be particularly useful in isolating the effects of paracrine and autocrine signals and in more precisely controlling the dosing and timing of pathogen inputs.

This work demonstrates the power of high-content single-cell techniques like RNA-seq in understanding pathogen-host interactions, but there is much left to do, both on the technical and biology sides. The researchers collected a very rich dataset on macrophage transcription and highlight the PhoPQ-IFN link, but there could be more to discover in this treasure chest of functional single-cell data.

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Helping the Help for CD8+ T Cell Responses

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Eickhoff et al. and Hor et al. use time-lapse intravital microscopy to show an unexpected choreography of CD4+ and CD8+ T cells "dancing" between different dendritic cell sub-populations during priming of cytotoxic immune responses to viruses.

The idea that clonal selection is at the basis of adaptive immune responses was first proposed in the Fifties by Nils Jerne and Sir Macfarlane Burnet. It was not, however, until the end of the Nineties that the field accepted that dendritic cells (DCs), first identified in 1973 by Ralph Steinman, are the antigen-presenting cells that support clonal selection and initiate adaptive immune responses in lymph nodes. The first visual in situ dynamic evidence of early interactions between naive T lymphocytes and DCs came in the early 2000s when two-photon intravital imaging methods were developed in immunology (reviewed in Pittet and Mempel, 2008). These early studies revealed a high degree of unexpected complexity in these interactions. First, clonal selection occurs within a complex tissue environment and within specific regions of lymphoid tissue. Second, the encounter of an antigen-presenting cell and a T cell specific for that particular antigen is a rare, non-random event. Dendritic cells accumulate in certain regions of lymph nodes and T cells migrate along preferential tracks, guided by combinations of chemokines. Third, the interactions between T cells and DCs have a specific controlled duration, which is critical for clonal expansion and T cell differentiation into effector and memory cells.

A key critical level in the initiation of immune responses, however, had not yet been addressed: DCs are a heterogeneous cell population that includes multiple cell subtypes with different functions. DCs fall into two main lineages (sometimes referred to as CD103+ and CD11b+ lineages), each including lymphoid tissue resident and migratory cells (Merad et al., 2013). One of the lineages (CD103+, CD8aa+, and XCR1+) specializes in the induction of CD8+ T cell responses and the presentation of internalized antigens on class I MHC molecules (cross-presentation) and will be referred to as XCR1+ DCs. The other subtype is more heterogeneous. CD11b+ DCs present internalized antigens on preferentially class II MHC molecules and induce CD4+ T cell and B cell responses (Merad et al., 2013). The current view of DC biology therefore underlines a repartition of antigen presentation to CD4+ and CD8+ T cells between DC subpopulations.

On the other hand, effective anti-viral cytotoxic immune responses by CD8+ T cells are strictly dependent on CD4+ T cells. In the absence of CD4+ T cells, CD8+ T cell responses are weak and lack long-lasting memory protection (Janssen et al., 2003; Shedlock and Shen, 2003). Different models have been proposed to account for these observations. The first proposed that CD4+ T cell help requires direct interactions between the three cell types (CD4+, CD8+ T cells, and DCs) (Ridge et al., 1998). The likelihood of this three-way cell interaction was questionable, and subsequent studies showed that the three cell types do not need to interact

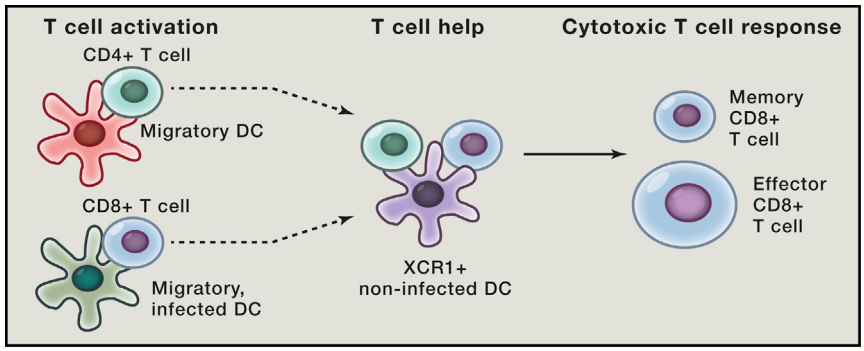

Figure 1. XCR1+ DC Platforms for T Cell Help during the Initiation of Anti-viral Immune Responses

