IL-15 ACTIVATED CD122 TRIGGERS JNK AND AKT TO REGULATE MOTILITY OF EFFECTOR CD8 T CELLS

by

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(Under the direction of Kimberly Klonowski)

ABSTRACT

Effector CD8 T cells (CD8 T_{eff}) are key mediators of pathogen clearance, but must migrate to the site of infection to be most effective. We previously reported that IL-15 could induce chemotaxis of CD8 T_{eff} after influenza infection, although how IL-15 signaling mediated migration, and whether this feature was specific to influenza was unclear. We reveal that IL-15 induces CD8 T_{eff} independently of the infection from which they were derived or their state of effector differentiation. Moreover, chemotaxis to IL-15 requires signaling through CD122, but not CD132 or IL-15Rα, and is dependent on Jak1 activation of PI3K and MAPK cascades. Additionally, we show that IL-15-induced migration of CD8 T_{eff} utilizes heterotrimeric G-protein signaling, suggesting involvement of G-protein signaling downstream of the IL-15 receptor. Together, these data postulate that IL-15-driven CD8 T_{eff} motility requires specific signaling cascades distinct from the proliferative and survival functions of IL-15.

INDEX WORDS: Influenza, CD8 T cells, IL-15, Migration, CD122

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DEDICATION

I would like to dedicate this document to my mother. She has always and unquestioningly supported me, through times both good and bad.

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I would first like to thank my major professor, Kimberly Klonowski, for her mentorship, guidance, and occasional tolerance of my shenanigans. I had no appreciation or even knowledge of immunology before joining your lab, and now I can't imagine doing anything else. I would also like to thank my committee members, Don Evans and Rick Tarleton. Don especially has always been both insightful and thoughtful, and I will be ever grateful for his help when times were difficult. I would also like to thank my lab mates current and past: Dave Rose, Mary Field, Hillary Shane, Josh Cole, Muaz Ibrahim, Kat Verbist, Lina Tibavinsky, Jeffery Tran, Chris Slade, and Katie Reagin, and I wish you all the best of luck. To Joseph Burch: thank you for being an awesome friend, landlord, and fellow scientist. I wouldn't have survived very long down here without you around. And to Jillian Fishburn: thank you for your support, your tolerance, your friendship. I could not have made it through my last year of graduate school without you.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The ability of the immune system to efficiently traffic cells to sites of inflammation is critical for the timely elimination of localized infections. Upon detection of infectious agents, the adaptive immune system generates large populations of lymphocytes that recognize antigens specific to the invading pathogen. Through production of both broad spectrum and site-specific chemoattractant molecules called chemokines, anatomically distinct tissues selectively recruit immune cells expressing corresponding chemokine receptors and tissue-specific adhesion molecules. In turn, recruited immune cells serve to limit pathogen spread, clear pathogen from infected tissues, and provide protective immunity.

Among the immune cells recruited to inflammatory foci are CD8 T cells. While CD8 T cells are key mediators of pathogen clearance [1, 2], they sometimes induce immunopathology that becomes detrimental to the host [3, 4]. Thus, understanding the factors guiding CD8 T cell recruitment to select tissues is pivotal for the development of effective therapeutic treatments. This review will highlight the features of CD8 T cell recruitment to the lungs and lung airways during influenza infection, with an emphasis on signaling through the common gamma chain cytokine IL-15. IL-15 provides a broad array of effector, survival, and chemotactic signals to CD8 T cells, and an important, yet

unclear role in the recruitment of CD8 T cells to the lungs and lung airways in respiratory infections [5, 6]. The corresponding findings presented here contribute to our understanding of IL-15-mediated recruitment of CD8 T cells.

1.2 INFLUENZA

Influenza is an enveloped, segmented RNA virus that is responsible for 3-5 million cases of severe respiratory illness and between 250,000 to 500,000 deaths annually. Symptoms of infection generally include fatigue, fever, and achiness, and can result in hospitalization and death in severe infections. Influenza primarily infects cells of the upper respiratory tract, including linings of the bronchi, trachea, and nasal passages. The resulting seasonal infections are typically only a significant health risk to the young, elderly, or immunocompromised [7]; however, some influenza strains, such as H1N1, are capable of infecting both the upper and lower respiratory tracts, resulting in highly transmissible and severe infections across all age groups. In addition to the expected annual burden, world-wide pandemics of influenza are not uncommon. Several such pandemics have occurred in the last century, including the infamous 1918 Spanish influenza, which killed an estimated 20-50 million individuals worldwide [8], and the more recent H1N1 pandemic in 2009. This annual health risk necessitates the generation of reliable, long term vaccine strategies that protect against multiple strains and subtypes of the virus, as well as therapeutics to help curb the danger posed to individuals who are particularly at risk.

Multiple vaccination strategies are readily available for immunization against influenza, including multivalent inactivated vaccines administered intramuscularly, and

live attenuated influenza vaccines (LAIV) administered via nasal spray (Flumist). Because inactivated virus vaccines do not contain replicating virus, they rely exclusively on antibody production by B cells for protective immunity, known as humoral immunity. Alternatively, because the LAIV is capable of infecting cells, it produces both humoral protection as well as T cell-mediated protection [9]. Importantly, vaccines relying on the generation of a strong humoral response against influenza do not ensure long-term protection, nor are they always efficacious against the current year's circulating strains [10, 11]. This failure to protect is attributed to the ability of influenza to undergo antigenic drift and shift. Inactivated virus vaccines traditionally rely on the generation of neutralizing antibodies against the influenza coat proteins hemagglutinin (HA) and neuraminidase (NA) [12]. Because the virus gradually but continually alters these proteins via antigenic drift, the generation of long term antibody based protection has proven difficult [13]. Furthermore, influenza is capable of antigenic shift, where, when a host is infected by multiple strains of influenza, exchange of whole coding segments of RNA can occur. This allows the virus to express a unique blend of proteins, many of which our immune systems have never encountered before, even with vaccination. Consequently, the novel strain is capable of generating a world-wide pandemic, causing millions of casualties.

While the generation of cross-protective vaccine strategies is vital to the reduction of the annual influenza threat, there is also a need to generate more effective therapeutics to help curb morbidity and transmission in infected individuals. In severe infections with influenza, the immune system itself contributes to immunopathology and disease [3, 14]. The production of an uncontrolled "cytokine storm" during severe infections is followed

by increased cellular infiltrate and tissue damage [3, 15, 16], and modulation of cytokine responses has been proposed as a potent therapeutic [17].

1.3 CD8 T CELL RESPONSE TO INFLUENZA

CD8 T cells play an integral role in the control and clearance of influenza, evidenced by the observation that CD8 T cells in mice lacking functional class I major histocompatibility complex (MHC-I) signaling capability exhibit little cytotoxic activity, and have delayed viral clearance [1]. In wild-type mice, initiation of the CD8 T cell response occurs within the first 24 hours post exposure to influenza. Shortly after infection, CD103+ dendritic cells begin pulling influenza-derived antigens from the lung environment, and migrate to the draining mediastinal lymph node [18]. Here they encounter circulating naïve CD8 T cells patrolling the blood and lymphatics for MHC-I presented cognate antigen. Upon MHC-I/peptide recognition, influenza-specific CD8 T cells activate and undergo metabolic changes that allow for their rapid clonal expansion [19]. Eventually, these activated cells begin to egress from the lymph node and migrate to the lungs and lung airways in a complex process involving specific chemokines and adhesion molecules (discussed in next section).

In as few as 4 days post infection, influenza-specific CD8 T cells are found in the infected lungs [20]. Here they interact with dendritic cells displaying IL-15 and MHC-I presented antigen, both of which are required for their continued expansion and survival [20, 21]. Over the next several days, CD8 T cells augment the innate and humoral responses to control and clear the virus [22]. This is accomplished through antigen-

specific elimination of virally infected cells and antiviral cytokine secretion, leading to the eventual viral clearance within 7-10 days in mouse influenza models [1].

During and after influenza infection, multiple populations of effector/memory CD8 T cells are found within the lungs: a population residing within the lung interstitium, and a population residing in the lung airways. These populations differ in their expression of several molecules associated with activation and differentiation, the latter of which expresses low levels of terminal differentiation marker KLRG1 and high levels of the activation marker CD69 [23]. Presumably, this small percentage of uniquely differentiated CD8 T cells recruited to the lung airways, or at least across the epithelial basement membrane, are those most critical to the clearance of influenza, as they are the only CD8 T cells in contact with the infected epithelium and therefore capable of killing infected cells. Currently, the signals dictating recruitment of these precious few cells to the lung airways are unclear.

After clearance of influenza infection, the majority of antigen-specific CD8 T cells undergo a contraction phase, and only a fraction of the peak effector population survives. These populations of memory CD8 T cells continue to reside in both the lung interstitium and lung airways, the latter of which are CXCR3+ and are thought to be protective against future infections [24]. However, this lung airway resident population is subject to decay over time, and protection against heterosubtypic stains of influenza is eventually lost [25]. Thus, for cross-protective CD8 T cell based vaccine design, it may be beneficial to recruit as many cells as possible to the lung airways to prolong their eventual decline to below protective quantities. Conversely, CD8 T cell effector functions during severe influenza infection can lead to significant immunopathology [3]. Thus,

striking a balance between protection and immunopathology is critical towards thoughtful vaccine and therapeutic design, and highlights the need to understand the factors responsible for inducing CD8 T cell recruitment to the lung airways.

1.4 INVOLVEMENT OF THE LUNG ENDOTHELIUM IN LYMPHOCYTE RECRUITMENT

The lung contains two major sources of circulation: pulmonary circulation and bronchial circulation. Pulmonary circulation includes the classically defined circulation of deoxygenated blood flowing to the alveoli, and leaving as oxygenated blood. In contrast, bronchial circulation serves as the main supply of blood to the major lung airways. Both circulations are lined with blood endothelial cells, although the mechanisms of lymphocyte trafficking are likely distinct for each.

The pulmonary circulation comprises the bulk of the blood flow to the lungs. It supplies blood to the distal airways, alveoli, and throughout the interstitium of the lung. The blood vessels lining much of this area are extraordinarily small in diameter, less than the width of a lymphocyte [26]. Thus, the traditional model of binding, rolling, and arresting associated with lymphocyte trafficking does not necessarily apply in the pulmonary capillaries. Instead, large numbers of lymphocytes are trapped and retained in the pulmonary capillary beds, initially due to physical limitations, but are retained there under homeostatic conditions in a lymphocyte function-associated antigen (LFA-1) dependent and vascular cell adhesion molecule 1 (VCAM-1) independent manner [27, 28]. This is not surprising given the constitutive expression of intercellular adhesion molecule-1 (ICAM-1), the ligand for LFA-1, by the pulmonary endothelium [29]. In

addition to LFA-1 expression, antigen-experienced CD8 T cells traversing the lung endothelium in the non-inflamed lung also have a requirement of heterotrimeric Gαi protein activity, specifically mediated through CCL5 [27]. In contrast to the homeostatic lung, one of the primary chemokines required for the recruitment of T cells in the inflamed lung is CXCL10, a ligand for the chemokine receptor CXCR3. Evidence for this is found in a variety of respiratory infection models. For example, in respiratory syncytial virus (RSV) infected mice, CXCR3 is required for efficient recruitment of antigen-specific CD8 T cells [30]. Furthermore, in respiratory parainfluenza infection, CD4 T cell recruitment to the lung airways and lung tissue is also dependent on their expression of CXCR3 [31]. Importantly, pulmonary endothelial cells are documented to make both CCL5 and CXCL10 under inflammatory conditions, as well as a variety of other cytokines, including IFNα, IFNγ, CCL2, CXCL2, and TNFα [32].

