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Pillars of Immunology

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Tuberculosis is the infectious disease of the human race, as it has been responsible for over one billion deaths in the last 200 years (1). Progress in controlling this disease is therefore a critical goal of the World Health Organization and other engaged, nongovernmental organizations (2). Despite significant efforts, there is still a need for an improved vaccine to limit lung disease. It is this need that has led to extensive research into the nature of the vertebrate immune response to the etiological agent of tuberculosis, *Mycobacterium tuberculosis*. Our understanding of this response is grounded in this month's *Pillars of Immunology* article that described fully, and for the first time using modern techniques, the nature of the T cells that respond to *M. tuberculosis* within a defined experimental system (3). This article, written in the author's characteristic clear and forthright manner, represents a substantial body of work that has informed and directed the tuberculosis T cell community for 30 y.

What was the genesis of this keystone article? One element was the environment the *Pillars of Immunology* author, Ian Orme, was working in at the Trudeau Institute in upstate New York. This was where, in the 1960s–1970s, George Mackaness developed and consolidated his theories on cell-mediated immunity, which culminated in his seminal work describing the need for the interaction between Ag-specific lymphocytes and macrophages for control of *Listeria monocytogenes* (4). Although Mackaness was not present at the Trudeau Institute during the period when the work described in the *Pillars of Immunology* article was conducted, his techniques and concepts were still strongly contributing to intellectual activity at the Institute, and he maintained an advisory presence, which directly influenced Orme. Another influence was Robert North, whose clear writing style provided the template for Orme's own papers. North was also using T cell transfers in infection and tumor models (5, 6), and this provided the impetus for Orme to use this technique to fully define the protective nature of T cells throughout *M. tuberculosis* infection (3). The final critical element that catalyzed Orme's work was the availability of superb technical expertise, which allowed for the performance of animal experiments with optimal and reproducible outcomes. This expertise has persisted at the Trudeau Institute and the person acknowledged for technical assistance at the end of the *Pillars of Immunology* article, Alan Roberts, is employed at the Trudeau Institute today.

What was the critical understanding that the *Pillars of Immunology* article provided? At the time of this article's publication, it was thought that sensitized T cells interacted with macrophages via MHC class II and released cytokines that activated macrophages to kill intracellular *M. tuberculosis*. It was also thought that T cells contributed to the inflammatory consequences of infection represented by granuloma formation and delayed type hypersensitivity. What was unknown was the nature of the T cells, what they did in terms of protection and immunopathology, and whether they could be characterized by their response to specific Ags. The goal of Orme's work was to determine the nature of the protective T cells and then induce them by vaccination. In this month's *Pillars of Immunology* article, Orme addressed the nature of the T cells, both in terms of function and surface phenotype, thereby providing the basis for much future work on T cell responses to *M. tuberculosis* infection. What was not addressed in this article was the nature of the Ags being recognized; but in a second article, Orme collaborated with Patrick Brennan at Colorado State University to determine for the first time which Ags of *M. tuberculosis* stimulated the T cells mediating protection (7). The theme in both articles was, and indeed has been in Orme's work throughout his career, that the critical activity to be measured in a T cell is its ability to mediate protection against *M. tuberculosis* growth in vivo. Although using this as a defining feature makes obvious sense, this measurement is not a simple thing to perform and requires clear and definitive in vivo experimentation as well as significant investment in containment facilities; something that Orme has worked to achieve at Colorado State University (http://csu-cvmbs.colostate.edu/academics/mip/Pages/Rbl.aspx).