Both CD4+ and CD8+ T cells are initially activated by distinct DCs in different anatomical sites of lymphoid organs. Activated T cells migrate to T cell zones and interact simultaneously in clusters containing the three-cell type. These three-cell interactions are indispensable for effector and memory anti-viral CD8+ T cell responses.

simultaneously. CD4+ cells deliver to DCs a "licensing" signal (through CD40-CD40L interactions) that activates DCs and permits the effective induction of CD8+ T cell responses (Schoenberger et al., 1998). This last model of T cell help does not predict simultaneous interactions between the three cell types.

Two studies now published in *Cell* (Eickhoff et al., 2015) and *Immunity* (Hor et al., 2015) investigate the nature of the cell-cell interactions that occur during the initiation of cytotoxcic CD8+ T cell responses using intravital imaging in different viral infection models. Eickhoff et al. (2015) show that, early after infection by ovalbumin or LCMV glycoprotein expressing Vaccinia virus (MVA), CD8+ T cells are found within a series of dynamic clusters around infected DCs. These clusters, as well as initial priming of antigen-specific CD8+ T cells, do not require macrophages or resident XCR1+ DCs but another population of unidentified, MVA-OVA-infected DCs. When OVA-specific CD4+ T cells are also present, they form separate clusters, which do not contain CD8+ T cells and are found in the white pulp, whereas the CD8+ clusters are present in the marginal zone. CD8+ and CD4+ T cell initial priming therefore occurs in different anatomical localizations and on different individual DCs.

The study by Hor et al. (2015) makes a similar initial observation using a model of cutaneous HSV-1 infection in which HSV-1-specific CD4+ T cells expand earlier than CD8+ T cells. They also show that early CD4+ T cell clusters in skin-draining lymph nodes are found in the medullary, the subcapsular sinus, and the B cell follicles rather than in the T cell zones. Using dynamic intravital imaging, they show that the early CD4+ cell clusters do not contain HSV-1-specific CD8+ T cells. Early CD8+ T cell clusters form around migratory skin DCs and not lymph node resident DCs. Therefore, like in the case of Vaccinia virus, after infection by HSV-1, the early priming of CD4+ and CD8+ T cells is physically segregated and seems to occur on distinct DC populations.

How then is CD4+ T cell help delivered to CD8+ T cells? The licensing model would have predicted that the DCs that had interacted with CD4+ T cells (the migratory ones in the case of HSV-1 and a CD11b+ population in the case of MVA) would then interact with CD8+ T cells to deliver the T cell help. This is not what was observed. In contrast, both studies show that, after infection (~40 hr in both the HSV-1 and the MVA models), dynamic clusters contain both CD4+ and CD8+ antigen-specific T cells. Eickhoff et al. (2015) show that these late "mix" clusters are present in the peripheral paracortex of the spleen. Hor et al. (2015) show that these clusters exclude migratory tissue-derived DCs and that the mix clusters form around XCR1+ DCs, suggesting that XCR1+ DCs present antigen on both class I and II MHC molecules. In the HSV-1 model, the same group showed previously that the activation of CD8+ T cells is not induced by infected migratory DCs, but rather by cross-presentation after antigen transfer to resident DCs (Allan et al., 2006). Eickhoff et al. (2015) go one-step further using XCR1-DTR mice, in which DTX injection results in the specific depletion of XCR1+ resident DCs. Depletion of XCR1+ DCs caused loss of the mix CD4+/CD8+ T cell clusters, but not of single CD4+ or CD8+ T cell clusters, suggesting again that initial activation of both CD4+ and CD8+ T cells occurs on XCR1-negative DCs (migratory DCs for CD4+ T cells, in the case of HSV-1, and probably other populations of resident DCs in the case of MVA). The results from both studies indicate that XCR1+ DCs represent a "platform" for dynamic interactions between CD4+ and CD8+ T cells.