The bronchial endothelium is the source of blood for the major airways of the lungs, including the trachea and various branches of the bronchi. Relatively little is understood about lymphocyte trafficking through this tissue, as the majority of pulmonary lymphocytes reside within the capillaries and interstitium of the lower lung. However, the anatomic position of tertiary lymphoid tissues, such as inducible bronchus associated lymphoid tissue (iBALT), that form during respiratory infections suggests that the lymphocyte recruitment through bronchial circulation does occur. iBALT is a major depot of immune cells, including CD4 and CD8 T cells, in the airways of the lungs, and is thought to serve as a makeshift priming site for T cells in the inflamed lung. This is evidenced by the fact that mice devoid of spleens and lymph nodes can still prime a T cell response in response to influenza [33]. In addition to harboring tertiary lymphoid

tissue, the upper respiratory tract of the lungs also harbors CD8 T cell resident memory cells that are important for control of heterosubtypic infections [34]. However, which circulation these cells truly originate from, and the factors that drive their migration to these sites, is presently unclear.

1.5 INTERLEUKIN 15

Introduction to IL-15

IL-15 is a 14-15kDa soluble protein belonging to the gamma chain family of cytokines. It is produced constitutively by subsets of dendritic cells (DC) and macrophages [35], as well as transiently by a multitude of other immune cells and non-hematopoietic tissues upon exposure to specific inflammatory stimuli, such as interferons (IFN) and toll like receptors (TLR). In lymphocytes, IL-15 is best known for its pro-survival effects, mediated primarily through induction of anti-apoptotic proteins such as a Bcl-2 [36, 37]. Indeed, both NK cells and some populations of CD4 and CD8 T cells require IL-15 for their survival and maintenance [38, 39]. Additionally, IL-15 also serves as a pro-inflammatory and activating signal for many immune cells during infection or injury, inducing recruitment, proliferation, chemokine production, and effector molecule secretion in many leukocytes (reviewed in [40]).

IL-15 production and regulation

The receptor of IL-15 is composed of three distinct components, including IL-15Rα, CD122 (IL-2/15Rβ), and CD132 (gamma chain, γc). Evidence suggests that majority of IL-15 signaling under homeostatic conditions occurs via trans-presentation

[41, 42] (Figure 1.1, middle). In this model, a cell produces both IL-15 and IL-15Rα, and complexes the pair in the endoplasmic reticulum. The complex is then transported to golgi and eventually to the cell membrane, where it signals either in an autocrine manner to the IL-15 producing cell, or in a paracrine fashion to adjacent cells [43]; however, trans-presentation is not limited cells proximal to the IL-15 producing cell. In some inflammatory settings, IL-15Rα can be cleaved by the enzyme A Disintegrin And Metalloprotease 17 (ADAM17) [44]. If already engaged in signaling with a cell expressing the CD122/CD132 dimer, the resulting IL-15/IL-15Rα complex is 'handed off' to the cell receiving the IL-15 signal, where it continues to transmit signals even when the original trans-presenting cell has disengaged [45]. Alternatively, if trans-presented IL-15 is not currently engaged in CD122/CD132 signaling, the IL-15/IL-15Rα dimer can be enzymatically cleaved into a soluble cytokine complex (Figure 1.1, bottom), as evidenced by the presence of IL-15 complexes in stimulated cultures of bone marrow-derived dendritic cells (BMDCs) [43], and in the serum of virally infected mice [5].

Trans-presented IL-15 is thought to be dependent upon the co-expression of IL-15Rα and IL-15 by the same cell. Mice lacking either component have nearly identical phenotypes, manifested primarily as immunodeficiency within the NK cell and memory CD8 T cell compartments [38, 46]. Correspondingly, irradiated mice reconstituted with a combination of IL-15Rα-/- and IL-15-/- bone marrow, where immune cells can produce either IL-15 or IL-15Rα, but not both, show similar deficiencies to their knockout counterparts [47]. Furthermore, co-cultures of dendritic cells expressing either IL-15 or IL-15Rα, but not both, are unable to coordinate to produce IL-15 complexes *in vitro* [43].

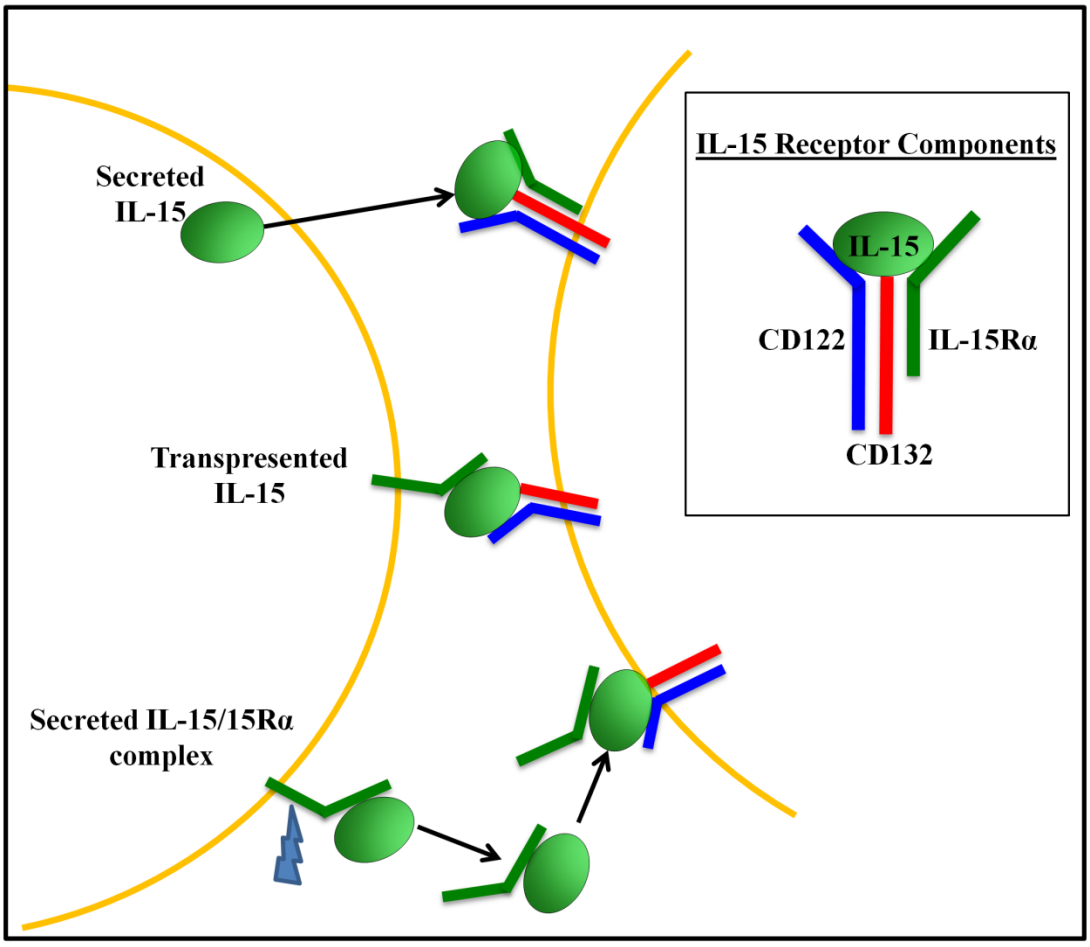


Figure 1.1: Models of IL-15 signaling between IL-15 producing and responding cells.

This dependency of IL-15 on IL-15Rα is highlighted further still by the fact serum taken from both mice and humans contains virtually all IL-15 complexes [48]. Still, IL-15 may have mechanisms for escaping its dependency on IL-15Rα in some inflammatory settings. In a mouse model of Imiquimod (toll-like receptor 7 agonist)-induced psoriasis, IL-15-/- and IL-15Rα-/- animals had distinct immune responses. Specifically, IL-15Rα-/- deficient mice had increased inflammation compared to either their wild-type or IL-15-/- counterparts. This inflammation was reduced after the addition of a dominant negative

recombinant IL-15Rα, suggesting that uncomplexed IL-15 existed in this model prior to the addition of IL-15Rα [49]. These data indicate that an *in vivo* mechanism likely exists by which IL-15 escapes cells in the absence of its receptor chaperone (Figure 1.1, top).

Regulation of both IL-15 and IL-15Rα is heavily dependent on interferons and other inflammatory stimuli. The promoter of IL-15 contains both an NFκB and interferon response factor (IRF) binding motif, and deletion of either of these components from IL-15 promoter constructs transfected into fibroblast cells resulted in a significant reduction in IL-15 message production [50]. Correspondingly, *in vitro* studies demonstrate the ability of IFNα, as well as toll-like receptor (TLR) ligands Poly:IC (TLR3 agonist) and LPS (TLR4 agonist), to trigger production of IL-15 in bone marrow-derived dendritic cells (BMDC) [51]. *In vivo* studies using emerald-GFP reporter mice also reported that MyD88 signaling was required in DC and monocytes, but not myeloid cells, for the efficient upregulation of IL-15 [52]. In contrast to the promoter of IL-15, the promoter of IL-15Rα contains a gamma activated site (GAS), which is triggered via IFNγ-mediated activation of Signal Transducer and Activator of Transcription (STAT)-1 homodimers. Accordingly, IL-15Rα was initially described as being inducible by IFNγ in several cell types [53]. In addition to type I and type II IFN, respiratory infections also induce robust type III IFN (IL-28/IL-29, IFNλ) [54]. Although the role of type III IFN in IL-15 regulation has not been explored, it is curious that mice deficient in type I IFN signaling are still capable of low level IL-15 production after respiratory infection [6], presenting a potential role for other inflammatory stimuli such as type III IFN in IL-15 production. Together, these data suggest that at least type I and type II IFN coordinate with other inflammatory signals to drive robust IL-15 production and regulation *in vivo* and *in vitro*.

IL-15 signal transduction in T cells

IL-15 is thought to signal primarily via trans-presentation under homeostatic conditions [55]. In this model, IL-15 bound to the high-affinity IL-15Rα on the surface of the trans-presenting cell is able to signal to responding cells expressing a CD122/CD132 dimer. Although many responding cells express IL-15Rα in addition to the CD122/CD132 dimer, it is thought to have little or no signaling capacity due to its short cytoplasmic tail. Moreover, despite the expression of IL-15Rα on IL-15 dependent lymphocytes, such as memory CD8 T cells and NK cells, the expression of this molecule on these cells is not required for their survival *in vivo* [56, 57].

Upon engagement of CD122 and CD132, several major pathways are triggered. CD122 and CD132 ligand binding induces the recruitment and activation of Janus Kinase (Jak) family members Jak1 and Jak3, respectively [58]. The recruitment of both Jak1 and Jak3 leads to the reciprocal phosphorylation and activation of one another, and results in the phosphorylation and activation of STAT3 and STAT5, respectively [58]. Additionally, Jak1 also phosphorylates several tyrosine residues within the cytoplasmic tail of CD122, including Y341, Y395, and Y498 [59]. Phosphorylation of Y341 on CD122 leads to subsequent recruitment of Shc and Grb2 adaptor proteins [60], followed by the recruitment and activation of phosphoinositide 3-kinase (PI3K) and mitogen activated protein kinase (MAPK) signaling cascades [61-64]. Meanwhile, phosphorylation of Y395 and Y398 appears to be vital for efficient STAT5, and potentially STAT3, activation [65].

In contrast to the multitude of pathways activated by Jak1, Jak3 activation through CD132 is markedly one dimensional, and primarily serves as an activator of

STAT5. However, Jak3 deficient cells do display altered migratory phenotypes. One study demonstrates that Jak3 is required for efficient activation of small GTPases Rac1 and RhoA in CD8 T cells [66], which are responsible for mediating actin cytoskeleton reorganization and leading edge formation. Accordingly, others have shown that Jak3 is important for mediating migration to chemotactic signals such as IL-8 in neutrophils [67], and CXCL12 and CCL25 in T cells [68]. Whether the requirement of Jak3 in these phenotypes involves direct signaling through Jak3 or is merely a developmental or bystander consequence remains to be elucidated.

