

The Effector T Cell Response to Influenza Infection

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Abstract Influenza virus infection induces a potent initial innate immune response, which serves to limit the extent of viral replication and virus spread. However, efficient (and eventual) viral clearance within the respiratory tract requires the subsequent activation, rapid proliferation, recruitment, and expression of effector activities by the adaptive immune system, consisting of antibody producing B cells and influenza-specific T lymphocytes with diverse functions. The ensuing effector activities of these T lymphocytes ultimately determine (along with antibodies) the capacity of the host to eliminate the viruses and the extent of tissue damage. In this review, we describe this effector T cell response to influenza virus infection. Based on information largely obtained in experimental settings (i.e., murine models), we will illustrate the factors regulating the induction of adaptive immune T cell responses to influenza, the effector activities displayed by these activated T cells, the mechanisms underlying the expression of these effector mechanisms, and the control of the activation/differentiation of these T cells, in situ, in the infected lungs.

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Abbreviations

APC	Antigen presenting cell
Bid	BH3 interacting-domain death agonist
Blimp-1	B lymphocyte-induced maturation protein-1
cRDC	Conventional RDC
CTL	Cytotoxic T lymphocyte
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DLN	Draining lymph node
FADD	Fas-associated death domain (FADD)
FasL	Fas ligand
IAV	Influenza A virus
IFN	Interferon
IL	Interleukin
IRF	IFN Regulatory factor
MIP-1 α	Macrophage inflammatory protein-1 α
MARCO	Macrophage receptor with collagenous structure
MAVS	Mitochondrial antiviral signaling
MHC	Major histocompatibility complex
Mo-RDC	Monocyte-like RDC
NLR	NOD-like receptor
PAMP	Pathogen-associated molecular pattern
pDC	Plasmacytoid DC
PRR	Pattern recognition receptor
RDC	Respiratory DC
REC	Respiratory epithelial cell
RIG-I	Retnoic acid-inducible gene 1
RLR	Rig-I-like receptor
TCR	T cell receptor
T _H	T helper
TipDC	TNF ⁺ iNOS ⁺ DC
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
TRAIL-DR	TRAIL-death receptor

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1 Introduction

In this section of the volume of Current Topics in Microbiology and Immunology on Influenza Pathogenesis and Control, we focus on the contribution of a specific subset of adaptive immune cells, that is activated T effector cells, to the control of viral replication in the host response to influenza A virus (IAV) infection. These activated T effector cells are classically categorized as CD8⁺ cytotoxic T lymphocytes (CTLs) and CD4⁺ T helper (T_H) cells. However, there is evidence for considerable heterogeneity of function among these T lymphocytes subsets, most notably among the T_H cells.

Both T cell subsets have been reported to have regulatory or suppressive activity against other adaptive or innate immune cell types. The most prominent cell type identified with such regulatory activity is the CD4⁺ T regulatory cell subset which can be directed to either self-constituents and/or foreign molecules such as the IAV gene products. Another important but only more recently appreciated distinct subset of CD4⁺ T cells is the subset of T cells which regulate B cell activation and germinal center formation in response to infection, the so-called T follicular helper T cell subset.

In this review, we will exclude the CD4⁺ (and CD8⁺) T regulatory cells as well as the T follicular helper T cell subset and restrict our focus to “conventional” CTLs and T_H cells which exhibit the capacity to migrate from draining lymph nodes (DLNs) to the site of IAV infection in the lungs. We will systematically review the factors regulating the induction of the effector cells from naïve precursors (and the role of respiratory dendritic cells in this process), expression of effector activities by these activated T cells, and the regulation of the activation and differentiation state of these T effector cells in the IAV-infected lungs.

2 Initiation of Adaptive Immunity

2.1 Dendritic Cell Networking in the Steady-State and Inflamed Lung

Because of its continuous encounter with the environment as it carries out its essential role in gas exchange, the respiratory tract is exposed to airborne foreign particles, such as pollutants, allergens, dusts, and microorganisms. The lungs have therefore evolved a variety of strategies to sense, respond to, and cope with these potential ‘dangers,’ including the establishment of a well-developed network of dendritic cells (DCs). DCs serve as the sentinels of the immune system at body surfaces (e.g., the lungs, skin, and gut), linking the response of innate immune cells and molecular sensors to the induction of adaptive immunity (Banchereau and Steinman 1998). DCs were once thought to be a homogenous population that was difficult to distinguish phenotypically from lung-resident alveolar macrophages. However, recent advances in the development of genetic tools to provide definitive information on DC biology now make it clear that DCs are a heterogenous cell population consisting of distinct DC subsets with discrete functions and with developmental pathways separate from the macrophage lineages (Helft et al. 2010). In the lung, DCs perform a range of tasks including recognition and acquisition of antigens derived from pathogens and allergens, antigen transportation to the regional lymph nodes, and perhaps most importantly, induction of CD4⁺ or CD8⁺ T cell immunity (Braciale et al. 2012; Lambrecht and Hammad 2012).

In the unperturbed lung, the DC network is composed of several distinct respiratory DC (RDC) subsets that differ in phenotype, anatomic localization, and function (Table 1). Of these, CD103⁺ and CD11b^{hi} RDC subsets exhibit several features characteristics of DC found in extralymphoid mucosal sites and are distributed at distinct anatomical sites: primarily intraepithelial localization for CD103⁺ RDC and submucosal/interstitial distribution for CD11b^{hi} RDC (Sung et al. 2006; del Rio et al. 2007; Edelson et al. 2010). In addition to these major populations, monocyte-like RDC (Mo-RDC) are also readily detectable in the uninfamed lung (Hao et al. 2008; Kim and Braciale 2009). In certain microenvironments within the lung parenchyma (i.e., alveolar septa), so-called conventional RDC (cRDC) (e.g., CD103⁺ and CD11b^{hi} RDC) and plasmacytoid DC (pDC) are both detectable. The human counterparts of murine CD103⁺, CD11b^{hi} RDC and pDC have recently been identified in the human lung (Table 1) (Villadangos and Shortman 2010; Neyt and Lambrecht 2013). Thus, several distinct DC subsets strategically positioned at the interface between the lung and the surroundings sense and sample the respiratory tract.

The aforementioned DC subsets are largely identifiable in inflammatory conditions such as viral infections; however, inflammatory mononuclear cells recruited to and infiltrating the inflamed tissues in response to infection (e.g., monocytes) add to the complexity of DC network in the infected lungs since many of them express/upregulate prototypical DC markers such as CD11c, major

Table 1 Major lung DC subsets in mice

DC subset	Mouse surface markers	Transcription factors	Human equivalent surface markers	Labor division	Anatomical distribution
CD103 ⁺ RDC	CD11c ⁺ CD103 ⁺	IRF8 Batf3	CD11c ⁺ BCDA3 ⁺ (CD141)	Surveillance of airway luminal surface Antigen uptake	Associated with epithelium Above basement membrane Alveolar septa
-	CD11b ^{+/-} Langerin ⁺ XCR1 ⁺ MHC II ⁺⁺	- - - -	XCR1 ⁺ MHC II ⁺⁺ - -	Antigen transport to the lymph node Activation of CD8 ⁺ T cells Cross-presentation -	- - -
CD11bhi RDC	CD11c ⁺ CD103 ⁻	IRF4 -	CD11c ⁺ BCDA1 ⁺ (CD1c)	Surveillance of parenchymal tissue Antigen uptake	Submucosa airways Beneath basement membrane Parenchyma
-	CD11b ⁺⁺ MHC II ⁺⁺ SIRP1 α ⁺	- - -	MHC II ⁺⁺ - -	Antigen transport to the lymph node Activation of CD4 ⁺ T cells Chemokine/cytokine production	Alveolar septa -
pDCs	CD11c ⁺ Siglec H ⁺ Ly6C ⁺ B220 ⁺ PDCA-1 ⁺	E2-2 -	CD123 ⁺ CD1c ⁻ BCDA2 ⁺ MHC II ⁺⁺ BCDA4 ⁺	Type I IFN production Regulating effectors in the lung -	Parenchyma Alveolar septa -
Monozytic RDC (Mo-RDC)	CD11c ⁺ CD103 ⁻ CD11b ⁺⁺ MHC II ^{+/-} Ly6C ^{+/-}	PU.1 - - - -	- - - -	Chemokine/cytokine production TH1 cell priming -	- Parenchyma 'Marginated' vasculature -

histocompatibility complex (MHC) II and costimulatory markers at various levels (Lin et al. 2008). The potential precursor-product relationship between CD11b^{hi} DCs and Mo-RDC in steady-state as well as inflamed lung remains at present speculative. A DC subset prominent in IAV-infected lung, but minimal in the normal lung, is TNF⁺iNOS⁺ DC (TipDC) (Aldridge et al. 2009). These DC are likely derived from the circulating Ly6C⁺CCR2⁺ monocyte subset that are rapidly mobilized to the infected lung upon viral infection and contribute to both the viral control and immunopathology.

