MHC class II binding site until cleaved in its N and C sides by proteases in this pathway. HLA-DM then mediated exchange of the loosely bound CLIP peptide with better binding antigenic fragments that were subsequently trimmed on exposed N and C ends to yield the peptides presented on the cell surface to T cells (26).

Thus, the study of Roche and Cresswell was a turning point in the field, although like most other advances of this type, it built on the work not only of the same laboratory over many years, but crucial observations of others working in parallel on this central issue in immunology. The control of class II Ag capture by Ii was a key advance in our understanding of how foreign Ags are made available to T cells for recognition, although one important implication of these data has been misunderstood for many years. Many still think of the CLIP region of Ii as preventing MHC class II from inappropriately binding the short peptides transported by TAP into the ER and meant for occupancy of MHC class I – β 2 microglobulin complexes. However, analysis of MHC class II molecules in Ii-deficient cell lines (27, 28) and cells from genetically engineered animals lacking Ii (29–31) has made clear that such short peptides do not stably occupy MHC class II molecules to an appreciable degree even when Ii is absent. So then what is the purpose of the binding site inhibition so cogently revealed in the studies of Roche and Teyton? Through the work of many laboratories, including our own, we now understand that the crucial role of Ii is to keep MHC class II molecules from binding longer sequences present in incompletely folded or floppy proteins undergoing biosynthesis in the ER, that is, from binding the same structures that are the preferred class II preferred substrates in endosomes (32). These substrates are not fully processed short peptides of the type that occupy MHC class I molecules, but rather the exposed segments of partially degraded proteins (33), consistent with the open ends of the MHC class II binding site in contrast to the closed binding site structure of MHC class I molecules.

Although there is a great emphasis presently on better understanding the innate immune system, it is important to remember that immunity to pathogens has for the longest time been recognized to have Ag specificity, and understanding the origin of such specificity in the adaptive response was a critical focus of immunological research for more than a century. The paper from Roche and Cresswell is a key building block of the intellectual edifice we now take for granted; it provided a key advance that integrated fundamental cell biological processes with the specificity of Ag responses involving conventional $\alpha\beta$ T cells. It is for this reason it remains a Pillar of Immunology.

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