IL-15 signal transduction in other immune cells

In addition to signaling to CD8 T cells, IL-15 also induces signaling cascades unique to other leukocyte populations. In similar fashion to CD8 T cells, neutrophils express IL-15 receptor components CD122 and CD132, and IL-15 enhances neutrophil survival *in vitro.* Moreover, activation of Jak3 in response to IL-15, presumably through CD132 signaling, enhances migration to chemotactic signals such as IL-8 [67]. Furthermore, MAPK signaling cascades are initiated by IL-15 in neutrophils, which may be attributed to CD122 engagement [62]. Unlike CD8 T cells, however, IL-15 does not influence STAT5 activation in neutrophils, and relies on Jak2 signaling rather than Jak1 [62]. Additionally, while IL-15Rα is thought to be dispensable for many CD8 T cell functions [56], the kinase Syk associates with IL-15Rα in human neutrophils, and signaling to this component by IL-15 increases neutrophil phagocytosis in a Syk dependent manner [69].

Another leukocyte population with unique IL-15 signal transduction is mast cells. Unlike neutrophils, mast cells from humans or mice do not express each of the components of the IL-15 receptor. Although they express CD132, they do not express CD122 protein or mRNA [53]. Moreover, although transfection with a CD122 expressing construct does allow mast cells to utilize IL-2 (which also signals through CD122), it is clear that CD122 does not normally participate in mast cell responses to IL-15. Instead mast cells reportedly express a unique IL-15 receptor that utilizes Jak2 and STAT6 as signaling components [70]. This receptor may have a role in mediating migration of mast cells *in vitro* [71], although it is unclear how this occurs.

Importantly, while neutrophils and mast cells may utilize IL-15 signals differently from CD8 T cells, it may be useful to examine these pathways and their effectors when considering how IL-15 signal transduction occurs in CD8 T cells, as our knowledge of these cascades in CD8 T cells may be incomplete

Transcriptional/translational changes induced by IL-15

IL-15 signal transduction induces an array of transcriptional activity. STAT3 and STAT5 in T cells and NK cells, and STAT6 in mast cells and neutrophils, undergo phosphorylation and dimerization with other STAT molecules, leading to their translocation from the cytoplasm to the nucleus. STAT5, which is activated by all common gamma chain cytokines in lymphocytes, upregulates several transcripts associated with proliferation and survival, including *c-myc, cyclin2D, bcl2, bclxL* [72, 73]. Unsurprisingly, STAT5 deficient T cells are incapable of proliferative responses in response to IL-2 stimulation [74]. Moreover, mice unable to form higher order structures

of STAT5 complexes have diminished responses to gamma chain cytokines, including IL-15 [75]. In addition to STAT5, STAT3 is also activated by IL-15 in T cells [58, 76]. In similar fashion to STAT5, STAT3 induces broad spectrum increases in transcripts important for survival and cell cycle induction. For example, STAT3 targets, such as *bcl2* and *ox40*, help mediate the survival of activated T cells [77]. Moreover T cells from STAT3 deficient animals exhibit elevated levels of pro-apoptotic transcripts, including *bad, bax,* and *caspase8*. This is thought to occur through STAT3 regulation of forkhead box transcription factors, including WAF1 and KIP1 [78], which regulate these transcripts.

Aside from controlling transcription via Jak/STAT pathways, activation of PI3K by IL-15 also induces robust changes in transcriptional and translational activity. Activation of PI3K triggers phosphorylation and activation of Akt. In turn, Akt regulates the transcription factors GSK3B, FOXO1, and FOXO3a, which help control cellular metabolism in non-terminally differentiated CD8 T_{eff} cells, as well as memory CD8 T cells [79]. Furthermore, Akt is the primary activator of mTOR, which inhibits the transcription factor e4EBP1, and activates the ribosomal kinase S6K. This results in the activation of eIF4E and S6, respectively, both of which increase ribosomal activity and the initiation of translation. Finally, activation of mTOR induces upregulation of T-bet expression, an important transcription factor for the development of CD8 T_{eff} cell functions [80].

Overall, between PI3K/Akt and Jak/STAT signaling pathways, IL-15 induces a wide variety of transcriptional changes involved with cell proliferation, survival, activation, and differentiation. Moreover, because mTOR positively regulates ribosomal

activity, signaling through IL-15 also poises cells for rapid translation of newly transcribed mRNAs. How these pathways might be integrated into cellular motility, adhesion, or trafficking downstream of IL-15 is currently unclear.

1.6 CD8 T CELL RESPONSES TO IL-15

CD8 T cells are dependent on IL-15 through several stages of their differentiation. IL-15-/- mice have a marked deficiency in their overall CD8 T cell numbers. This deficiency likely results from the partial dependence that several populations of CD8 T cells have on IL-15. In the thymus, CD4 and CD8 double positive CD8 T cells are partially dependent on IL-15 (along with IL-7) for differentiation into the CD8 single positive lineage [81], and mice deficient in signaling with either cytokine have significant deficiencies in their mature CD8 T cell pool. This is not wholly surprising considering that thymocytes within the T cell zone are one of the few cell populations that are known to constitutively produce IL-15 [82], suggesting a crucial role for the cytokine at this anatomic location.

Once mature, naïve CD8 T cells are still IL-15 sensitive both *in vivo* and *in vitro.* In response to IL-15, naïve CD8 T cells from both humans and mice upregulate various markers for survival and proliferation, including BcL2 [83, 84]. Furthermore, at high concentrations, IL-15 induces the formation antigen-independent memory, also known as virtual memory [85]. This is thought to be a result of IL-15 trans-presented by CD8α+ dendritic cells (DC) found in the lymph nodes of normal mice, and may partially explain why unimmunized mice contain significant populations of memory CD8 T cells as defined by CD44, especially as the mice age [86].

After activation, CD8 T_{eff} cells continue to rely on IL-15 for their survival and function. In influenza infection, mice depleted of IL-15 expressing DC have numerically inferior CD8 T cell responses in the lungs, which is recovered through administration of exogenous IL-15 expressing DC [20]. Moreover, both terminally differentiated short lived effector cells (SLEC) and developing memory cells are dependent on IL-15 for their continued survival [79, 87]. In particular, IL-15 is heavily implicated in the transition from effector to memory phenotype CD8 T cells [80].

Although IL-15 clearly influences CD8 T cells throughout their development, maturation, and differentiation, the reliance of memory CD8 T cells on IL-15 is perhaps the best characterized. Mice deficient in IL-15 signaling gradually lose their CD8 T cell memory populations generated after systemic infections, such as lymphochoriomeningitis virus (LCMV) and vesicular stomatitis virus (VSV) [46, 88, 89]. This is thought to be primarily due to poor homeostatic proliferation of memory CD8 T cells in the absence of IL-15, demonstrating that in addition to mediating survival, IL-15 induces proliferation in certain contexts. One exception to the IL-15/CD8 T cell memory dogma is that CD8 T cell memory developed from respiratory infections is uniquely independent of IL-15 for maintenance [90]. These cells express lower levels of the CD122 than memory CD8 T cells derived from systemic infections, suggesting memory derived from respiratory infections rely on other signals for their homeostatic maintenance.

In addition to aiding in the development, differentiation, and maintenance of CD8 T cells, IL-15 also induces a wide variety of changes in CD8 T cell migration and effector function. *In vitro* studies have demonstrated the ability of IL-15 to induce direct CD8 T cell migration [5], as well as to cause activation and rearrangement of various

adhesion molecules. CD11a in particular is up-regulated in response to IL-15 [91], and plays a role in the retention of CD8 T cells in the lungs [28]. Additionally, IL-15 induces changes in cellular morphology. IL-15 treated CD8 T cells undergo uropod formation, followed by redistribution of key adhesion molecules to the newly formed structure, including ICAM-1, ICAM-2, ICAM-3, CD44, and CD43 [92]. Others have also noted that IL-15 induces distinct changes in the migratory potential of T cells. In particular, chemokine receptors CCR1-CCR5, which allow T cells to respond to a wide variety of chemokines, are all up-regulated in response to IL-15 [93]. Moreover, surface core 2 O-glycan synthesis in response to IL-15 also influences migration potential of CD8 T cells to inflamed tissues [94]. Finally, IL-15 promotes the effector functions of activated CD8 T cells. Activated human CD8 T cells produce more IFNγ and tumor necrosis factor alpha (TNFα) in response to IL-15 than their untreated counterparts, and similar effector functions have been reported in murine CD8 and NKT cells [95, 96]. Together, these data clearly demonstrate roles for IL-15 signaling on CD8 T cells outside of the classically defined roles of survival and proliferation. It is likely that many of these functions are context dependent, and factors such as IL-15 concentration, the context of IL-15 (trans-presented or soluble), the stage of differentiation of the responding cell, or other extrinsic signals such as integrin engagement, chemokines, and co-stimulation, all influence the outcome of IL-15 signaling. Determining how the context of IL-15 may influence the outcome of signaling will be an important factor in developing its use as a therapeutic or adjuvant going forward.

1.7 IL-15 IN RESPIRATORY INFECTIONS

IL-15 is produced in response to multiple respiratory viral infections in mice, including rhinovirus and multiple strains of influenza. In both mice and humans infected with rhinovirus, IL-15 mRNA production and soluble IL-15 complex formation peaked and subsided in the first two days of infection, although cell associated IL-15 protein remained for several days in mouse lung tissue [6]. In accordance with studies demonstrating the dependence of IL-15 on type I IFN [50], type I IFN receptor deficiency in rhinovirus infected mice lead to partial reduction IL-15 expression, as well as a corresponding loss in NK cell and CD8 T cell recruitment and/or survival in the lungs [6]. Administration of intranasal IL-15 complexes in this model boosted CXCL9 and CXCL10 production and enhanced lymphocyte recruitment to the lungs [6]. Curiously, IL-15 complex administration to type I IFN receptor-deficient mice also induced both IL-15 and IL-15Rα expression, suggesting that IL-15 may function in an autocrine loop independently of type I IFN, though this was not further characterized [6].

After influenza infection, both the production of IL-15 and the role IL-15 plays in lymphocyte recruitment draws several parallels with observations from rhinovirus infection. Soluble IL-15 complexes are detected in the lungs of influenza infected mice early after infection, with the highest observed production occurring around 72 hours [5]. Accordingly, IL-15 mRNA production in the lungs and lung airways of influenza infected mice was upregulated in the first 24-48 hours, although this upregulation continued out to at least day 10 post infection after influenza in these tissues [5]. Also in accordance with rhinovirus infection, virus-specific CD8 T cells were dependent on IL-15 for their optimal recruitment to the lung airways after influenza infection. Although an

exact mechanism for this recruitment was not identified, IL-15 was found to be a chemoattractant for influenza-specific CD8 T cells *ex vivo,* suggesting that IL-15 has a potential role in the direct recruitment of influenza-specific CD8 T cells after influenza [5]. Notably, the role of IL-15 in modulating the CD8 T cell response to influenza infection is not limited to recruitment, evidenced by the observation that IL-15 produced by lung DC was required for optimal survival of infiltrating CD8 T cells within the lung parenchyma [20]. Additionally, IL-15 also played a role in CD8 T cell-induced pathology after influenza infection [3], highlighting that IL-15 plays a role in CD8 T cell effector function as well.

That IL-15 controls aspects of CD8 T cell recruitment, survival, and effector function after respiratory infections begs the question of whether these differential outcomes are independent of one another. It is likely that contextual expression of IL-15 induces differential outcomes such as migration and survival in CD8 T cells. Understanding when and where IL-15 is produced during respiratory infections, as well as differentiating how IL-15 triggers multiple outcomes in responding CD8 T cells, will be key to the development of effective therapeutics and vaccine strategies. IL-15 has already been utilized as an adjuvant for a vaccinia-based influenza vaccine [97, 98] and IL-15 blockade results in improved outcomes in models of chronic inflammation [99, 100]. Therefore, knowledge gained regarding IL-15 biology will be of utmost importance to its use in clinical settings.