2.2 Activation of DCs and Antiviral Innate Immunity in the Lung

Prompt activation of innate antiviral immunity at the site of virus replication is a crucial step toward ultimate control of pathogen replication and a successful host defense against invading pathogens such as IAV. In particular, DC activation is pivotal to initiate adaptive immunity and to ultimately clear infectious virions from the infected lung (Braciale et al. 2012; Neyt and Lambrecht 2013). The recent discovery of several DC subsets in the lung implies that these distinct RDC subsets likely respond differently (i.e., display distinctive as well as overlapping functions) to a given virus; however, most studies investigating respiratory virus infection have not yet fully assimilated the impact of this DC heterogeneity into the cellular/molecular processes underpinning the induction and expression of the host response: an area meriting further detailed investigation.

Resting DCs in the lung can be stimulated by several mechanisms upon IAV infection. Foremost, DCs are equipped with a various innate immune recognition receptors that recognize conserved pathogen-associated molecular patterns (PAMPs). Specific pattern recognition receptors (PRRs) for these PAMPs include Toll-like receptors (TLRs), retinoic acid-inducible gene-1 (RIG-1)-like receptors (RLRs), and NOD-like receptors (NLRs). Several forms of IAV nucleic acids can be recognized by TLR3 (dsRNA) and TLR7/8 (ssRNA) (Yoo et al. 2013). Within the DC compartments, gene array data compiled in the Immunological Genome Project reveal that CD103⁺ RDCs predominately express TLR3, whereas CD11b^{hi} RDCs express TLR2/7. Although the contribution of PRR recognition of viral PAMPs has been well-established *in vitro*, the relevance of PAMP/PRR interaction to the development of innate and adaptive immunity *in vivo* after IAV infection is less clear. Neither the absence of TLR3 (Le Goffic et al. 2006) or the RIG-I signaling adaptor mitochondrial antiviral signaling (MAVS) (Koyama et al. 2007) diminishes virus clearance and the adaptive immunity to IAV infection. Furthermore, *Tlr7*^{-/-} or *Tlr7*^{-/-}*Mavs*^{-/-} mice are able to mount an effective CTL response and efficiently clear IAV (Heer et al. 2007; Pang et al. 2013a). Paradoxically, TLR7 and RIG-1 signals are required for efficient IAV replication *in vivo* (Pang et al. 2013b). These studies suggest a considerable complexity among different

PAMPs/PRRs (and the cell types in the lung expressing these PRRs) in their ability to support the induction of antiviral immunity.

Although direct recognition of viral nucleic acids and proteins by PRRs is the primary stimulatory pathway, DC activation through the recognition of nonmicrobial danger signals associated with cellular stress or damage as a result of infection has also been increasingly recognized. Damage-associated molecular patterns (DAMPs) are the host cell constituents released from damaged/dying cells as well as intact cells located within sites of viral replication. Notable DAMPs include nucleotides, heat shock proteins, nuclear proteins (e.g., HMGB1), mitochondrial DNA, cytokines, and reactive oxygen species (Said-Sadier and Ojcius 2012). Virus propagation within the respiratory tract results in the leak of or stimulates the release of DAMPs from intracellular compartments as well as extracellular sources (e.g., extracellular ATP, extracellular matrix components, and uric acids) (Pang and Iwasaki 2011). These DAMPs (also known as ‘alarmins’ or ‘danger signals’) serve to alert the host immune system and along with viral PAMPs, are believed to play a crucial role in activating innate immune sentinels such as DC in the airway mucosa in part via an inflammasome-dependent mechanism.

Inflammasomes are large intracellular multiprotein complexes consisting of NLR family members, such as NLRP3 and IPAF. The DAMP-activated NLR protein complex recruits the inflammasome-adaptor protein ASC, which in turn engages caspase-1 leading to caspase-1 activation. Once activated, caspase-1 promotes the maturation of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-18 (Said-Sadier and Ojcius 2012). By virtue of their ability to detect and respond to a large range of PAMPs and DAMPs, inflammasomes are an integral part of the host defense to pathogens like IAV. Indeed, IAV-derived ssRNA can, in an NLRP3-dependent manner, activate the inflammasome complex in a variety of cell types including bone marrow-derived DCs or macrophages (Kanneganti et al. 2006). In addition, the IAV-M2 viral protein, a proton-selective ion channel, can serve as an ionophore to promote nucleotide transport into the cell cytosol and directly trigger caspase-1 activation and secretion of IL-1 β (Ichinohe et al. 2009). The importance of inflammasome activation to anti-IAV immunity is evident in mice deficient in inflammasome complex-associated molecules, such as NLRP3, IL-1R, caspase-1, or ASC. These mice exhibit sustained, elevated lung viral titers, reduced infiltration of neutrophils and monocytes into the infected lung, an impaired adaptive immune response, and reduced cytokine/chemokine levels compared to infected inflammasome-sufficient mice (Ichinohe et al. 2009; Thomas et al. 2009; Allen et al. 2011). Although reports differ in the extent to which inflammasome impairment affects the host response to respiratory virus infection, the accumulating data overall strongly suggest that activation of the NLRP3-dependent inflammasome and the ensuing activation of the IL-1/IL-1R-mediated signaling defense are critical for the establishment of antiviral innate and adaptive immunity (Pang et al. 2013a). It is likely that additional DAMPs will be identified which are involved in modulating lung DC activation and antiviral immunity during IAV infection.

2.3 Antigen Acquisition and Migration of Lung DCs

After activation in the lung, RDCs carry out two distinct roles in the host response to IAV infection. On one hand, perhaps the most important and unique function of RDC in the infected lung is the capture of antigen derived from IAV for delivery to the DLN. Upon arrival here, the migrant RDCs present the processed viral peptides to IAV-specific T cells to initiate adaptive immunity. On the other hand, the activated DCs release various pro-inflammatory mediators in the infected lung which serve to limit viral replication and spread but when released in excess, enhance disease severity.

The acquisition of IAV antigen in the lung by cRDC prior to their egress from the inflamed lung is most likely achieved by direct infection of RDC. Different DC subsets in both mice and humans differ in susceptibility to IAV infection. In case of IAV H1N1 PR/8 infection in mice, CD103⁺ RDCs, unlike CD11b^{hi} RDCs, are highly susceptible to infection with this strain *in vivo* (Hao et al. 2008; Manicassamy et al. 2010; Hargadon et al. 2011). This differential susceptibility among the RDC subsets to IAV infection is in part dependent on DC-intrinsic sensitivity to type 1 interferons (IFN) (Molledo et al. 2011). DC infection by human IAV fails to release infectious virions likely due to lack of hemagglutinin cleavage by furan enzymes. Furthermore, phagocytic engulfment of cell-free virions or dying/dead infected cells harboring viral antigen (e.g., respiratory epithelial cells and neutrophils) is another feasible mechanism. In addition, antigen acquisition by a membrane nibbling process, known as 'trocytosis' (Desch et al. 2011), can be employed by activated DCs, although this latter remains to be formally demonstrated during IAV infection. Viral antigen transfer from migrant antigen-bearing RDC to LN-resident DC (i.e., CD8 α ⁺ DC) for presentation to naïve T cells (i.e., cross-presentation) has also been reported (Belz et al. 2004). The relative contribution of these various mechanisms of antigen uptake and their impact on antigen presentation efficiency and the subsequent antiviral immune response is currently ill-defined.