What, then, does the *Pillars of Immunology* article tell us about T cells in the defined mouse infection model? The first important observation is that the nature of the T cell response is temporally associated with the kinetics of bacterial activity within the target organs (figure 2 in Ref. 3). This is critical and demonstrated that T cell responses do not comprise a fixed entity, but rather constitute an integration of changing

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in vivo events, thereby underpinning future work highlighting the role of Ag availability, the inflammatory context, and even the role of the microbiome in defining individual T cell responses. The article uses a deceptively simple approach, in that splenocytes from infected mice were purified by panning on Ab-coated plates (no easy magnetic bead kits were available at this time) and then transferred into irradiated (500 rad, 24 h earlier) recipient mice, who then received an *M. tuberculosis* infection. The level of bacterial burden was then compared between mice that had received various lymphoid populations and a $\log_{10}$ protection value was determined. This model had been developed by Orme and Frank Collins in previous work at the Trudeau Institute, which resulted in the first report of a T cell requirement for protection against aerosol *M. tuberculosis* infection (8). The differential ability of T cells to mediate protection over time was shown in the *Pillars of Immunology* article by complement and anti-Thy1.2 treatment of donor T cell populations taken from mice that had been infected with *M. tuberculosis* for various periods prior to harvest. In addition, the relative roles of $CD4^+$ and $CD8^+$ T cells were also determined over time by treating the donor populations with anti-L3T4 (−CD4) or anti-Lyt2 (−CD8) Ab and complement. Using this technique, Orme identified the biphasic capacity of the T cell response to mediate protection, peaking firstly post the peak bacterial growth and thereafter 80 d into infection (figure 2 in Ref. 3). By breaking the T cell contribution into that provided by $CD4^+$ and $CD8^+$ subsets, Orme identified for the first time that although both subsets exhibited the biphasic response, the protective $CD8^+$ subset was delayed relative to the $CD4^+$ subset and was also less substantial (figure 3 in Ref. 3). Using T cells from the first peak, Orme addressed the relative capacity of $CD4^+$ and $CD8^+$ T cells to protect against aerosol infection and found that whereas $CD4^+$ T cells were efficient in controlling low dose infection, the $CD8^+$ T cells were actually able to allow mice to survive a normally lethal infection (figure 4 in Ref. 3). Using the low-dose challenge, it was also clear that the biphasic nature of the T cell response and the relative levels of protection mediated by $CD4^+$ and $CD8^+$ T cells seen in the i.v. challenge also occurred in the aerosol challenge (figure 5 in Ref. 3). These distinct differences between the protective capacities of the T cell subsets in *M. tuberculosis* infection have been investigated further, with new developments showing the importance of specific cell-trafficking patterns in mediating protection (9–11). Interestingly, the inability of protective T cells to mediate early protection in the aerosol model shown in this article was seen in several later studies and has prompted a focus on ensuring the presence of T cells within the lung for effective early protection (12).

The *Pillars of Immunology* article also determined the impact of the proliferative state of the T cells on their ability to mediate protection by treating the donor mice with cyclophosphamide to deplete actively dividing cells. Again, a clear difference was observed between the $CD4^+$ and $CD8^+$ T cells, with $CD8^+$ T cell protection always being ablated by prior cyclophosphamide treatment, whereas the $CD4^+$ T cells appeared to be highly sensitive immediately postinfection but increasingly resistant as the infection progressed (figure 6 in Ref. 3). Orme ascribed this difference in cyclophosphamide sensitivity to different levels of proliferation among the protective T cells. He also suggested that this T cell proliferation was dependent upon the activity of the bacteria; i.e., when bacteria were growing, the T cells were also being driven to expand, whereas as bacterial burden was reduced, the T cells became more quiescent and memory-like. This concept was expanded and integrated with studies of the Ag specificity of these cells in a subsequent important article (13).

While the *Pillars of Immunology* article defined for the first time the dynamic nature of the T cell response to *M. tuberculosis*, the subsequent articles published in *The Journal of Immunology* by Orme and colleagues demonstrated the Ag specificity (7) and mixed effector/memory nature of T cells (13) present in the *M. tuberculosis*–infected host. In combination, these articles provided the basis for our understanding of the T cell response to *M. tuberculosis* and are as relevant today as they were when they were first published.

Disclosures

The author has no financial conflicts of interest.

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