CHAPTER 2: IL-15 ACTIVATED CD122 TRIGGERS JNK AND AKT TO REGULATE MOTILITY OF EFFECTOR CD8 T CELLS

2.1 Introduction

IL-15 is a pluripotent common gamma chain cytokine responsible for the development and homeostatic maintenance of NK, NKT, and both CD4 and CD8 T cells [36, 38, 39, 95]. Additionally, the activation and survival of NK cells and CD8 T cell effector and memory subsets after infection is dependent on IL-15 [42, 101, 102]. As such, integration of IL-15 into vaccines for infectious diseases and cancer is under current exploration to potentiate both innate and adaptive immunity [97, 98, 103, 104]. However, IL-15 is also enriched in psoratic lesions [105], as well as the joints [106] and intestines [107] of patients with rheumatoid arthritis and celiac disease, respectively. Moreover, blockade of IL-15 in animal models of these diseases improves disease outcome [99, 100]. This would suggest that variations in the contextual expression and/or function of IL-15 may tip the immunological balance between immunity and pathology. Therefore, understanding how IL-15 regulates specific cellular functions will be important to determine prior to utilization of IL-15 in clinical settings.

IL-15 has well-defined roles in CD8 T cell activation and survival; however, a less well understood property of IL-15 is its ability to regulate lymphocyte trafficking. This function has been characterized as being indirect, via induction of the adhesion molecules such as CD11a on NK cells [91], as well as C-C chemokine receptors 1-5 on

CD8 T cells [93]. However, IL-15 also directly regulates lymphocyte chemotaxis in some contexts. We previously reported that IL-15 deficient mice are unable to efficiently recruit virus-specific CD8 T cells to the lung airways after influenza infection, despite harboring equivalent frequencies of these cells peripherally [5]. Importantly, this deficiency in recruitment of virus-specific CD8 T cells to the respiratory tract in IL-15-/- mice was recovered following intranasal delivery IL-15/IL-15Rα-Fc complexes (IL-15c), which mimic the natural state of IL-15 *in vivo* [5, 108]. Corroborating with *in vivo* results, this chemotactic feature of IL-15 was recapitulated using virus-specific CD8 T cells *in vitro*, a finding which has also been observed in other lymphocytes [71, 109, 110]. Production of IL-15 is dependent on local inflammatory signals such as type I interferons [50], and as such is enriched at sites of viral infection [5]. Moreover, IL-15 can exist in both a cell-bound and secreted state (reviewed in [55]) that would allow for the establishment of cell-bound or soluble cytokine gradients at endothelial surfaces or within tissues, respectively. Taken together, these data suggest that IL-15 serves as an important chemotactic signal *in vivo;* however, the signaling cascades through which IL-15 mediates these migratory effects, and whether identical or divergent signaling pathways confer the diversity of IL-15 functions, is largely unknown.

IL-15 signals to T cells primarily via a mechanism referred to as trans-presentation *in vivo* [42]. In trans-presentation, IL-15 and IL-15Rα are produced and complexed within a single cell, which then co-translocate to the cell membrane [43]. *In vitro*, T cells respond to trans-presented IL-15 through their expression of IL-2/15 receptor beta (CD122) and the common gamma chain (γc, CD132), initiating Jak1/STAT3 and Jak3/STAT5 signaling, respectively [58]. Additionally, phosphorylation

of CD122 by Jak1 recruits the adaptor protein Shc, which binds to phospho-tyrosine 341 of the CD122 molecule [111]. In turn, Shc recruits both PI3K and MAPK family members to CD122, where they are subsequently activated by Jak1. PI3K regulates aspects of lymphocyte metabolism and survival by signaling to the PI3K effector Akt, which modulates mTOR, β-catenin, and multiple forkhead box transcription factor activities [87, 112]. Similarly, IL-15 activation of MAPK signaling also plays a role in the activation and survival lymphocytes, as evidenced by the IL-15 dependent increase of 4-1BB (CD137) expression and enhanced survival of 4-1BB expressing effector CD8 T cells after influenza infection [64]. Importantly, PI3K and MAPK pathways also have well-defined roles in cellular migration (reviewed in [113] and [114]). However, whether these pathways modulate T cell migration in response to IL-15 is unknown.

The current study examines the signaling pathways required for IL-15-mediated lymphocyte motility. We show here that CD122, but not CD132, is both required and sufficient to induce migration of effector CD8 T cells *in vitro*. Moreover, downstream CD122 signaling required both Jnk and Akt signaling for efficient migration of CD8 effector T cells to IL-15. Additionally, we note that IL-15-mediated migration required heterotrimeric G-protein signaling, despite the receptor components of IL-15 having no known heterotrimeric G-protein association. To our knowledge, this is the first examination of the IL-15 signaling cascade that incorporates lymphocyte motility, and provides insight as to how IL-15 signaling produces individual outcomes in CD8 T cells.

2.2 Materials and Methods

Mice and viruses

C57Bl/6 mice were obtained from Charles River (Bethesda, MD) via the NCI program. Influenza virus A/HKx-31 (x31) was generously provided by Dr. S. Mark Tompkins (University of Gerogia, Athens, GA). Vesicular stomatitis virus (VSV) (New Jersey strain) expressing recombinant ovalbumin (ova) (VSV-ova) was originally provided by Leo Lefrançois (University of Connecticut, Farmington, CT). Mice were infected with either 10^3PFU x31 intranasally (i.n) in 50uL of PBS or $5x10^5$ VSV-OVA intravenously (i.v.) in 200uL of PBS as indicated.

Cell lines and culture

1G11 murine lung capillary endothelial cells were a generous gift from A. Mantovani (Inst. Humanitas, Milan, Italy). Cells were maintained in DMEM with 20% FBS, 1% penicillin/streptomycin solution, 1% non-essential amino acids, 10mM HEPES, 100ug/mL endothelial cell growth factor (Sigma, St Louis, MO), and 100ug/mL heparin sulfate (Sigma St Louis, MO). Cells were grown and maintained in gelatin coated tissue culture flasks prior to use.

For IL-15 and IL-15Rα detection, 1G11 cells were seeded at $2.5x10^5$ cells/well in a 24 well gelatin coated plate and stimulated with following stimuli for 24 hs: 100ng/mL human IFNα4 (PBL Assay Science, Piscataway, NJ), 20ng/mL murine IFNγ or 10ng/mL murine TNFα (Peprotech, Rocky Hill, NJ), Poly:IC, LPS, flagella (Invivogen, San Diego, CA). Post treatment, cells were de-adhered using PBS+10mM EDTA.

IL-15/IL-15Rα detection

For determining IL-15 mRNA expression, RNA was first isolated from cells using the RNeasy kit (Qiagen, Germantown, MD) and subsequently cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA). IL-15was quantified by RT-qPCR using probes against IL-15 and GAPDH (Taqman assay numbers Mm00434210_m1 and Mm99999915_g1), using an Applied Biosystems 7500 Real-Time PCR System. Samples were analyzed in duplicate and normalized to GAPDH expression. Results are expressed as fold induction using delta-delta-CT values. For IL-15Rα protein detection, 1G11 cells were stimulated with the indicated inflammatory stimuli, harvested, and stained with biotinylated anti-IL-15Rα (R&D Systems, Minneapolis, MN) for 30 minutes, followed by streptavidin-APC or PE for 15 minutes. IL-15Rα expression was determined using a BD LSRII cytometer (San Jose, CA) and analyzed using Flowjo software (Ashland, OR).

Cell isolation and migration assays.

Bronchoalveolar lavage (BAL), lungs, spleens and mediastinal/cervical lymph nodes were harvested from x31 or VSV-OVA infected mice. BAL was collected via intratracheal administration and recovery of 1mL PBS three times. Lungs were perfused with PBS and heparin (Sigma, St Louis, MO). Single cell suspensions were obtained from mechanically disrupted spleens and lymph nodes followed by straining through nytex. Erythrocytes were removed from BAL and splenocyte suspensions using a lysis buffer containing Tris-buffered ammonium chloride. For single cell isolation from the lung parenchyma, tissues were incubated in 1.25mM EDTA for 30 minute, followed by

collagenase digestion (6mg/mL) for one hour. Lymphocytes were further enriched through resuspension in 44% percoll underlaid with 67% percoll followed by centrifugation, and lymphocytes at the interface were collected.

For migration assays, splenic CD8 T cells were enriched to at least 85% of lymphocytes using the Untouched CD8 enrichment kit (Life Technologies, Carlsbad, CA). Approximately 300,000 CD8 T cells were resuspended in 100uL migration media (RPMI+5% FBS) and added to the top chamber of a 5μM Transwell (Corning, Corning, NY), and 600uL of migration media in the bottom. Cells were pre-incubated in Transwells at 37 degrees for 45 minutes, followed by 90 minutes to allow for migration to IL-15 complexes IL-7, or CCL5. IL-15 complexes were generated by mixing 1:1.5 ligand:receptor of IL-15 [R&D Sytems or Tonbo (Minneapolis, MN and San Diego, CA)] and IL-15Rα-Fc (R&D Systems, Minneapolis, MN). In some experiments, inhibitors of IL-15 signal transducers were placed in the top and bottom chambers Transwells at the following concentrations [10μM Ruxolitinib, 1μM Akt inhibitor VIII, 25μM S3I-201 (Cayman Chemical, Ann Arbor Michigan); 15μM UO126; 15μM SB202190, 15μM SP600125; 15μM LY2940002, and 1μM Rapamycin [each generously provided by W. Watford (University of Georgia, Athens, GA)]. In some cases, cells were pretreated with 1ug/mL pertussis toxin (PTX) (Sigma, St Louis, MO) or vehicle for 90 minutes prior to migration assays. For receptor blocking experiments, T cells were pre-treated with 20ug/mL anti-CD122 or anti-CD132 (ebioscience, San Diego, CA) for 30 minutes prior to migration assays.

Post migration, migrated cells were collected from the bottom chambers of Transwells, and stained with anti-CD8 and anti-CD44 (ebioscience, San Diego, CA), and

MHC class I tetramers reactive to the nucleoprotein of either influenza (ASNENMETM) or VSV (RGYVYQGL) (NIAID Tetramer Facility, Emory University, Atlanta, GA). In some experiments, cells were also stained with anti-CD122 or anti-CD132-biotin (ebioscience, San Diego, CA) followed by streptavidin PE or APC (Life Technologies, Carlsbad, CA).

Statistics:

Unpaired two-tailed student's t test was applied utilizing Prism Graphpad software (La Jolla, CA). p values are indicated in figure legends and statistical significance indicated where appropriate.

2.3 Results

Murine lung endothelial cells are a potential source of trans-presented IL-15 for responding CD8 T_{eff}

We previously reported that influenza-specific CD8 T cells partially depend on IL-15 for their migration to the lung airways of influenza infected animals [5]. We sought to examine potential sources of IL-15 that may contribute to migration during influenza infection to better understand how IL-15 directs CD8 T cell trafficking *in vivo*. Under homeostatic conditions, IL-15 is produced primarily by dendritic cells and macrophages [35]. During inflammation, however, a wide variety of non-hematopoietic cells including endothelial cells [109] and epithelial cells [115] upregulate IL-15 and IL-15Rα, which is required for IL-15 trans-presentation. Moreover, endothelial cells in particular are known to produce chemokines and inflammatory cytokines during influenza infection [32], and

are anatomically unique in their ability to establish gradients of surface-bound cytokines exposed to the circulation. As such, we hypothesized that the pulmonary endothelium may play a role in the production and regional presentation of IL-15 after influenza infection. We examined the murine pulmonary capillary endothelial cell line 1G11 (described in [116]) for IL-15 and IL-15Rα expression in response to inflammatory stimuli typically associated with IL-15 and IL-15Rα production, such as TLR agonists and type I IFN for IL-15 [43], and type II IFN for IL-15Rα [53]. We found that 1G11 cells expressed IL-15 message in response to both type I and type II IFN by 2.8 fold and 2.4 fold, respectively (Figure 2.1), but not in response to other classic inducers of IL-15 such as LPS and Poly:IC [43]. Additionally, type I and type II IFN induced IL-15Rα protein by nearly 6 fold and 25 fold, respectively, suggesting that the pulmonary endothelium is a viable source of trans-presented IL-15.