Following an inflammatory stimulus like virus infection (see below), the viral antigen-bearing activated RDC migrate to the DLN along a chemokine gradient dependent on expression of the chemokine receptor CCR7 (Kim and Braciale 2009). Although the detailed molecular mechanisms underlying CCR7 upregulation by RDC after IAV infection remains elusive, stimulation through receptors for PAMPs and DAMPs likely play an integral role in promoting DC migration. Stimulation by DAMPs and inflammasome activation can recruit immature DCs and induce their functional maturation, leading them to take up antigens and home to secondary lymphoid organs. After IAV infection of mice deficient in IL-1R signaling, the migration of CD103⁺ RDC to the DLN is severely impaired, along with a reduction in the total accumulation of inflammatory/immune cells into the DLN and the subsequent impairment of the antiviral CTL and T_H cell responses in the infected lungs (see below). On the other hand, it is noteworthy that persistent or unchecked release of DAMPs (i.e., S100A9) into the local milieu can lead to

excessive stimulation of innate immune cells including DCs (Tsai et al. 2014), which can trigger aberrant uncontrolled T cell proliferation (perhaps even self-reactive T cells). Thus, net effects of DAMPs to host immunity are determined by the microenvironment and DAMP concentration.

The complement system is an essential component of innate immunity and bridges between the innate and adaptive immune systems. Similar to viral PAMPs and cellular DAMPs, components of the complement pathway in the lung provide additional immunological cues to DC activation and antiviral immunity to IAV infection. Complements, however, affect DC migration rather than the DC's T cell stimulatory activity (Kandasamy et al. 2013). Mice deficient in complement following IAV infection resulted in diminished lung DC migration (notable in CD103⁺ RDC compartment) and accumulation into the DLN, contributing to the subsequent impaired anti-IAV T cell response in the infected lungs. Of interest, CD103⁺ RDC possesses a unique capacity to both sense and produce complement, thereby controlling both its own migration and the migration of CD11b⁺ RDCs from the infected lung into the MLN.

2.4 Antigen Presentation and T Cell Activation in the DLNs

Efficient viral clearance from the site of infection and establishment of lifelong protective immunity against intracellular pathogens such as viruses requires the activation of CTLs and T_H cells. Naïve T cells undergo several distinct phases of immune responses including clonal expansion, acquisition of effector function, migration to the site of infection, and self-renewal (Williams and Bevan 2007). Initial programming of T lymphocyte responses occurs sequentially in lymphoid tissues draining sites of infection where IAV-specific naïve T cells are selected by professional antigen presenting cells (APCs) to proliferate, yielding functionally distinct activated T cell subsets with different functions and fates (Lawrence and Braciale 2004; Yoon et al. 2010).

Among the DC populations present in the DLN of IAV infected mice (i.e., migrant CD103⁺, CD11b^{hi} and Mo-DC, pDC, CD8 α ^{+/-} LNDC), the lung-derived migrant CD103⁺ and CD11b^{hi} RDCs serve as the primary APCs for naïve IAV-specific T cells albeit with different capacities (Kim and Braciale 2009). CD103⁺ migrant RDCs are the most potent APC for activating CTLs following IAV infection among the DC subsets in the DLN. CD103⁺ RDCs (but not CD11b^{hi} RDCs) also have the capacity to capture noninfectious virus preparations delivered into the RT, then process and present IAV antigens CTLs (i.e., cross-presentation) (Kim and Braciale 2009; Helft et al. 2012). In keeping with its prominent role in priming antiviral CTLs, selective depletion of langerin-expressing CD103⁺ RDCs prior to IAV infection (GeurtsvanKessel et al. 2008) or mice constitutively lacking CD103⁺ RDCs (Batf3-deficient mice) (Helft et al. 2012) results in a severely diminished CTL response and impaired virus clearance from the lungs with subsequent infection. While the mechanistic basis for the potency displayed by

CD103⁺ RDC to prime CTL activation are under intense investigation, its superior cross-presenting ability (Kim and Braciale 2009; Moltedo et al. 2011; Helft et al. 2012) along with enhanced loading of processed antigen onto MHC I molecules (Ho et al. 2011) have been described. In support of this view, CD103⁺ DCs (and CD8 α ⁺ DC) preferentially express the DC receptor DNGR-1 (CLEC9A), which is implicated in cross-priming (Schreibelt et al. 2012; Zelenay et al. 2012).

Consistent with its dominant role in CTL priming, CD103⁺ RDC migration and accumulation in the DLN is rigorous at an earlier phase (i.e., up to day 4 post-infection in mice) (Kim and Braciale 2009). Following this initial wave of migration and antigen presentation to CTLs by CD103⁺ RDC, IAV antigen is continuously replenished and presented to T cells in the DLN by migrant CD11b^{hi} RDCs. This temporal transition of APC to CD11b^{hi} RDC in a greater quantity at later phases could serve as an amplification loop for generating CTLs until the resolution of the acute infection (Ballesteros-Tato et al. 2012). In contrast to migrant cRDC subsets, pDC has been reported to capture viral antigen in the lung, but antigen positive pDCs in the DLN fail to induce CTL responses during sub-lethal IAV infection (GeurtsvanKessel et al. 2008; Wolf et al. 2009). However, pDCs appear to play a role in eliminating virus-specific CTLs in the infected lung via Fas/Fas ligand (FasL) interaction and enhance mortality during lethal infection (Langlois and Legge 2011). Likewise, antigen-bearing Mo-RDC are detected in the DLN; however, they exhibit a minimal, if any, activity to stimulate CTL proliferation (Kim and Braciale 2009).

In contrast to their differing ability to activate naïve IAV-specific CD8⁺ T cells, migrant CD103⁺ and CD11b^{hi} RDC subsets both robustly trigger naïve CD4⁺ T cell activation with an equal efficiency (Nakano et al. 2009). In addition, other DC subsets such as Mo-RDC can drive CD4⁺ T cell differentiation into T_H1 cells, albeit with lower efficiency (Nakano et al. 2009). Thus, lung DC subsets accumulated in the DLN display a differential hierarchy in APC function for the activation of anti-IAV CTL and T_H1 cells. The distribution of distinct activities among DC subsets in the regulation of the host immune response and IAV pathogenesis is poorly characterized and is of great interest in discovering novel therapeutics and vaccinations.

3 Lymphocyte Migration into the IAV-Infected Lung

T lymphocyte migration into the lung has relatively defined, albeit overlapping, steps: selectin interactions between lymphocytes and the vascular endothelium mediate rolling and tethering stages; firm lymphocyte adhesion onto the lung vascular network is mediated by integrin interactions; and coordinated migration into the respiratory tract is regulated by integrins and chemokine gradients.

IAV-specific lymphocyte migration into nonlymphoid tissues has both specific and nonspecific components (Masopust and Schenkel 2013). The integrin CD11a, which is only upregulated in activated T cells, is required for optimal lung vascular

adhesion and retention within the respiratory tract allowing preferential recruitment over naïve cells (Thatte et al. 2003). In addition, RDCs preferentially upregulate the chemokine receptor CCR4 on effector T cells, allowing selected recruitment to the IAV-infected lung, wherein the corresponding ligands CCL17 and CCL22 are produced (Mikhak et al. 2013). However, ICAM-1, the corresponding ligand for CD11a is constitutively expressed in secondary tissues allowing nonspecific recruitment of effector cells (Thatte et al. 2003), and during influenza infection, a large number of IAV-specific CTLs are detected in noninflamed tissues (Lawrence and Braciale 2004). This dichotomy of nonspecific and specific recruitment of activated lymphocytes may allow heightened systemic immunosurveillance on one hand while allowing preferentially, but not stringent, enrichment at the site of infection on the other.

T cell migration into the airways, the principal site of IAV replication, may occur either through T cells transversing the respiratory epithelium from the lung interstitium or direct entry from the circulation. The ability of T cells to migrate into the airways is selective, with CTL recruitment favored over T_H cells due to an unknown mechanism. CTL entry into the airways from the circulation is dependent on IL-15 (Verbist et al. 2011), which is regulated by type I IFN (Mattei et al. 2001). IL-15 can increase CD11a expression (Allavena et al. 1997), and IL-15 itself is chemotactic (Verbist et al. 2011). It is currently unclear which signals are required to promote migration from the lung interstitium into the airways or whether this is a major avenue of migration into the airspace environment.