The final set of experiments in Eickhoff et al. (2015) addresses the physiological relevance of XCR1+ DCs for T cell help to cytotoxic effector and memory responses. They show that depletion of XCR1+ DCs deprives CD8+ T cells from CD4+ T cell help. These "helpless" CD8+ T cells display reduced expansion and differentiation after Vacinia virus infection, and memory cytotoxic responses are compromised. Importantly, Eickhoff et al. (2015) show that selective KO of class II MHC molecules on XCR1+ DCs is sufficient to reproduce the phenotype, suggesting that antigen presentation to CD4+ T cells is required.

These studies make a critical contribution to our understanding of the initiation of cytotoxic immune responses to virus. They show that the initial priming of CD8+ T and CD4+ T cells occurs on different DC populations and that a secondary three-cell relationship is orchestrated by XCR1+ DCs (Figure 1). These XCR1+ DCs are not directly infected and must present viral antigens on both class I and II MHC molecules. They are absolutely required for effective cytotoxic primary and memory T cell responses. Previous studies showed that XCR1+ DCs are the most efficient antigen cross-presenting DCs (Merad et al., 2013). Their role as "platforms" for delivering CD4+ T cell help suggests that cross-presentation is not their only critical contribution to the initiation of cytotoxic immune responses. The production

of IL12, which is far higher in XCR1+ DCs than in other DC types, may also be important. It is also likely that XCR1+ DCs express a unique set of chemokines and chemokine receptors, maybe including XCR1 itself, that determines their function. It will be important to investigate how this new model for T cell help applies to other CD8+ T cell responses, including anti-tumor responses.

A key step for future research will certainly be to unravel the role of DC subpopulations during the initiation of immune responses in humans. Both T cell and DC lineage organizations are conserved between human and mice. However, in both cases, the functions of the subsets seem to have evolved differently. CD4+ human T cells are often cytotoxic, and it is still unclear if the human lineage homologs of XCR1+ DCs, which express BDCA3+ and CD141+, cross-present antigens more efficiently than other DC subsets. In addition, the production of IL-12 is clearly not restricted to the human CD141+ DCs. Determining which human DC, if any, functions as a "T cell help platform" will certainly be a major challenge in the next years and an essential step toward designing effective CD8+ T cell vaccines.

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Melatonin Lulling Th17 Cells to Sleep

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In this issue, Farez et al. report that the circadian hormone melatonin, whose levels vary with seasonal changes in night length, shifts the immune response toward an anti-inflammatory state that may explain the seasonal variability of multiple sclerosis disease activity.

An imbalance between the inflammatory and regulatory responses of the immune system can lead to chronic immune cell activation and autoimmunity. Mounting evidence implicates the Th17 subset of T helper cells, characterized by the production of the proinflammatory cytokine interleukin-17 (IL-17), with playing a central role in autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (IBD) (Gaffen et al., 2014). Predominantly located at barrier tissues that interface with the external world, Th17 cells maintain a high degree of flexibility to respond rapidly to constant fluctuations in environmental conditions and stimuli. The mechanisms that allow the adaptation of Th17 cells to the ever-changing environment include the ability to respond to changing nutrient status through the aryl hydrocarbon receptor (AHR) (Quintana et al., 2008), to oxygen sensing pathways including HIF1α (Dang et al., 2011), and to changes in osmotic pressure through serum/glucocorticoid regulated kinase 1 (SGK1) (Wu et al., 2013). In this issue of *Cell*, Farez et al. (2015) uncover another environmental cue—seasonal changes in daylight—that modulates the development of pathogenic Th17 cells. The daylight effect is mediated by the hormone melatonin, produced by the pineal gland and involved in the regulation of the circadian rhythm (Brzezinski, 1997). Melatonin inhibits the development of proinflammatory Th17 cells and shifts the balance of the immune response toward immunosuppression.

It has been known for some time that the latitudinal gradient—the greater the distance from the equator— correlates with increasing occurrence of multiple sclerosis (Alonso and Hernán, 2008). One of the phenomena linked to latitude is seasonal variation in exposure to UV radiation. There are convincing epidemiological data supporting the role of UV radiation-dependent vitamin D in reducing the disease course of MS (Munger et al., 2004). Yet, this correlation does not explain the increase in MS