CD8 T_{eff} cells from multiple viral infections and divergent stages of differentiation migrate to IL-15 in vitro

Having establishing a relevant source of IL-15 for migrating CD8 T cells, we next sought to explore how IL-15 regulates CD8 T cell motility. Previous work from our laboratory demonstrated that influenza-specific CD8 effector T cells (T_{eff}) migrated towards IL-15/IL-15Rα-Fc complexes *in vitro* [5]. Moreover, IL-15 also participated in the selective recruitment of antigen-specific T_{eff} to the lung airways after influenza infection *in vivo*. As respiratory infections generate an inflammatory milieu which includes local IL-15 production [5, 20], the influenza model facilitated our ability to track T cell movement from a known source (the blood) to a final destination (the lung).

However, it is unclear whether CD8 T_{eff} cells derived from respiratory infections are uniquely programmed to migrate towards IL-15 or if this migratory potential is a broad feature of all activated CD8 T_{eff}.

To determine whether CD8 T_{eff} derived from systemic infection also migrate to IL-15 *ex vivo*, we collected and enriched splenic CD8 T_{eff} derived from vesicular stomatitis virus (VSV) infected animals and compared their migration to IL-15 with that of influenza-specific CD8 T_{eff} near their respective peak expansion, 6 and 9 days post infection (dpi), respectively. Splenocytes from both VSV and influenza infected animals contained a significant proportion of antigen-experienced cells, denoted by high CD44 expression, a fraction of which were specific for N-tetramer (VSV, 10-20% positive) or NP-tetramer (influenza, 2-5% positive). We found that antigen-specific CD8 T_{eff} for either virus were IL-15 responsive as measured by pSTAT5 staining (Figure 2.2A), which is activated by IL-15 signaling through CD132. Moreover, IL-15 readily induced migration of CD8 T_{eff} from both influenza (~3.1 fold) and VSV (~4.0 fold) compared to media controls (Figure 2.2B), suggesting that migration to IL-15 was not unique to circulating CD8 T_{eff} primed by respiratory infections. However, it was still possible that subsets of alternatively differentiated CD8 T_{eff} generated from each infection might migrate differentially to IL-15, as effector subsets differ in their ability to respond to some chemotactic factors [117].

To examine whether unique populations of CD8 T_{eff} differentially migrate to IL-15, we characterized antigen-specific T_{eff} based on expression of CD127 and KLRG1, which delineate CD8 T_{eff} into populations of short lived effector cells (SLEC), early effector cells (EEC), and memory precursor cells (MPEC). Despite strikingly different

differentiation patterns based on CD127 and KLRG1 expression (Figure 2.2C), antigen-specific CD8 T_{eff} from both sources expressed similar levels of CD122 and CD132, the two signaling components of the IL-15 receptor. CD122 in particular was upregulated in all CD8 T_{eff} subsets (5.5-6.7 fold increase in VSV CD8 T_{eff} subsets, 4.0-4.9 fold increase in influenza), relative to their naive counterparts (Figure 2.2D). Together, these data suggest that circulating virus-specific CD8T_{eff} broadly possess the capacity to migrate to IL-15, and that IL-15 induced chemotaxis is not a unique characteristic of CD8 T_{eff} cells derived specifically from respiratory infections. Additionally, since VSV-infected animals generated larger and more concentrated populations of antigen-specific cells, we used VSV-derived CD8 T_{eff} to answer subsequent questions about IL-15c induced migration.

CD122 engagement is both required and sufficient for migration of CD8 T_{eff} cells to IL-15 in vitro

The two major signaling components of the IL-15 receptor during trans-presentation are CD122 and CD132, which activate the Jak1/STAT3 and Jak3/STAT5 pathways, respectively. Additionally, IL-15Rα has also been reported to possess some signaling capacity independently of CD122 and CD132 [118], and is expressed on the surface of antigen-experienced CD8 T cells [56]. Using mice deficient in IL-15Rα or blocking the CD122 or CD132 chains of the IL-15R using mAbs, we sought to determine the contribution of the individual IL-15 receptor components on IL-15-mediated migration.

IL-15Rα is expressed on activated CD8 T cells, and readily binds soluble IL-15 *in vitro* [119]. Traditionally thought to be devoid of signaling capacity, recent evidence demonstrates the ability of IL-15α to signal through Jnk and NFκB in myeloid cells [118], and may have a role in the internalization process bound IL-15 [119]. Although the expression of IL-15Rα on CD8 T cells is largely inconsequential for development of effector and memory CD8 T cell responses [56], the role of IL-15Rα in IL-15-mediated migration has not been explored. To determine whether expression of IL-15Rα on CD8 T_{eff} facilitates their IL-15 induced migration, we infected wild-type (WT) or IL-15Rα-/- animals with VSV and compared the ability of resultant CD8 T_{eff} derived in each host to migrate in response to IL-15. As seen in Figure 2.3A, IL-15Rα deficiency on CD8 T_{eff} did not alter migration rates to IL-15.

As IL-15Rα did not participate in IL-15-driven migration of CD8 T_{eff}, we next examined the role of CD132 in this process. Among the antibodies reactive to CD132, the clone tugm2 specifically blocks this receptor component without influencing CD122 signaling, at least with respect to IL-2 [120], which also signals through CD122 and CD132. We pre-incubated CD8 T_{eff} with this mAb clone to test whether CD132 was required for IL-15 induced migration of CD8 T_{eff}. Blockade of CD132 did not impede the IL-15-induced movement of the CD8 T_{eff} over the untreated controls (Figure 2.3B) suggesting that CD132 does not participate in IL-15c-mediated migration of CD8 T_{eff}. To verify that the tugm2 antibody specifically blocked CD132 in the context of IL-15, we compared CD122 expression prior to and after antibody treatment, as well as phospho-STAT5 activation after IL-15 exposure. Treatment with antibody alone had no effect on CD122 expression, nor did it inhibit CD122 engagement by IL-15, as evidenced by

internalization of CD122 in response to cytokine (Supplemental Figure 2.1). Additionally, tugm2 pre-treated cells reduced pSTAT5 induction by 85% in response to IL-15 treatment (Supplemental Figure 2.1). This suggests that tugm2 specifically blocked CD132/Jak3/STAT5 signaling without influencing CD122 signaling, validating that CD132 is not involved in IL-15-induced migration of CD8 T_{eff}.

In contrast to IL-15Rα and CD132, CD122 signal transduction includes a multitude of distinct cascades, including Jak1/STAT3, PI3K, and MAPK in CD8 T cells [58, 63]. After ruling out the contribution of CD132 and IL-15Rα, we asked whether IL-15-mediated migration of CD8T_{eff} required CD122 signaling. To this end, we utilized the blocking mAb clone TM-β1, which specifically inhibits CD122 signaling [121]. In contrast to CD132 blockade, incubation of CD8 T_{eff} with TM-β1 prior to migration assessment completely abrogated the movement of CD8 T_{eff} towards IL-15 (Figure 2.3C), highlighting CD122 as the key IL-15 receptor component required for CD8 T_{eff} migration.

Although CD122 blockade fully inhibited CD8 T_{eff} migration to IL-15, it was important to consider that IL-15 may not interact with CD132 alone due to the very low affinity of this interaction [122]. As such, it was plausible that CD132 could potentiate CD8 T_{eff} migration to IL-15 independently of CD122. To test this possibility, we assayed the ability of CD8 T_{eff} to migrate to the cytokine IL-7, which signals through CD132, but not CD122. Although naïve and some effector cells were IL-7 responsive as measured by loss of surface CD127 (IL-7Rα) expression (Supplementary Figure 2.2), IL-7 was unable to induce migration of CD8 T_{eff} (Figure 2.3D). Together, these data suggest that CD122, but not IL-15Rα or CD132 is required and sufficient for migration of T_{eff} IL-15c.

Jak1, but not STAT3 is required for migration of CD8 T_{eff} to IL-15

CD122 activation by IL-15 initiates Jak/STAT, PI3K, and MAPK signaling cascades in T cells [63]. Specifically, IL-15 signaling to CD122 activates the Janus kinase Jak1, which phosphorylates and activates STAT3 [58]. In T cells, STAT3 regulates transcription of forkhead box transcription factors required for survival [78], and as such, is largely implicated for its role in preventing apoptosis. However, STAT3 also influences aspects of cellular motility, and plays a role in migration of both fibroblasts and squamous cell carcinoma cells by regulating small GTPase activity [123-125]. As such, we hypothesized that the Jak1/STAT3 pathway serves as an important link between IL-15 engagement of CD122 and CD8 T_{eff} mobility. Using specific inhibitors of both Jak1 and STAT3, we blocked the activity of these signaling components prior to and during assessment of IL-15 induced migration of CD8 T_{eff} *in vitro*. The Jak1 inhibitor Ruxolitinib potently abrogated migration of CD8 T_{eff} to IL-15c (Figure 2.4A) whereas vehicle treated cells efficiently migrated to IL-15c. In contrast, blockade of STAT3 with the inhibitor S31-201 had no significant effects on migration (Figure 2.4B). These results suggest that although Jak1 is required for CD8 T_{eff} migration to IL-15, it may be targeting pathways downstream of CD122 that are independent of STAT3. Of note, Ruxolitinib treatment of CD8 T_{eff} did reduce migration of CD8 T_{eff} to media alone by ~50%, implying that Ruxolitinib may have off-target or detrimental effects, although no differences in T cell viability were noted (data not shown).

Akt and Jnk signaling are required for IL-15-mediated migration of CD8 T_{eff}

In addition to STAT3, Jak1 also phosphorylates the tyrosine residue Y341of murine CD122 [111] which allows for recruitment of the adaptor protein Shc. Subsequently, members of PI3K and MAPK families are recruited to CD122 and activated [59]. PI3K and MAPK pathways carry out a variety of roles in cytoskeletal regulation (reviewed in [126] and [114]). Moreover, deletion of specific PI3K isoforms in many leukocytes, including T cells, results in inefficient migration to chemotactic stimuli [113]. As such we hypothesized that one or both of these pathways would be required for IL-15c-driven migration of CD8 T_{eff}. We first examined the contribution of PI3K using the pan-PI3K inhibitor LY2940002. Blockade of this pathway prior to and during migration assessment completely inhibited all IL-15c-driven migration of CD8 T_{eff} (Figure 2.5A). We did note that in this assay, background levels of migration also dropped slightly, albeit significantly, with treatment of this inhibitor. This was not due to loss of cell viability after treatment with LY2940002 (data not shown), suggesting instead that PI3K cascades globally regulate cellular motility in CD8 T cells.

To more narrowly define the signaling cascade regulating IL-15 induced CD8 T_{eff} migration, we examined whether Akt, one of the primary effectors of IL-15 activated PI3K [112], was required for migration. Akt regulates several aspects of leukocyte migration, both directly and indirectly. In CD8 T cells, the strength of Akt signaling regulates egress of CD8 T cells from the lymph nodes, primarily through inhibition of FOXO transcription factors, which in turn down-regulate expression of the lymph node homing molecules sphingosine-1-phosphate receptor 1 (S1P1), CD62L, and CCR7 [127]. Additionally, Akt directly regulates chemotaxis in other leukocytes, such as neutrophils,

through activation of cytoskeletal rearrangement proteins [128]. Whether IL-15 activated Akt in CD8 T_{eff} is essential to their migration to IL-15 is unclear. To examine this possibility, we utilized Akt inhibitor VIII to specifically block Akt signaling during *in vitro* assessment of CD8 T_{eff} migration to IL-15c. Treatment of CD8 T_{eff} with Akt VIII prior to and during migration assessment abrogated all migration to IL-15c without significantly affecting background levels of migration (Figure 2.5A), highlighting the importance of this pathway in IL-15 induced CD8 T_{eff} migration.