It is noteworthy that a significant fraction of T lymphocytes (as well as innate immune cells (e.g., neutrophils, monocytes)) localized to the normal/unperturbed lungs are not located within the pulmonary interstitium but rather remain “marginated” within the circulation of the pulmonary vasculature even following lung profusion (Anderson et al. 2012). Therefore following infection, the frequency of innate immune and antigen-specific adaptive immune cells within the “lungs” will need to take into account cell numbers within the airspaces, interstitium, and pulmonary vasculature.

4 Adaptive Immune-Mediated Antiviral Activity in the Lung

The arrival of IAV-specific T_H cells (more precisely CD4⁺ effector T cells) and CD8⁺ CTLs into the respiratory tract coincides with a significant impact on overall virus titer. Adaptive immune cells and their products (e.g., immune-modulating cytokines and chemokines, neutralizing antibodies, etc.) act in concert, and in a partly redundant fashion, in ultimately eliminating infectious virus. In the murine model of IAV infection, efficient viral clearance is not solely dependent on T_H cells or CTLs (Eichelberger et al. 1991; Topham et al. 1996a; Topham and Doherty 1998); however, the removal of both cell types (i.e., via acute depletion or

genetic deficiency) renders mice incapable of clearing IAV from the respiratory tract (Wells et al. 1981; Topham et al. 1997). Thus, the adaptive immune response to primary IAV infections implements a multifaceted approach to virus elimination not dependent on any one cell type.

The relative contribution of CTLs to optimal IAV clearance has been highlighted with the use of β_2 -M deficient mice (i.e., defective in MHC I display and presentation), which have a severe reduction in the number of CTLs. These mice are able to clear IAV in the absence of CTLs albeit with a delay in viral clearance depending on viral strain (Eichelberger et al. 1991; Bender et al. 1992). More so, infection of β_2 -M deficient mice with a highly virulent strain of influenza results in reduced survival (Bender et al. 1992). These data indicate IAV-specific CTL effector activity has a significant impact on viral clearance, with the requirement for this effector cell type in experimental models, dependent on the virulence of the influenza stain. While CTLs produce robust levels of cytokines and chemokines upon antigen encounter, the primary contribution of CTLs to IAV clearance is through the cytotoxic elimination of IAV-infected respiratory epithelial cells (RECs) (Lukacher et al. 1984; Hou and Doherty 1995; Topham et al. 1997; Hufford et al. 2011). Due to unhindered access to the respiratory epithelium and their concentration in the airways, CTLs localized to the airspace are likely the primary mediators of eliminating infected RECs; however, there is evidence MHC I is localized basolateral on the respiratory epithelium (Walters et al. 1999), and it may be that CTLs eliminate IAV-infected RECs as they transverse the basement membrane into the airways.

IAV-specific T_H cells are the dominant adaptive immune cell type localized in the lung during the time of IAV clearance. T_H cells express a wide array of soluble mediators (e.g., $IFN\gamma$, IL-2, IL-10) (Sun et al. 2009, 2011) and can be cytotoxic (Graham et al. 1994; Brown et al. 2006; Hua et al. 2013). Although T_H cytotoxic mechanisms may contribute to the control of virus replication and elimination (Brown et al. 2006; Hua et al. 2013), T_H cell anti-IAV roles may be primarily indirect through cytokine production and B cell help (Topham et al. 1996a, b; Topham and Doherty 1998).

4.1 T Lymphocyte Cytotoxic Mechanisms

T lymphocytes employ cytotoxic mechanisms to eliminate cells infected with intracellular pathogens such as IAV. The exquisite specificity of T cell-mediated killing is dependent on the interaction between the T cell receptor (TCR) and requisite antigen complexed with MHC on the infected cell. Unlike cytokine production, TCR signaling strength required to elicit cytolysis is minimal (Valitutti et al. 1996; Faroudi et al. 2003; Purbhoo et al. 2004), and killing is unilateral allowing the T effector cell to survive following target cell engagement (Kupfer et al. 1986). This allows a given T lymphocyte to serially eliminate a number of virally infected cells in a short manner of time. Despite this rapid killing, T cell-mediated

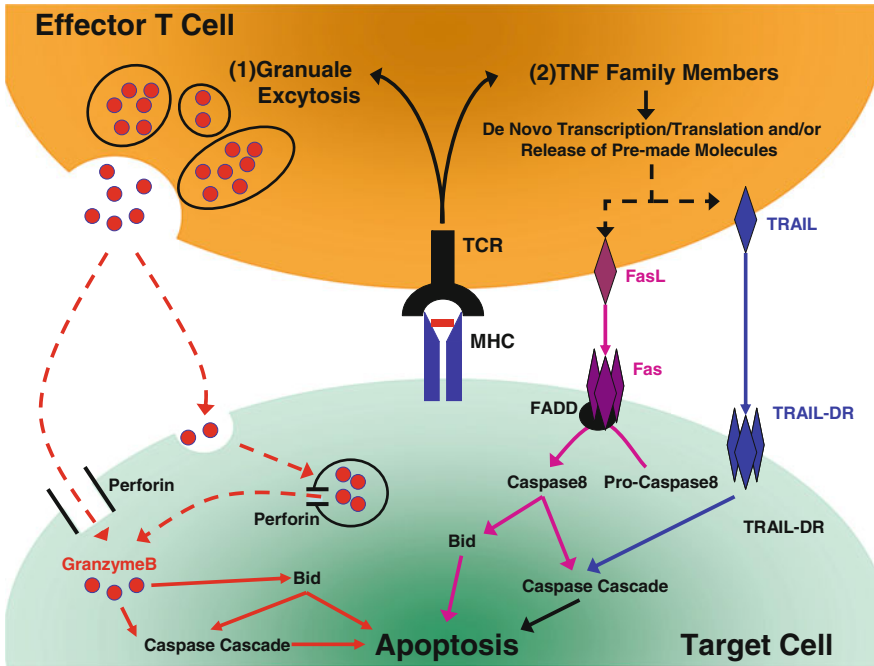


Fig. 1 Effector T cell-mediated cytotoxic pathways during influenza infection: (1) Granule exocytosis is initiated following TCR stimulus resulting in the release of premade cytotoxic granules onto target cells. The subsequent release of the pore-forming protein perforin and serine proteases (granzymes) activate caspase cascades resulting in target cell apoptosis. (2) TNF-related proteins are upregulated upon TCR stimulus. Binding to their respective ligands on target cells recruits ligand associated death domains which ultimately activate caspase cascades resulting in target cell apoptosis

cytotoxicity exhibits little nonspecific killing. The requirement of specific antigen to elicit cytotoxicity and the polarized release of cytolytic material towards the target cell with the formation of a tight ring of adhesion molecules around the point of release are all thought to limit potential damage in uninfected neighboring cells (Lukacher et al. 1984; Dustin and Long 2010).

T lymphocyte-mediated cytotoxicity is mediated by two distinct mechanisms: (1) granule exocytosis which utilizes the pore-forming protein, perforin, to facilitate the entry of serine proteases and (2) the engagement of tumor necrosis factor (TNF) family members with their respective ligands (Fig. 1). Both pathways initiate apoptotic cascades within the target cell culminating in target cell death (Russell and Ley 2002). In several murine viral infection models, granule exocytosis alone is the principal means of immune-mediated cytotoxic clearance (Kagi et al. 1994; Walsh et al. 1994; Mullbacher et al. 1999); however, perforin-deficient mice have no defect in clearing IAV infection (Topham et al. 1997). Only when RECs lacked the expression of Fas, a TNF family member involved in cell

death, and T cells were deficient in granule exocytosis (perforin deficiency) were mice unable to efficiently clear IAV infection (Topham et al. 1997; Hufford et al. 2011). Therefore, T cells employ both granule exocytosis and TNF family member pathways to eliminate IAV-infected cells. Importantly, these studies also highlight that the elimination of virally infected RECs, the cell type propagating infectious IAV, is a critical step in T cell-mediated influenza virus clearance.

