T Cells Stop to Smell the (Antigenic) Roses

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In 1971, prehistory for many contemporary readers of The Journal of Immunology, MHC restriction was yet to be outlined, TCR structure was unexplored, and T cell maturation remained shrouded in mystery. However, elegant work in currently unfashionable species such as sheep and rats had clearly established that lymphocytes recirculate, traveling from the blood via lymph nodes to the lymph, and returning to the blood by way of the thoracic duct (1, 2). All recirculating lymphocytes eventually traverse the thoracic duct. There were also data to suggest that lymphocyte trafficking was altered by Ag exposure, and the notion that Ag-specific lymphocytes could be transiently recruited from the recirculating pool was being bandied about (3). It was within this setting that Jon Sprent joined Graham Mitchell in the lab of Jacques Miller at the Walter and Eliza Hall Institute in Melbourne and decided it was time to investigate lymphocyte recirculation in the frustratingly small but inexpensive and genetically malleable species of laboratory mice (4).

Sprent learned to cannulate the minute thoracic duct of anesthetized mice, a daunting surgical feat that involved jeweller’s forceps, 0.5-mm bore nylon tubing, tissue adhesive, a fine horse hair to remove lymph clots, and a steady hand coupled with infinite patience. In these pre-animal-care-committee days, cannulated mice were left for days to run on the top of a hamster wheel, where they churned out around 20 ml of lymph daily (5, 6).

To determine whether Ag-specific T cells are recruited from the circulation by Ag exposure, Sprent and Mitchell injected CBA mice i.v. with SRBC and collected thoracic duct lymphocytes over the course of the next 5 days. Why use sheep erythrocytes? Sprent, Miller, and Mitchell reasoned that using a multicomponent Ag would be more likely to yield a generic answer, rather than one that applied only to that particular antigenic response. SRBC also offer an easy means of enumerating Ab-producing cells, using a modification of a viral plaque-forming assay. In this assay, spleen cells are seeded over a lawn of SRBC or HRBC in agar on a microscope slide and scanned some time later after the addition of complement for holes (or plaques) in the lawn. Holes formed in the presence of antigen-specific Abs are called indirect plaques, and result from red cell cytolysis upon secretion of SRBC- or HRBC-specific IgM or IgG Abs by an Ab-forming cell, usually visible in the center of the plaque. Cytolysis in the absence of cross-linking antoglobulin (direct plaques) requires secretion of pentameric IgM Ab. This simple method allows quantification of Ag-specific IgM- and IgG-secreting cells.

The crystal clear data of Sprent et al. (4) showed that thoracic duct lymphocytes were depleted of SRBC-specific T cells during the first 1 to 2 days of Ag exposure (whereas HRBC-specific T cells were as abundant as they were before SRBC injection). Conversely, the thoracic duct lymph was specifically enriched for SRBC-reactive T cells by day 5 of Ag encounter. Upon Ag injection, the tissues of the primary donor animal efficiently filtered Ag-reactive T cells, first depleting and then enriching their numbers within the pool of recirculating lymphocytes. Sprent and Mitchell showed this process of “biological filtration” applies equally to alloreactive lymphocytes withdrawn from recirculation 1 to 2 days after injection of histoincompatible lymphocytes. Thus, 2 days after injection of (CBA x C57BL/6)F1 spleen cells, thoracic duct lymphocytes from CBA mice were unable to cause graft-vs-host response (measured as splenomegaly) when transferred to (CBA x C57BL/6)F1 neonates, but their ability to respond to (CBA x BALB/c)F1 mice remained unabated (4).

From this foundation, only minor technical embellishments were required to negatively select (MHCa x MHCb)F1 T cells in Ag-injected parental MHCa hosts and show that Ag-specific F1 T cells able to collaborate with MHCb B cells remained. These experiments, performed in 1978, revealed that (MHCa × MHCb)F1 T cells are comprised of largely nonoverlapping sets of Ag-specific T cells able to help MHCb and MHCb B cells (7). This strongly implied that each T cell expresses a single type of TCR, whether comprised of a single receptor or dual receptors separately recognizing MHC and Ag. Additional experiments, in which MHCa T cells were negatively selected for MHCb alloreactivity by filtration through an MHCb host, exposed no or few residual Ag-specific MHCb-restricted T cells (8). Such acute negative selection contrasted with the developmental tolerance induced in parent-into-F1 radiation bone marrow chimeras (MHCb bone marrow reconstituted (MHCa x MHCb)F1 mice), which revealed that MHCb T cells developing in an MHCa-expressing environment could be restricted to recognizing

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2 Abbreviation used in this paper: HRBC, horse RBC.

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Ag on MHC\(^{a}\) APCs (9). Acute negative selection and developmental tolerance had their vocal opponents and proponents in the 1970s, but we now accept that both techniques expose underlying properties of T cell development and Ag recognition. Parent-into-F\(_{1}\) bone marrow chimeras revealed that both tolerance and MHC restriction specificity are developmentally acquired (as we know now, in the thymus through positive and negative selection). Negative selection by filtration exposed some inherent crossreactivity of MHC restriction specificity by depleting alloreactive cells.

Our knowledge of the mechanisms by which T cells recirculate has also greatly expanded since 1971. We now understand that T cells carried through the blood into the spleen can enter the white pulp and exit via the marginal sinuses. Alternatively, T cells can pull themselves from the blood into the lymph nodes by means of a carefully controlled multistep process of adherence to and extravasation through high endothelial venules, coupled with chemokine-regulated microenvironmental homing and exit through the efferent lymph (10). Recent advances in intravital microscopy have allowed visualization of trafficking T cells in live mice (11).

The discovery of biological filtration clearly provided the foundation for our understanding of lymphocyte homing and the basis for experiments detailing the MHC restriction of T cell function. It also served as the foundation for the prolific scientific career of Jon Sprent, being one of the first of more than 260 articles he has published to date. The work is typical of Sprent’s experimental approach, largely restricted to in vivo protocols, a result, Sprent claims, of his inability to use a tissue culture hood while smoking his pipe. His legacy of elegant in vivo experiments, such as those described in this “Pillars of Immunology” article, may be one of the unexpected benefits of pipe smoking.

References