One major target of Akt is the mechanistic target of rapamycin (mTOR). Similar to Akt, mTOR is a key regulator of T cell metabolism, but also influences CD8 T cell migration indirectly through modulation of chemokine receptors CCR5, CCR7, and CXCR3 and 4, as well as adhesion molecules CD62L and S1P1 [129]. Recently, a role for mTOR in direct regulation of CD8 T cells was discovered in response to the chemokine CXCL12 through modulation of cytoskeletal activity [130]. This suggests that mTOR activation is intimately linked with CD8 T cell motility. We sought to determine if IL-15-mediated CD8 T_{eff} migration also required mTOR signaling. Using the drug rapamycin to target mTOR signaling, we observed that blockade of this particular Akt substrate did not affect CD8 T_{eff} migration (Figure 2.5B).

In addition to PI3K signaling, the MAPK family members Jnk, p38, and MEK1/2 are all reported to signal downstream of IL-15 or IL-2 bound CD122 in T cells [111, 131, 132]. Moreover, motility of primary T cells and T cell lines in response to the cytokine IL-6 and α2β1 integrin signaling is dependent on MAPK signaling [133, 134]. To determine the role of MAPK signaling in IL-15-mediated migration CD8 T_{eff}, we utilized specific inhibitors against MAPK family members. We found that inhibition of Jnk using

the inhibitor SP600125 completely abrogated migration of CD8 T cells to IL-15c (Figure 2.5C). In contrast, neither U0126 (MEK1/2 inhibitor) nor SB203580 (p38 inhibitor) had any effect on migration. This suggests that although IL-15 activates multiple MAPK family members, only Jnk is required in the context of CD8 T_{eff} cell migration.

Migration of CD8 T_{eff} cells to IL-15c requires the involvement of heterotrimeric G-protein activity

Prior reports show that IL-15 signaling induces migration of mast cells that is dependent on heterotrimeric G-protein signaling [71], though curiously neither CD122 nor CD132 contain the heterotrimeric G-protein binding motif E/DRY. Because mast cells reportedly express a unique IL-15 receptor component, IL-15R-X [135], it was proposed that IL-15 induced G-protein signaling must occur through this receptor [70]. We challenged the concept that G-protein activation in response to IL-15 is unique to mast cells by asking whether CD8 T_{eff} cell migration was also dependent on heterotrimeric G-protein signaling. To this end, we pretreated effector CD8 T cells with pertussis toxin (PTX) to block heterotrimeric G-protein signaling before allowing them to migrate to either IL-15c or the control chemokine RANTES (CCL5). We found that blocking heterotrimeric G-protein signaling completely negated the chemotactic effects of IL-15c as well as the CCL5 chemokine control (Figure 2.6). Background migration to media alone was unaffected by pertussis toxin treatment, demonstrating that neither cell viability nor motility in general was affected by this condition.

2.4 Discussion

Proper tuning of the T cell response to localized infections is vital to the survival of the host. There often lies a delicate balance between pathogen control and tissue damage, particularly in sensitive locations such as the brain and lungs where excess inflammation can result in fatality. The cytokine IL-15 plays a pivotal role in this balance, dictating aspects of T cell recruitment, activation, and survival at multiple stages of the immune response to different pathogens. We previously determined a role for IL-15 in the recruitment of CD8 T cells to the lung airways during influenza infection [5]. *In vivo*, antigen-specific CD8 T cells were reliant on IL-15 for their migration to the lung airways, while i*n vitro,* IL-15 served as a direct chemotactic agent for influenza-specific CD8 T cells. However, neither the source of IL-15 *in vivo* nor our understanding of how IL-15 signaling dictated T cell migration was examined.

During inflammation, the blood endothelium produces a plethora of chemokines [32] which establish a gradient on the luminal surface of the endothelium, and serve to recruit nearby crawling/rolling T cells [136]. The ability of IL-15 to exist as a trans-presented dimer with IL-15Rα would similarly allow the establishment of such a gradient. Moreover, others have highlighted that both blood [137] and lymphatic endothelia [82] are viable producers of IL-15. We demonstrated that cells representative of the pulmonary capillary endothelium produce IL-15 and IL-15Rα in response IFNα or IFNγ (Figure 2.1). The ability of IFNγ to upregulate IL-15 is in contrast to other cell types, as IL-15 expression is generally thought to be reliant on MyD88 signaling and type I interferons for induction in hematopoietic cells [52]. This suggests that alternate signals may be responsible for inducing IL-15 in non-hematopoietic tissues. While our attempts

to verify that pulmonary endothelial cells from influenza infected mice produce IL-15 were inconclusive (data not shown), this does not rule out the participation in IL-15 production by this tissue, as reagents for murine IL-15 detection are notoriously difficult to use [55].

The route and type of infection have broad impacts on the quality and quantity of the CD8 T cell response (unpublished results, [138]), which in turn influences the chemotactic properties of different T cell subsets [117, 139]. However, we observed that IL-15 induced the migration of CD8 T cells indiscriminately of infection type or state of differentiation based on CD127 and KLRG1 expression (Figure 2.2B-D). Rather, all observed phenotypes of CD8 T_{eff} expressed similar levels of CD122 and CD132, and responded similarly to IL-15 as measured by pSTAT5 (Figure 2.2A), suggesting that IL-15 can potentially recruit these cells. This is corroborated by studies correlating IL-15 and number of T cells in the synovial fluid of patients with rheumatoid arthritis (RA) [106], and is further supported by the reduction of site-specific T cells after local neutralization of IL-15 in murine models of RA and psoriasis [49, 99, 109].

Numerous reports have highlighted that NK cells, CD8 T cells, and other leukocytes readily migrate to IL-15 *in vitro* [5, 71, 110]; however, the signaling cascades that dictate IL-15 induced migration have been unclear until now. This study shows that CD122 is both required and sufficient to induce migration of CD8 T_{eff} to IL-15. CD132 signaling alone was insufficient to induce migration (Figure 2.3D), and blockade of CD132 had no impact CD8 T_{eff} migration (Figure 2.3D). In contrast, blockade of CD122 completely abrogated IL-15 induced CD8 T_{eff} migration (Figure 2.3C), as did inhibiting the primary effector of CD122, Jak1 (Figure 2.4A). We did note that inhibition of Jak1 by

the Ruxolitinib did result in blockade of background levels of migration, suggesting it may have off target or detrimental effects to the cell, although viability of cells exposed to Ruxolitinib was not affected (data not shown). Alternatively, as other Janus kinases such as Jak2 and Jak3 are required for motility of leukocytes to some chemokines [66, 67], the requirement of Jak1 signaling in leukocyte motility may be universal. In addition to observations with CD8 T cells, it was also observed that NK cells and CD4 T cell subsets migrate to IL-15 at rates based on their relative expression of CD122 (Appendix A). This suggests that IL-15 may broadly recruit all CD122 expressing cells. That IL-15 may act simultaneously as a pluripotent chemoattractant and inducer of effector function could explain why IL-15 expression is so very tightly regulated. IL-15has mechanisms of regulation in the cell at the DNA [50], RNA [140], and protein level [88], as well as its own extracellular neutralizing agent in the form of a soluble dominant negative IL-15Rα [49]. This could explain why loss of control of IL-15 expression is often detrimental in many autoimmune disorders.

IL-15 signaling in T cells triggers activation of PI3K and its downstream effectors Akt and mTOR [61]. Akt and mTOR signaling are intimately linked with one another. Full activation of either requires reciprocal activation by the other, although each carries independent functions as well (reviewed in [141]). This study found that Akt but not mTOR signaling was required for CD8 T_{eff} migration to IL-15 (Figure 2.5A), suggesting that Akt drives migration to IL-15 independently of mTOR activity. As phospho-Akt reportedly binds directly to actin [142], and actin is a substrate for Akt phosphorylation [143], it is feasible that IL-15-driven Akt directly modulates cytoskeletal activity required for migration. Of note, PI3K activation in lymphocytes also targets Rho-GTPase activity

[144]. Rho-GTPases are critical modulators of the actin cytoskeleton, and help define cell polarity and leading edge formation [145]. This explains why cells treated with the pan-PI3K inhibitor LY294002 failed entirely to migrate through Transwell chambers, regardless of the presence of IL-15 (Figure 2.5A), while inhibition of Akt had no effect on background levels of migration, but specifically blocked IL-15 induced migration.

In addition to PI3K and Akt, this study determined that Jnk is required for IL-15-driven motility of CD8 Te_{ff}. The most thoroughly characterized target of Jnk is the transcription factor c-Jun, which is part of the AP-1 transcription factor complex; however, activated Jnk targets other substrates, including serine 178 of the integrin tail binding protein Paxillin in T cells [146]. Paxillin is involved in the formation of focal adhesions through regulation of the actin binding protein FAK (focal adhesion kinase), which provides insight as to how Jnk might migration in response to IL-15.

Most chemotactic stimuli signal through G-protein coupled receptors (GPCR), which are dependent on heterotrimeric G-protein signaling for their function. We observed that IL-15-mediated migration of CD8 T_{eff} was also dependent on heterotrimeric G-protein signaling (Figure 2.6). Typically, heterotrimeric G-proteins interact with chemokine receptors via a DRY motif on the cytoplasmic portion of the receptor. As no component of the IL-15 receptor contains a DRY motif, this suggests that IL-15 is able to trigger G-protein signaling independently of such a motif. Instead, IL-15 may utilize non-receptor associated heterotrimeric G-proteins, such as the ASG family of heterotrimeric G-proteins to induce migration [147]. Further studies should attempt to characterize this quality of IL-15 signaling.

Our data provide a model by which IL-15 signaling cascades drive the migration of CD8 T_{eff} (Figure 2.7). Engagement of CD122 by IL-15 triggers Jak1 phosphorylation. Subsequently, Jak1phosphorylates the cytoplasmic tail of CD122, recruiting the adaptor molecule Shc. In turn, Shc recruits and activates effectors of PI3K and MAPK signaling cascades, which are capable of interacting both directly and indirectly to regulate cytoskeletal rearrangement.

IL-15 is already well established for its role in guiding T cell activation, differentiation, and survival. This study provides an additional role for IL-15 in modulating the CD8 cell response to pathogens through direct regulation of migration. Here we explored the mechanism by which IL-15 mediates CD8 T cell motility. We provide both a potential source for IL-15 and a signaling mechanism by which IL-15 may directly regulate CD8 T_{eff} recruitment during infections. Going forward, understanding how IL-15 differentially modulates survival, proliferation, and migration should be given strong consideration. The ability to dictate each is likely reliant on the context in which IL-15 is encountered by responding T cells. For example, activated dendritic cells provide strong co-stimulatory signals to T cells, which could explain the ability of IL-15 to enhanced proliferation and effector function. Likewise, IL-15 production from steady state dendritic cells and macrophage subsets may not provide optimal stimulatory signals, but rather only enough stimulation to induce low level homeostatic proliferation in response to IL-15. In the context of endothelial-derived IL-15, it is likely that the presence of chemokines and activated integrins on the inflamed endothelium also influence the outcome of IL-15 signaling. Pathways downstream of chemokines, integrins, and IL-15 have several points of overlap, and could allow for crosstalk to

optimize endothelial adhesion/rolling/arrest on endothelial cells, or trans-endothelial migration. As IL-15 is being tested as both a vaccine adjuvant and a target for regulation of inflammation in several models, evaluation of each of the pluripotent roles for IL-15 will be vital to our ability to modulate it effectively.