4.1.1 Granule Exocytosis

Granule exocytosis involves the coordinate release of secretory lysosomes upon TCR engagement with cognate antigen/MHC complexes on a target cell. These cytotoxic granules contain a variety of serine proteases termed granzymes and the pore-forming protein perforin. Upon granule tethering and fusion with the plasma membrane, perforin complexes form on the target cell allowing the transfer of pro-apoptotic granzymes into the target cell, which rapidly undergoes programmed cell death. Despite the presence of perforin pores on the target cell, very little cell lysis (and subsequent release of inflammatory DAMPs) has been observed (Lopez et al. 2012), potentially limiting the inflammation resulting from granule exocytosis.

A variety of granzymes have been identified in both human and mouse, and their individual functions are ill-defined and controversial (Susanto et al. 2012). Granzyme B, which is well studied, initiates target cell apoptosis by two distinct mechanisms (Waterhouse et al. 2005; Kaiserman et al. 2006; Cullen et al. 2007). In mice, granzyme B preferentially cleaves pro-caspases directly into their pro-apoptotic active form. Human granzyme B mediated apoptosis is largely dependent on cleavage of the BH3 interacting domain death agonist (Bid), thereby initiating the mitochondrial apoptotic pathway and caspase activation. Other granzymes can also initiate cell death but with distinct mechanisms. For example, granzyme K-induced apoptosis is dependent on Bid cleavage but independent of downstream caspase activation (Zhao et al. 2007; Guo et al. 2010).

Granzyme activity is not limited to triggering apoptotic cell death. Granzyme K can disrupt influenza nucleocapsid protein importation into the nucleus thereby inhibiting virus replication *in vivo* (Zhong et al. 2012). In addition, granzymes have the potential to modify the extracellular matrix (Buzza et al. 2005) and trigger target cell cytokine production (e.g., TNF, IL-1 β) (Metkar et al. 2008; Joeckel et al. 2011); however, it is unclear whether these latter functions are pivotal during IAV clearance and subsequent tissue damage.

While individual granzymes have distinct functions, granzyme-mediated cell death is dependent on perforin as evidenced by the inability of perforin-deficient mice to exhibit granule exocytosis (Kagi et al. 1994). Perforin functions by binding to the target cell membrane in a receptor-independent mechanism and forming multimeric complexes (Blumenthal et al. 1984; Voskoboinik et al. 2005). The subsequent pore in the target cell membrane permits the transfer of the constituents of the cytotoxic granules. It is currently unclear whether granzymes passively diffuse through large perforin pores on the target cell membrane surface or through

a more active process within endocytic membranes (Lopez et al. 2012; Susanto et al. 2012). Thus, the precise role of perforin in this process remains to be elucidated.

During IAV infection, both T_H cells and CTLs utilize granule exocytosis. As mentioned previously, the principal target for CTL-mediated cytolysis is the IAV-infected RECs (Topham et al. 1997; Hufford et al. 2011), whose elimination is essential for successful IAV clearance. The capacity for T_H cells to engage in granule exocytosis has only been recently appreciated. Few RECs express MHC II (Gereke et al. 2009), and MHC II respiratory epithelium deficiency does not influence IAV clearance in murine models (Topham et al. 1996b). However, passive transfer of cytotoxic T_H cells into IAV-infected wild type or B cell-deficient mice can improve host morbidity (Brown et al. 2006; Hua et al. 2013). In addition, the presence of IAV-specific T_H cells correlated with improved disease protection in patients with heterologous influenza infections (Wilkinson et al. 2012), indicating T_H cell-mediated cytolysis may be of significant benefit during subsequent infections.

4.1.2 TNF Family Members

Cytotoxicity can be elicited by a variety of TNF family members, the prototypical example being FasL. Interaction of FasL with its cognate receptor, Fas, is vital in regulating T cell homeostasis, and human deficiencies in Fas signaling result in autoimmune lymphoproliferative syndrome (Roths et al. 1984; Legge and Braciale 2005; Stranges et al. 2007; Imai et al. 2012); however, these interactions can also be critical in eradicating virally infected cells, notably during IAV infections (Topham et al. 1997; Hufford et al. 2011).

TCR engagement with peptide complexed MHC upregulates FasL expression in T lymphocytes. The nature of FasL trafficking to the outer membrane is debatable with evidence indicating FasL deposition is dependent on vesicular traffic of preformed FasL molecules onto the outer membrane (Isaaz et al. 1995; Li et al. 1998; Lettau et al. 2006) or de novo transcription/translation following TCR engagement (Lowin et al. 1996). Nonetheless, FasL-mediated cytotoxicity occurs via the oligomerization of the target cell's Fas molecules, which are expressed on the cellular membranes of most cell types, by membrane-bound FasL (Rouvier et al. 1993). FasL is sensitive to metalloproteinase cleavage on the cell surface (Kayagaki et al. 1995), thereby limiting surface exposure and minimizing non-specific cell killing. This soluble form of FasL is a chemoattractant and can induce nonspecific cell death, albeit at much less efficiency as its membrane-bound counterpart (Schneider et al. 1998; Ottonello et al. 1999).

Fas signaling on the target cell's surface requires Fas trimerization, which may or may not precede binding to FasL (Siegel et al. 2000; Dockrell 2003). Upon FasL/Fas engagement, Fas-associated death domain (FADD) binds the death domain of Fas and recruits pro-caspase-8. Caspase 8 proceeds to cleave pro-apoptotic Bid, thereby initiating the mitochondrial apoptotic pathway, and in

parallel, converts pro-caspase-3 to caspase 3 leading to a subsequent apoptotic caspase cascade.

Additional TNF family molecules can initiate cytotoxicity, either as soluble factors (i.e., TNF—as discussed later) or membrane-bound ligands (i.e., TNF-related apoptosis-inducing ligand (TRAIL)). TRAIL-induced apoptosis is commonly associated with tumor clearance (Benedict and Ware 2012); however, there is evidence of its importance in viral models (Lum et al. 2001; Kotelkin et al. 2003). TRAIL and its cognate ligand, TRAIL-death receptor (TRAIL-DR), are similar to FasL/Fas in that TRAIL engagement with TRAIL-DR recruits a death complex, thereby initiating an apoptosis cascade (Benedict and Ware 2012). Like FasL, TRAIL also can be present in a soluble form. Nonspecific cell death may be limited because high TRAIL-DR expression is limited to IAV-infected cells compared to noninfected cells (Brincks et al. 2008; Herold et al. 2008), and TRAIL expression in CTLs is partially regulated by TCR stimulus (Ishikawa et al. 2005; Brincks et al. 2008). In murine models, neutralization of TRAIL or the use of TRAIL deficient mice leads to delayed IAV clearance and increased morbidity (Ishikawa et al. 2005; Brincks et al. 2008); however, the significant role for TRAIL in regulating CTL expansion and migration during influenza infection must also be taken into account (Brincks et al. 2011)

4.2 *T Cell Soluble Mediators*

CTLs and in particular, T_H cells produce a wide array of cytokines and chemokines upon their infiltration into the IAV-infected lung (Fig. 2). The TCR-mediated production of these soluble mediators is important in ultimately orchestrating the elimination of infectious IAV while also minimizing the extent of the resulting inflammation. While impossible to describe all the T cell-derived products, the following subsections highlight pivotal cytokines/chemokines shaping IAV disease.

4.2.1 $IFN\gamma$

$IFN\gamma$ is the most prominent cytokine produced by both IAV-specific T_H cells and CTLs upon entry into the infected lung. While other cell types can produce $IFN\gamma$, the peak $IFN\gamma$ production coincides with the arrival of IAV-specific T_H cells and CTLs into the respiratory tract, and acute removal of both subsets with depleting antibodies effectively eliminates detectable $IFN\gamma$ (Hufford et al. 2011).

