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CD4 on the Road to Coreceptor Status

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The advent of monoclonal antibody technology in the late 1970s initiated a wave of discovery that lead to the identification of many important cell surface proteins that defined unique cellular populations with restricted functions (1). In the early 1980s, antibodies to T4 (now known as CD4) and T8 (now known as CD8) were shown to identify unique subpopulations of T cells with either helper or cytotoxic activity, respectively (2–6). Importantly, antibodies to CD4 (T4/Leu3 in humans; L3T4 in mice) were shown to block the MHC class II-mediated proliferation and function of T cells leading to the suggestion that CD4 was intimately involved in the cooperative recognition of MHC class II associated foreign antigen by T cells (5,7,8). Early studies by the Marrack/Kappler and Burakoff labs had suggested that CD4 binding to MHC class II molecules might increase the overall avidity between T cells and APCs (9–11). However, direct evidence for such an interaction had remained frustratingly elusive and it wasn't clear if additional molecules were required for this interaction. Furthermore, the function of this interaction was controversial.

In the 1987 *Pillars of Immunology* article discussed in this commentary (12), Doyle and Strominger provided the first direct evidence that CD4 bound to MHC class II and that this interaction could mediated cell adhesion. In this study they generated recombinant simian virus 40 (SV40) encoding human CD4 which was used to infect CV1 fibroblasts. A distinct advantage of this approach was that high expression of CD4 was obtained, which would prove crucial (10– 15 times more that the HPB-ALL human T cell line). A cell binding assay was then established to study CD4:MHC class II interaction using the $CD4^+$ CV1 fibroblast monolayer and the human B lymphoblastoid cell line, Raji, that expressed very high levels of MHC class II. Importantly, they showed that three other MHC class II positive B cells also bound to the $CD4^+$ CV1 fibroblast monolayer but that two MHC class II negative B cells did not. As one might expect there was a direct correlation between the extent of cell adhesion and the level of MHC class II expressed by the various B cell lines. Furthermore, they showed that antibodies to CD4 or pooled antibodies to HLA-DR and DP blocked cell adhesion, while antibodies to MHC class I had no effect. While these experiments might seem almost simplistic by modern standards, it should be emphasized that this was a very significant observation and achievement at the time. This study was also important because it was performed using a heterogonous system devoid of potential TCR:MHC interaction, thus ruling out the possibility that CD4:MHC class II 2 interaction required the T cell receptor or another T cell protein. This study served to further galvanize interest in this important interaction.

A factor that was instrumental in the success of this study was the use of two cell populations that expressed very high levels of their respective proteins. Indeed, in discussion the authors commented that "the high level of CD4 expression obtained in this study was crucial for demonstrating this high affinity, stable interaction". However, the authors acknowledged that at more physiological levels of expression this CD4:MHC class II interaction likely helps to mediate low affinity interactions and that "these molecules must

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work in parallel with other specific and accessory adhesion molecules to allow functional cell-cell interactions". Indeed, subsequent studies independently confirmed this interaction (13) and showed that CD4:MHC class II affinity was in fact very weak and barely measureable by surface plasmon resonance (14), making the key role played by CD4 even more remarkable. We should not forget that while this story was evolving similar studies were demonstrating a direct interaction between CD8 and MHC class I molecules (15–18).

The discovery of CD4 as the receptor for HIV further enhanced interest in the structure-function analysis of CD4:MHC class II interaction and how this compared with the CD4:gp120 association. Mapping studies reveled that multiple residues on one face of the D1 and D2 membrane-distal domains of CD4 (which has four extracellular domains) recognize MHC class II (19–22). This appeared to only partially overlap with the more confined gp120 binding site. Reciprocal analysis showed that the primary binding site for CD4 was in the MHC class II β2 domain (23–25). However, subsequent analysis implicated the involvement of other domains (26,27).

Subsequent studies revealed that CD4 had additional interactions which appeared to contribute to its function (28,29). Insight into the mechanism of CD4 signaling was first revealed by studies demonstrating the association of the *Src* tyrosine kinase, $p56^{lck}$, with the cytoplasmic tail of CD4 (30–32). Physical association between CD4 and the TCR was also demonstrated (33–35) and mapped to the D3 domain (36). These observations led Janeway to propose that CD4 acted as a co-receptor for potentiating TCR signaling and function (37,38). Importantly, subsequent studies showed that CD4 had to bind with the same MHC class II molecules as the TCR supporting this notion (39).

The critical importance of CD4 in mediating helper T cell development and function was realized following the analysis of CD4 deficient mice (40). This also facilitated the generation of mice that only expressed a tailless CD4 mutant, which could not associate with $p56^{lck}$, demonstrating that T helper cell development could be rescued in the absence of CD4:$p56^{lck}$ signaling (41). While this suggested that CD4:MHC class II interaction alone could mediate T cell development, this only occurred when tailless CD4 was overexpressed. More recent microscopic analysis of TCR:MHC class II interaction within the immunological synapse has suggested that CD4 initially associates with the TCR before being segregated to the periphery of the contact zone (42,43). Thus, the current consensus is that the CD4:MHC class II interaction doesn't impart a significant adhesion benefit to the T cell:APC interaction *per se* (44), but rather plays an instrumental role in bringing p56lck to the TCR:CD3 complex. Of course some measure of affinity for MHC class II 3 is required for this process, which was confirmed by the original work of Doyle and Strominger (12).

Despite the substantial number of studies that have attempted to dissect the function of CD4 and how this is regulated, it is possible that important details remain to be elucidated. It is evident in biology that key molecules continue to reveal surprising observations despite decades of examination; p53 and $NF_{lck}B$ being prime examples. Given the central importance of CD4 in T cell biology is remains possible that some of its secrets remain to be revealed. Indeed many questions remain, for instance, the contribution and kinetics of CD4 in TCR:MHC microcluster formation and migration, how these events are regulated by CD4 palmitoylation, and structural insight into the precise docking of CD4 to the TCR:CD3:MHC complex.

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Abbreviations used in this paper

SV40 simian virus 40

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