Figure 2.1

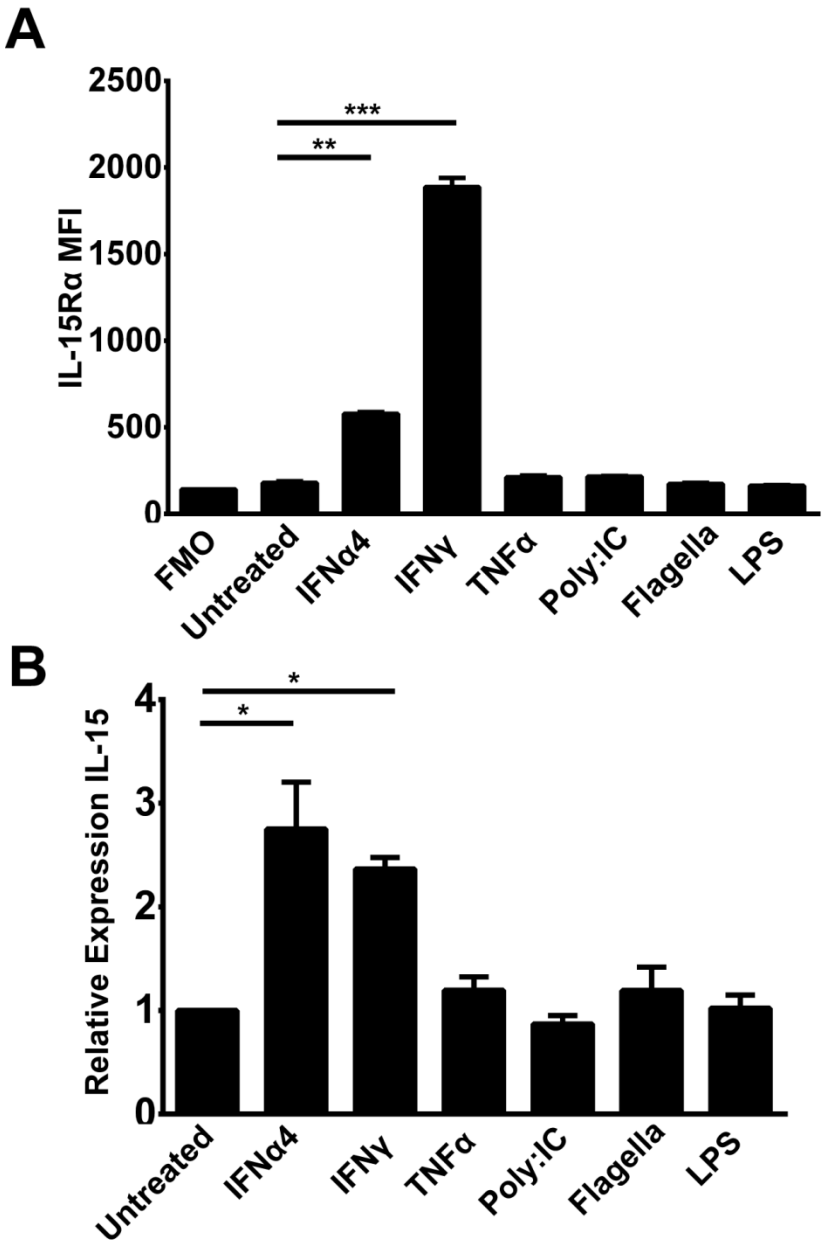

A
IL-15Rα MFI
2500
2000
1500
1000
500
0
***
**
FMO
Untreated
IFNα4
IFNγ
TNFα
Poly:IC
Flagella
LPS
B
Relative Expression IL-15
4
3
2
1
0
*
*
Untreated
IFNα4
IFNγ
TNFα
Poly:IC
Flagella
LPS

Figure 2.1: IL-15 and IL-15Rα are produced by murine lung endothelial cells in response to type I and type II interferons. (A and B) 1G11 murine lung capillary endothelial cells were treated with the indicated cytokines and inflammatory stimuli for 12 hours. A) RT-qPCR detection of IL-15 in stimulated 1G11 cells. Data are expressed as fold change in expression over untreated cells ± SEM. B) IL-15Rα surface expression on stimulated 1G11 cells. Data are expressed as MFI ± SEM (**$p < 0.05$, ***$p < 0.005$), and is representative of two independent experiments (n=3-4 mice/group).

Figure 2.2

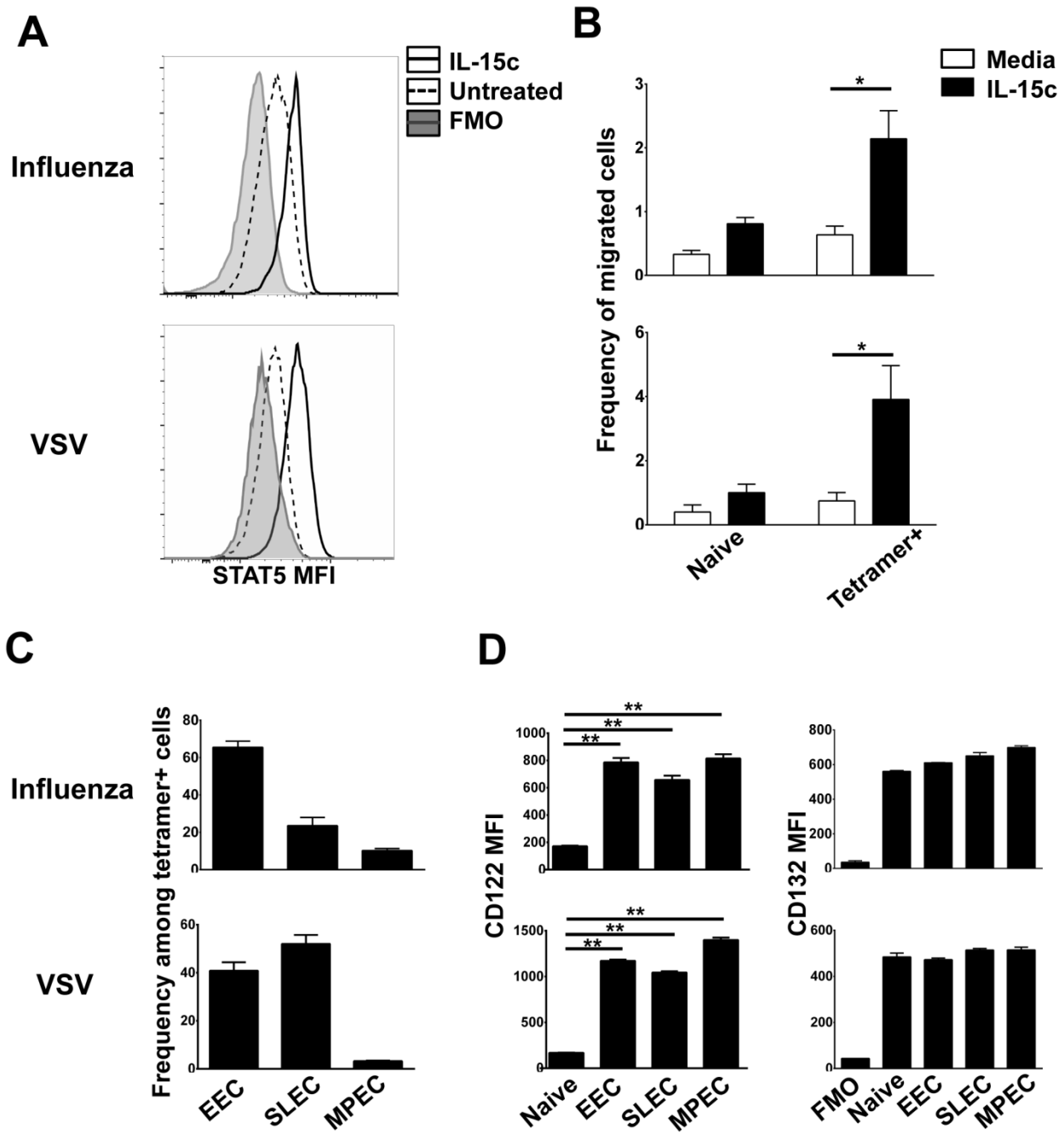

A
Influenza
VSV
IL-15c
Untreated
FMO
STAT5 MFI
B
Media
IL-15c
Frequency of migrated cells
Naive
Tetramer+
C
Influenza
VSV
Frequency among tetramer+ cells
EEC
SLEC
MPEC
D
CD122 MFI
CD132 MFI
Naive
EEC
SLEC
MPEC
FMO

Figure 2.2: CD8 T_{eff} derived from multiple viral infections and divergent stages of differentiation migrate to IL-15 complexes *in vitro*

Pathogen-specific $CD8^{+}CD44^{hi}$ T_{eff} isolated 9 dpi with influenza (top) and 6 dpi with VSV (bottom) were assayed for IL-15 responsiveness after identification with the virus-specific MHCI class tetramer. A) Histograms of pSTAT5 expression in $CD8^{+}CD44^{hi}$ T cells in response to 100ng/mL IL-15c (dark histogram) or media (dashed histogram). An FMO was used as a negative control (shaded histogram). B) Enriched splenic CD8 T cells derived from either influenza (top) or VSV (bottom) were placed in the upper well of a Transwell apparatus with either 100ng/mL IL-15c or media alone in the bottom chamber. After 90 minutes, the number of migrated naïve or tetramer-positive cells recovered from the bottom well was determined using counting beads, and compared to the number of input tetramer-positive cells, giving a frequency of migrated tetramer-positive cells. Average frequency ± SEM (*$p < 0.05$) are displayed. C) Relative frequencies of the various tetramer-specific CD8 T_{eff} pools derived from the indicated infections based on CD127 and KLRG1 expression. The mean ± SEM is displayed. D) CD122 (left) and CD132 (right) expression within each subset of $CD8T_{eff}$ defined based on CD127 and KLRG1 expression. The mean ± SEM is displayed. Naïve CD8 T cells or FMO were used as negative controls for CD122 and CD132, respectively. All data are representative of at least two independent experiments (n=3 to 5 mice/group).