$IFN\gamma$ can activate immune cells (e.g., macrophages), upregulate immune-modulating molecules (e.g., MHC), and modulate antibody isotype switching. Despite these processes, $IFN\gamma$ deficiency has no impact on IAV clearance (Graham et al. 1993). It is likely that $IFN\gamma$ contributes to viral clearance but is not essential due to overlapping antiviral mechanisms. This concept is illustrated in nitric oxide

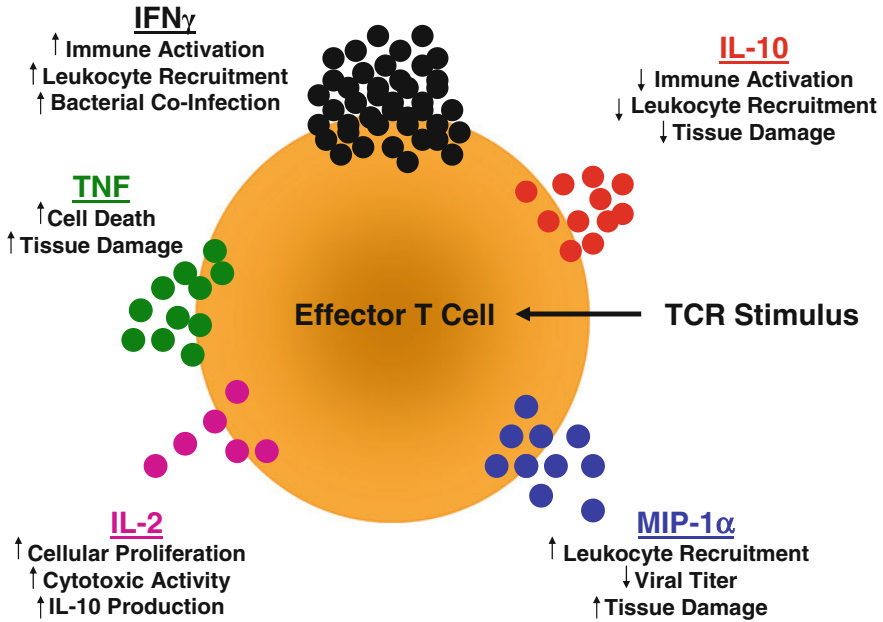


Fig. 2 The major types and functions of soluble mediators produced by effector T cells during IAV infection. Upon entry into the lung and subsequent TCR stimulation, IAV-specific T cells can produce an array of cytokines and chemokines. Major T cell derived soluble factors involved in influenza disease are depicted along with a brief summary of function

synthase knockout mice (unable to synthesize the inflammatory signaling molecule nitric oxide), which require $IFN\gamma$ for the elimination of IAV (Karupiah et al. 1998). Additionally, absence of $IFN\gamma$ during murine influenza infections has been demonstrated to affect leukocyte recruitment (Baumgarth and Kelso 1996; Turner et al. 2007), isotype switched antibody levels (Baumgarth and Kelso 1996; Bot et al. 1998), and immunopathology (Wiley et al. 2001).

There is sparse evidence to suggest that $IFN\gamma$ is a significant contributor to immune-associated tissue damage during IAV infection. Rather, its absence can be linked with reduced tissue damage, likely due to its impact in regulating leukocyte recruitment to the lung (Wiley et al. 2001). However, $IFN\gamma$ may contribute to the susceptibility to secondary bacterial infections during IAV infection. During IAV infection, alveolar macrophages exhibit decreased expression of the scavenger molecule MARCO (macrophage receptor with collagenous structure) and reduced capacity to phagocytize bacteria (Sun and Metzger 2008). Subsequently, it was demonstrated that acute $IFN\gamma$ neutralization restores MARCO expression and bacterial ingestion in alveolar macrophages. Furthermore, IAV-infected mice receiving $IFN\gamma$ neutralizing antibody demonstrated increased resistance to secondary bacterial infection (Sun and Metzger 2008).

4.2.2 TNF

TNF is produced by both T_H cells and CTLs, usually in conjunction with $IFN\gamma$ production. Unlike other T cell-derived effector molecules (e.g., $IFN\gamma$, granzymes) TNF release is nonpolarized, rather than directionally towards the stimulating target cells (Huse et al. 2006), potentially allowing potent nonspecific effects. In addition, many cell types can produce TNF upon PAMP recognition (Parameswaran and Patial 2010). It is likely that macrophages, rather than T cells, are the most significant source of TNF during influenza infection.

TNF is a pleiotropic cytokine, whose function is dependent on the manner of presentation, the target cell type engaged, and the local inflammatory environment (Parameswaran and Patial 2010). Upon stimulation, TNF can be presented in both soluble and membrane forms. In addition, there are two TNF receptors: (1) TNFR1, which is expressed in all cell types, can bind both forms of TNF and contains a death domain; and (2) TNFR2, which is predominantly expressed in immune cell types, only binds membrane-bound TNF and does not contain a death domain (Grell et al. 1995). TNF signaling can be also affected by the presence of metalloproteases, which can cleave the extracellular domains of TNFR1 and TNFR2, thereby prohibiting further TNF signaling (Van Zee et al. 1992).

Because of the overall complexity of TNF signaling, TNF may promote anti-inflammatory (e.g., diminish cytokine production, inhibit phagocytosis, trigger apoptosis) or pro-inflammatory (e.g., stimulate cytokine production, augment cellular proliferation, trigger cellular necrosis) outcomes. A similar dichotomy in function is present during IAV infection. TNF has been demonstrated to reduce CCL2, a chemokine which promotes infiltration of inflammatory monocytes/macrophages into the lung (Damjanovic et al. 2011). In contrast, TNF has been demonstrated to be a significant contributor to immune-mediated tissue damage during IAV infection without any discernable impact on viral clearance (Peper and Van Campen 1995; Hussell et al. 2001; Belisle et al. 2010). It is currently unclear, however, whether the loss of TNF signaling in murine studies, like $IFN\gamma$, is compensated by other antiviral mechanisms.

4.2.3 IL-10

Potential sources of IL-10 in the lung during IAV infection are CTLs, T_H cells, and regulatory T cells; however, IL-10 reporter mice (which effectively mark cells undergoing IL-10 gene expression) indicate lung CTLs are the predominant source (Sun et al. 2009). Despite the capacity to make IL-10, these CTLs infiltrating the respiratory tract are not a strict regulatory subset because they readily produce inflammatory cytokines (e.g., $IFN\gamma$) and are cytotoxic (Sun et al. 2009). Thus, IL-10 production is concomitant with T cell-mediated antiviral activity, likely to limit rather than effectively prohibit subsequent inflammation.

IL-10 is a potent regulatory cytokine which, upon binding to its receptor, IL-10R, on innate and adaptive immune cells, can downregulate MHC and co-stimulatory

molecule expression, modulate inflammatory cytokine expression, and inhibit cellular proliferation (Couper et al. 2008). During IAV infection, acute blockade of IL-10R signaling during T cell-mediated virus clearance results in excessive pulmonary inflammation and injury (Sun et al. 2009). In contrast, mice with IL-10 deficiency have reduced viral titers, likely due to enhanced antibody production and T_H cell responses (McKinstry et al. 2009; Sun et al. 2010). Thus, the timing and location of IL-10 production can hamper antiviral immunity (i.e., during adaptive immune activation) or prohibit excessive pulmonary inflammation (i.e., during viral clearance).

4.2.4 MIP-1 α

Macrophage inflammatory protein (MIP)-1 α (i.e., CCL3) is readily produced by CTLs and T_H cells upon activation (Obaru et al. 1986). Other potential sources include B lymphocytes, natural killer cells, and myeloid cells (e.g., macrophages, neutrophils, etc.). MIP-1 α is a chemokine which, upon binding to its receptors CCR1, CCR3, or CCR5, exerts potent chemotactic and pro-inflammatory effects (Menten et al. 2002). MIP-1 α can enhance lymphocyte cytokine production and is a potent recruiter of monocytes, lymphocytes, immature DCs, and activated neutrophils (Karpus et al. 1997; Menten et al. 2002). In IAV-infected MIP-1 α deficient mice, viral clearance was delayed, albeit eventually achieved (Cook et al. 1995). Inefficient viral clearance was accompanied with deficient leukocyte recruitment in the lung and reduced tissue damage. Thus, MIP-1 α is likely a significant pro-inflammatory soluble mediator necessary for efficient IAV clearance.

5 Regulation of Effector T cell Responses in the Lung

5.1 *Local Differentiation of Effector T Cells in the Lung*

As discussed previously, IAV-specific CTLs and T_H cells are activated in the DLN. The classical view in immunology is that T cell activation and differentiation into effector T cells are programmed and completed in the DLN; however, recent murine studies have found that upon migration to the IAV-infected lungs, both IAV-specific CTLs and T_H cells undergo extensive phenotypic changes during IAV infection, suggesting that local lung environments can further shape the differentiation of effector T cells (Fig. 3). CTLs activated in the DLN are capable of producing high levels of IFN γ upon antigenic stimulation. Interestingly, compared to CTLs residing in the DLN or spleen, CTLs in the lung downregulate their capacity to produce IFN γ following antigenic stimulation (Fulton et al. 2008), suggesting that lung environment factors restricts the extent of IFN γ production by CTLs to avoid potential collateral damage caused by exuberant IFN γ (Fulton and Varga 2010).

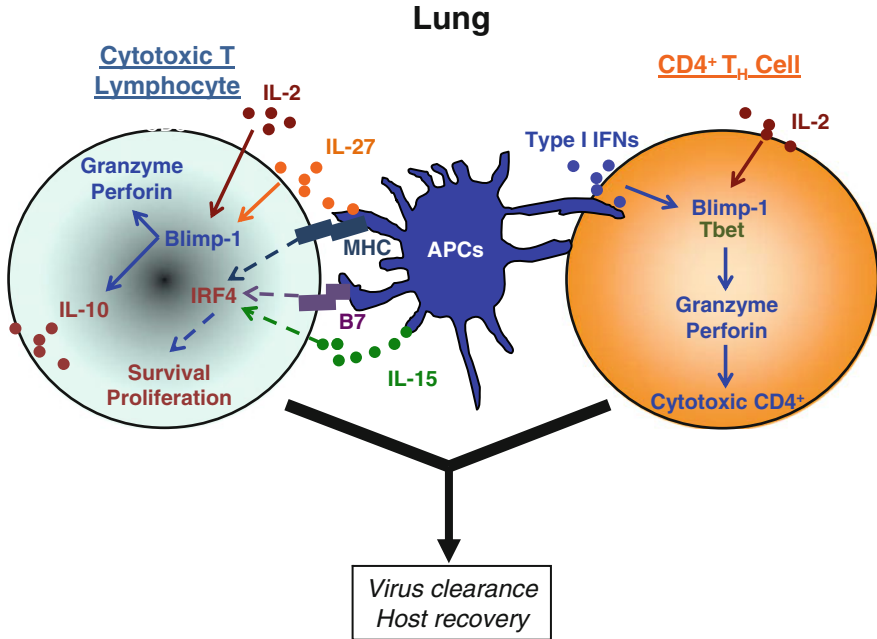


Fig. 3 Lung APCs shape local CTL and CD4⁺ T_H cell responses by providing essential differentiation, survival, and proliferation signals. Within the DLN, professional APCs and local inflammatory milieu control the early stages of antigen-specific T cell proliferation and differentiation. During influenza infection, there is significant evidence demonstrating that upon arrival into the infected lung after activation in the DLN, effector T cells undergo additional rounds of differentiation and proliferation regulated by APCs present in the respiratory tract and locally produced soluble mediators

Coinciding with their downregulation of effector cytokine IFN γ , CTLs also acquire the ability to produce IL-10 upon migrating into the lung (Sun et al. 2009, 2011). It was demonstrated that lung CTLs, but not LN effector CTLs, produce significant amount of IL-10 following antigenic-stimulation in vitro (Sun et al. 2009, 2011). As discussed previously, IL-10 functions to inhibit exuberant pulmonary inflammation during virus-induced diseases. Thus, these data suggested that CTLs acquire potent regulatory functions (i.e., capacity to produce IL-10) in the lung in response to the IAV-infected environment. The inflammatory cytokine IL-27 plays a critical role in the local instruction of IL-10 production by CTLs (Sun et al. 2011). Compared to DLN, IL-27 message is highly enriched in the infected lungs and the cellular sources of IL-27 appear mainly to be local inflammatory phagocytes including DCs, macrophages, and neutrophils, which are recruited to the lung following IAV infection (Sun et al. 2011). Therefore, lung inflammatory responses caused by virus infection are able to further instruct the continuous differentiation of CTLs for the acquisition of regulatory features.

T_H cells also upregulate IL-10 production upon migration into the lungs, suggesting the same IL-27-dependent mechanisms relates to lung T_H cells as well (Sun et al. 2009). More strikingly, T_H cells in the lungs also acquire the ability to eliminate IAV-infected MHC II⁺ cells via granule exocytosis (Brown et al. 2006, 2012; Hua et al. 2013). T_H cells in the DLN, however, express minimum granzyme B and perforin (Brown et al. 2012; Hua et al. 2013), suggesting that the acquisition of cytotoxic molecules in T_H cells requires signals from the local lung environments during IAV infection. Type I IFN (expressed at higher levels in the IAV infected lung compared to the DLN) and IL-2 are required for the expression of cytotoxic molecules in lung T_H cells (Hua et al. 2013). Thus, lung environmental cues modulate the in situ differentiation of T_H cells into cytotoxic T_H cells through type I IFNs.

5.2 Local Regulation of Proliferation and Survival of Effector T Cells

Besides shaping the phenotype of T_H cells and CTLs, local APCs and the lung environment also provide essential survival and proliferation signals for lung T_H cells and CTLs. Early studies have identified that CTLs undergo extensive proliferation in the lung following IAV infection (Lawrence and Braciale 2004; McGill and Legge 2009). Interestingly, acute depletion of mononuclear phagocytes (mostly DCs) in the lung following T cell priming abrogated CTL proliferation and impaired antiviral T cell responses (McGill et al. 2008), suggesting that local DC and T cell interaction provides signals for the continuous proliferation of effector T cells in the lung during influenza infection. Interestingly, like the proliferation of T cells during naïve T cell activation, the in situ proliferation of lung CTLs also requires both signal 1 (MHC-peptide stimulation) and signal 2 (B7-CD28 and CD70-CD27 co-stimulation) during IAV infection (Dolfi et al. 2011; van Gisbergen et al. 2011). In addition to providing signals licensing the in situ proliferation of effector T cells, RDCs also provide critical surviving signals for CTLs. Mechanistically, the trans-presentation of IL-15 by RDCs plays a critical role in sustaining the viability of lung antiviral CTLs (McGill et al. 2010). Interestingly, IL-15 trans-presentation to effector T cells is regulated by TSLP signaling in DCs (Yadava et al. 2013). Taken together, these recent data have extended our conventional lymphoid organ-centered view of T cell responses during infection and established that local environmental cues can continuously shape tissue effector T cells phenotype and constantly modulate their function.

5.3 Molecular Mechanisms Regulating Effector T Cell Responses in the Lung

5.3.1 Transcriptional Control of Effector T_H Cell Responses in the Lung

T cell activation and effector differentiation are subject to intensive transcriptional control, and various transcription factors have been demonstrated to regulate the transition from naïve T cells to effector T cells (O'Shea and Paul 2010; Zhang and Bevan 2011). Emerging evidence also suggests various transcription factors regulate the extensive phenotypic changes effector T cells exhibit upon their migration into the lung, as well.

B lymphocyte-induced maturation protein-1 (Blimp-1) is a transcription repressor that plays an important role in regulating T and B cell function (Crotty et al. 2010). The expression of Blimp-1 has previously been shown to promote effector T cell migration to the lung and modulate memory T cell generation during IAV infection (Kallies et al. 2009). Interestingly, Blimp-1 is highly expressed in lung T_H cells, compared to their counterparts in the DLN (Hua et al. 2013). Importantly, Blimp-1, whose expression can be orchestrated by IL-2 and type I IFN, is required for the expression of granzyme B and perforin in lung T_H cells as well as their ability to kill target cells (Hua et al. 2013).

Besides Blimp-1, the T-box transcription factor, T-bet, also plays an important role in the development of lung cytotoxic T_H cells during IAV infection (Hua et al. 2013). Like Blimp-1, T-bet expression is upregulated in lung T_H cells by local type I IFN signaling (Hua et al. 2013). Mechanistically, T-bet and Blimp-1 cooperate to regulate the differentiation of cytotoxic T_H cells as T-bet directly binds to cytotoxic molecules and Blimp-1 controls the accessibility of the cytotoxic molecule loci to T-bet (Hua et al. 2013). Thus, local cytokine milieu controls the development of cytotoxic T_H cell development through the upregulation of the transcription factors T-bet and Blimp-1 during IAV infection.

5.3.2 Transcriptional Control of CTL Responses in the Lung

Besides regulating local T_H cell differentiation, Blimp-1 also modulates local CTL responses in the lung during IAV infection. Similar to its function in T_H cells, Blimp-1 promotes cytotoxic molecule expression in lung CTLs (Shin et al. 2009; Sun et al. 2011). Moreover, the production of IL-10 by lung CTLs is critically dependent on Blimp-1 during IAV infection. The ablation of Blimp-1 expression in CTLs specifically abrogate IL-10 but not IFN γ production by CTLs following antigen restimulation (Sun et al. 2011), suggesting that Blimp-1 is uniquely required for the requisition of regulatory function of effector CTLs. Consistent with this result, T cell specific Blimp-1 deficiency is associated with enhanced host inflammation in the airway (Sun et al. 2011). Notably, IL-27, which is highly

produced in the lung compared to the DLN, cooperates with IL-2 to induce Blimp-1 expression in CTLs (Sun et al. 2011). Thus, Blimp-1 appears to be a transcription factor that translates local environmental cues into the phenotypic and functional traits of lung effector T_H cell and CTLs during IAV infection. Recently, interferon-regulatory factor (IRF) 4 was shown to play an essential role in regulating both the quality and quantity of CTL responses during IAV infection (Man et al. 2013; Yao et al. 2013). IRF4 acts as a transcriptional activator to promote effector differentiation and as a transcriptional repressor to regulate cell cycle progression and survival, thereby sustaining both the expansion and effector differentiation of CTLs during IAV infection (Yao et al. 2013). Interestingly, the conditional deletion of IRF4 expression in CD8⁺ T cells specifically impairs the proliferation and survival of lung CTLs (Yao et al. 2013), suggesting that IRF4 is a critical regulator of CTL responses in the lung in vivo. As discussed previously, RDCs can sustain CTL proliferation and survival through the provision of antigen, co-stimulatory molecules, and cytokines. Thus, it is tempting to speculate that local DCs exert their function by promoting IRF4 expression in lung CTLs.

5.4 In Situ Control of Effector T Cell Activities by Different Types of APCs

Effector T cells express high levels of mRNAs encoding various effector cytokines and cytotoxic molecules and thus are able to promptly produce these cytokines and cytotoxic molecules following antigenic or mitogenic stimulation. However, it is important to note that effector T cells do not spontaneously secrete cytokines or release cytotoxic molecules in vivo without further stimulation due to additional layers of translational control of cytokine expression, possibly to avoid potential self-destruction. Classically, the activities of effector T cells (i.e., production of cytokines and release of cytotoxic molecules) were often monitored through in vitro cytokine staining and degranulation assays following maximally antigenic or mitogenic stimulation. Such methods are efficient to measure the potential of effector T cells to produce cytokines and release cytotoxic molecules but fail to faithfully report the in vivo activities of effector T cells. In an in vivo setting, effector T cells likely interact with multiple target cell types and the strength of antigenic stimulation they receive from these cells could vary drastically. In particular, as noted above, early in vitro analysis demonstrated an expressional hierarchy in the regulation of T cell effector activities in which high-strength antigenic signaling induces the production of cytokine and release of cytotoxic molecules, while weak antigenic stimulation only induces the release of cytotoxic molecules but not the production of cytokines (Valitutti et al. 1996).

During IAV infection, the infected lungs contain many cell types that potentially bear IAV antigen (Kohlmeier and Woodland 2009; Braciale et al. 2012). These cells include both infiltrating lung CD45⁺ APCs as well as CD45⁻ RECs.

Interestingly, it was recently demonstrated that the interaction of CTLs with RECs and APCs resulted in differential effector activities by CTLs (Hufford et al. 2011). Using techniques that measure the *in vivo* release of cytokines through the injection of a reagent inhibiting T cell secretion, Hufford et al found that effector T cells releasing IFN γ primarily reside in the lung interstitium rather than the airways (Hufford et al. 2011). Since the lung interstitium is enriched with APCs, the authors further demonstrated that the interaction of the APCs with effector CTLs triggers the release of both cytokines and cytotoxic molecules, while the interaction of RECs with CTLs primarily induce the release of cytotoxic molecules but not effector cytokines by CTLs (Hufford et al. 2011). One crucial difference between the APC and CD45⁻ target is the expression of the co-stimulatory ligands, B7-1 and B7-2. Lung APCs express high levels of B7 molecules (which ligate CD28 in the effector CTLs) while RECs express minimal B7 expression (Hufford et al. 2011). Indeed, blockade of the B7-CD28 interaction suppresses IFN γ production by effector CD8 T cells but not their cytotoxic activity or virus clearance *in vivo*. Thus, the engagement of co-stimulatory receptors on effector CD8⁺ T cells by APCs provide the additional signal strength necessary for the production of pro-inflammatory cytokines by the T cells (Hufford et al. 2011). In further analysis, the principal population stimulating *in vivo* release of effector cytokines by CTLs were the lung CD11c⁺ inflammatory DCs or TipDCs (Aldridge et al. 2009; Neyt and Lambrecht 2013). The acute depletion of CD11c⁺ cells in the lung drastically abrogate *in vivo* effector T cell cytokine production (Hufford et al. 2011). Thus, lung DCs controls all the aspects of CTL responses in the lungs including survival, proliferation, differentiation, and effector activities.

Surprisingly, another significant population of lung APCs was neutrophils. Neutrophils were previously demonstrated to be required for the innate defense against IAV infection. Despite their condensed nuclei and low levels of gene transcription, neutrophils can be directly infected with IAV transcribe viral genes, and thus contain significant influenza antigen. *In vivo* depletion of neutrophils diminished CTL IFN γ production (Hufford et al. 2012). These data demonstrated that neutrophils are required for triggering the maximal effector activities of CTLs *in vivo*.

The aforementioned results have revealed a potential new layer of effector T cell regulation *in vivo*: effector T cell activities in the lung are differentially regulated by the strength of interaction with their target cell types. This uncoupling of the release of inflammatory cytokines and the release of cytolytic molecules by effector T cells may be employed in the future to design novel therapeutics for influenza infection (Sun and Braciale 2013). For example, the specific blockade of certain co-stimulatory signals such as B7 may specifically dampen unwanted tissue inflammation by inhibiting the release of pathogenic cytokines from effector T cells but not perturb viral clearance since this blockade regimen specifically retain the ability of effector T cells to clear virus through cytotoxic molecule-dependent mechanisms.

6 Conclusion and Future Avenues of Research

This report has reviewed the current state of knowledge concerning the effector T cell response to IAV infection in the experimental setting. As the findings within demonstrated, our knowledge of the factors regulating the induction of adaptive immune T cell responses to IAV, the effector activities displayed by these activated T cells, the mechanisms underlying the expression of these effector mechanisms, and the control of the activation/differentiation state of these T cells in the infected lungs has progressed dramatically in recent years. While of necessity much of this information has come from studies in experimental nonhuman IAV infections, this work has provided both insight and a focus for current and future research in human IAV infection (Lee et al. 2011).

Although we now understand the broad outline of the events regulating the response of CD8⁺ and CD4⁺ T cells to respiratory virus infections like IAV, there remains both larger fundamental issues concerning the course of the host response during and following virus clearance as well as specific questions related to the role of specific cell types within the uninfected and infected respiratory tract as inducers and regulators of T effector cell activity and as targets for immune recognition. A major gap in our knowledge is the process of restoration of normal pulmonary function following a severe the respiratory tract IAV infection and the contribution (if any) from effector T cells remaining in the lungs following infectious virus clearance. Likewise, the role of specific cell types such as the alveolar macrophages and distinct subsets of RDC both as inducers of effector T cell differentiation and as targets and regulators of T cell effector activity need to be understood. Equally importantly is the need for a deeper understanding, in particular in the human, of the impact of differential susceptibility of various cell types in the respiratory tract to infection by different IAV strains (i.e., both seasonal and pandemic) on disease severity and the outcome of infection. As we look to the prospect of a “universal influenza vaccine,” we must also be cognizant of the need to develop novel strategies to control excess inflammation in those individuals unfortunate enough to develop severe life-threatening IAV infection.

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