Figure 2.3

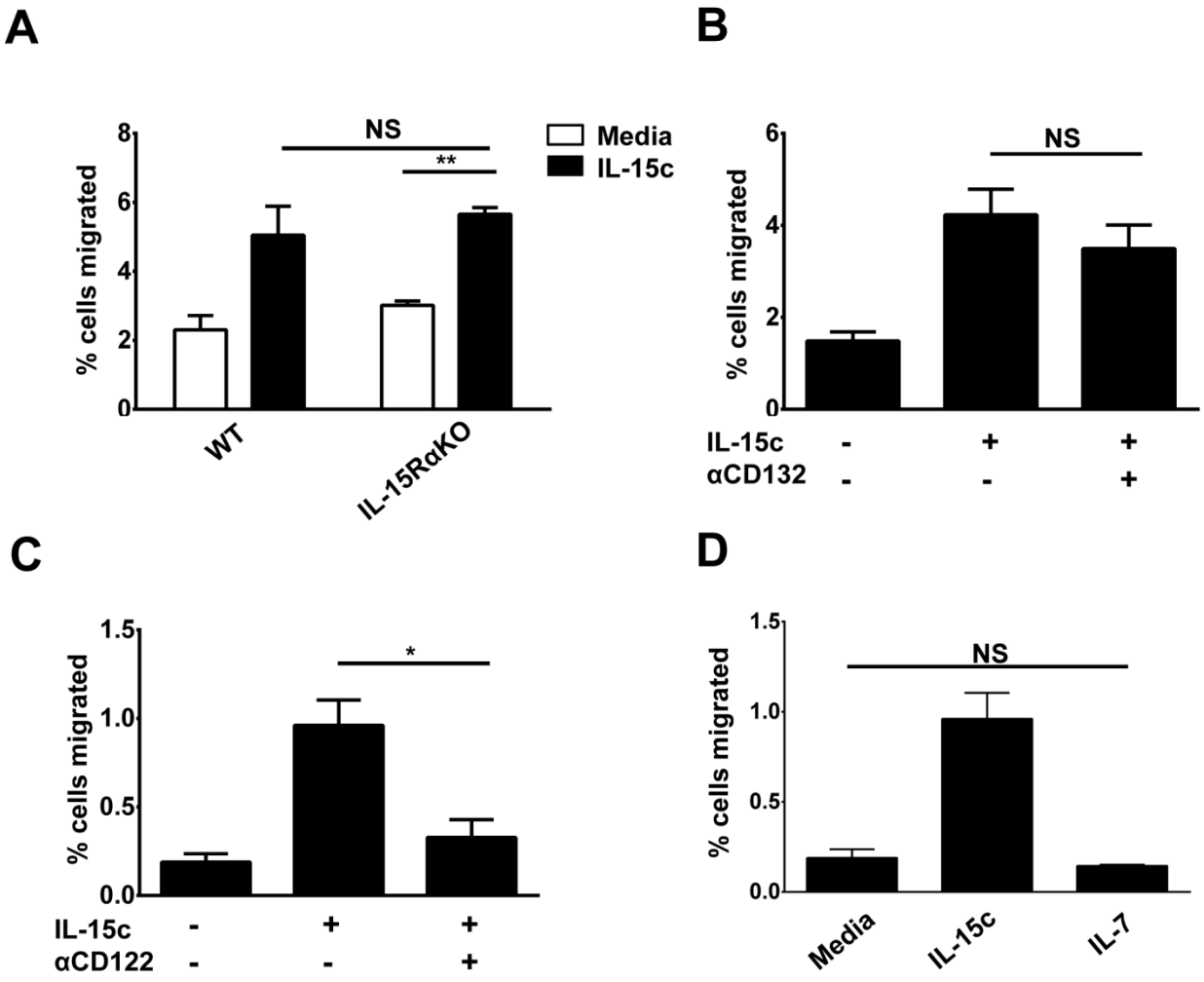

A
B
C
D
NS
**
Media
IL-15c
% cells migrated
WT
IL-15RαKO
IL-15c
αCD132
*
αCD122
Media
IL-15c
IL-7

Figure 2.3: CD8 T_{eff} cell migration to IL-15c is dependent on CD122 but not γ chain or 15Rα signaling. A-D) Enriched splenic CD8 T cells isolated 6 dpi with VSV were allowed to migrate to cytokines in Transwell chemotaxis chambers for 90 minutes. After that time, migrated cells were collected and assayed for CD44 expression and tetramer reactivity. A) CD8 T cells isolated from WT or IL-15Rα-/- infected mice assayed for migration to100ng/mL IL-15c (black bars) or media (white bars). B and C) Cells were pre-incubated with anti-CD132 (B), anti-CD122 (C), or control mAbs prior to migration to 100ng/mL IL-15c. D) CD8 T cells were allowed to migrate to 100ng/mL IL-15c or 100ng/mL IL-7. All data are expressed as an average frequency of migrated tetramer-positive cells relative to input ± SEM (*$p < 0.05$, **$p < 0.005$, NS=not significant) and are representative of at least 2 independent experiments (n=3 to 4 mice/group).

Figure 2.4

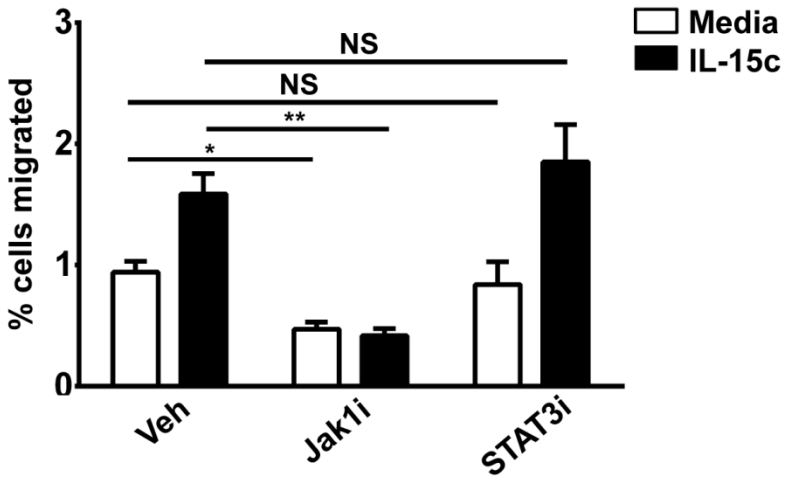


Figure 2.4: CD8 T_{eff} migration to IL-15c is dependent on JAK1, but not STAT3 signaling. Effector CD8 T cells 6 dpi with VSV were treated with vehicle (DMSO), Ruxolitinib (Jak1i) or S3I-201(STAT3i) for 45 minutes prior to 90 minute migration assessment to 100ng/mL IL-15c (black bars) or media (white bars) in Transwell chemotaxis chambers. Inhibitors were also added to the top and bottom chambers of the Transwell during migration to IL-15c. Migrated cells were assayed for CD44 expression and tetramer reactivity. Data are expressed as frequency of migrated cells of total input ± SEM, and is representative of at three independent experiments (n=4 mice/group). (*$p<0.05$, **$p < 0.005$, NS=not significant)

Figure 2.5

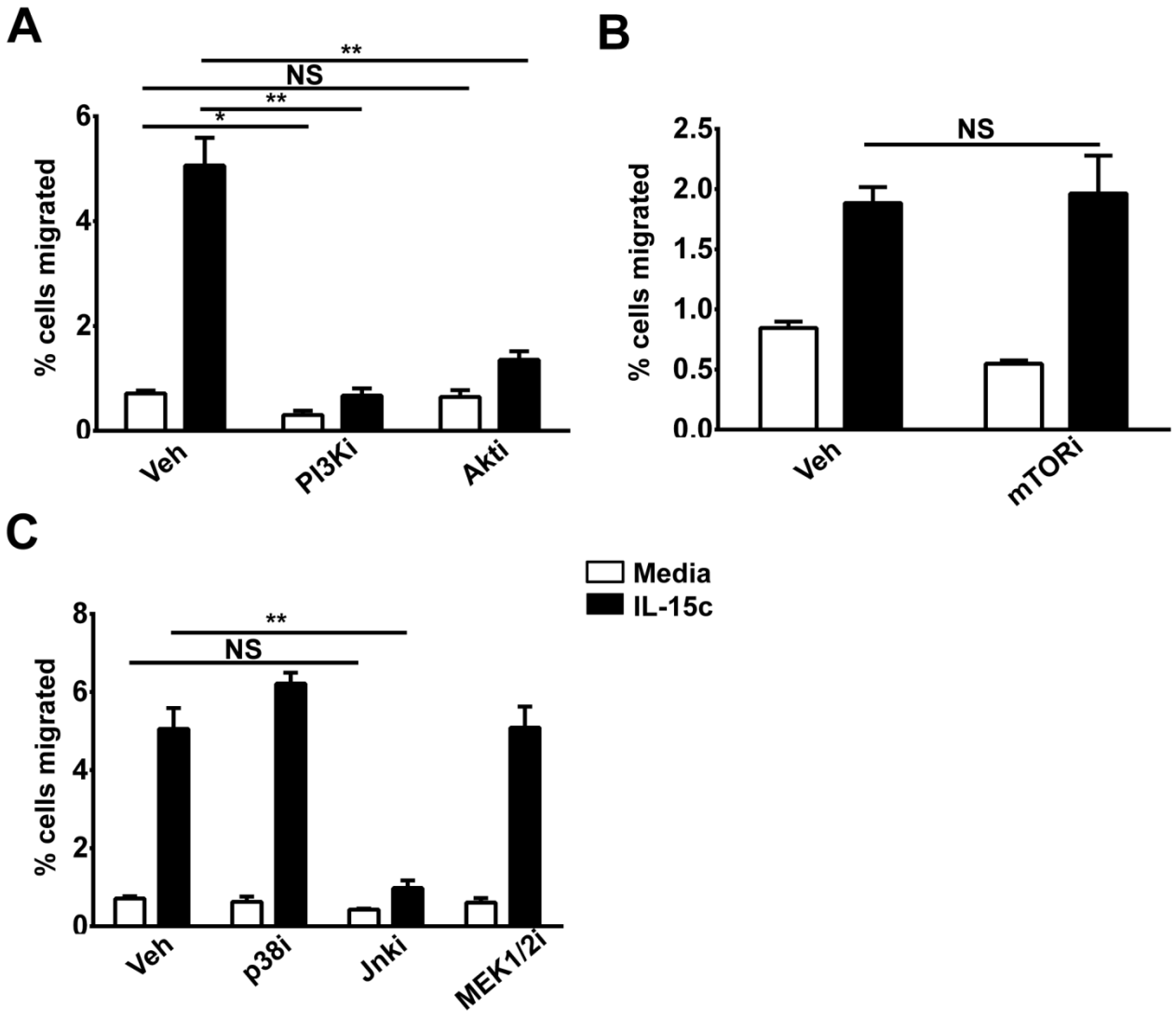

A
**
NS
**
*
% cells migrated
6
4
2
0
Veh
PI3Ki
Akti
B
NS
% cells migrated
2.5
2.0
1.5
1.0
0.5
0.0
Veh
mTORi
C
Media
IL-15c
**
NS
% cells migrated
8
6
4
2
0
Veh
p38i
Jnki
MEK1/2i

Figure 2.5: Akt and Jnk signaling are required for IL-15-mediated migration of CD8 T_{eff}.

A-C) Effector CD8 T cells isolated 6dpi with VSV were treated with vehicle (DMSO) or drug for 45 minutes prior to a 90 minute migration to 100ng/mL IL-15c (black bars) or media (white bars) in Transwell chemotaxis chambers. Drugs were added to the top and bottom chambers of the Transwell during migration to IL-15c. Migrated cells were assayed for CD44 expression and tetramer reactivity. A) CD8 T cells were treated with LY294002 (PI3Ki), Akt inhibitor VIII (Akti) or vehicle prior to and during migration assessment. (B) CD8 T cells were treated with rapamyacin (mTORi) prior to and during migration assessment. C) CD8 T cells were treated with SB202190 (p38i), SP6000125 (Jnki), or U0126 (MEK1/2i) or vehicle prior to and during migration assessment. Data are expressed as frequency of migrated cells of total input ± SEM (*$p<0.05$, **$p < 0.005$, NS=not significant), and is representative of at least two independent experiments (n= 4 mice/group).

Figure 2.6

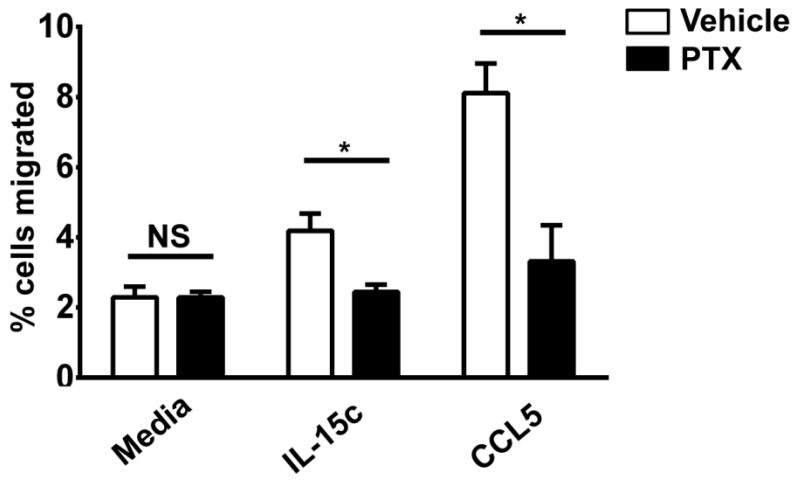


Figure 2.6: IL-15c-driven migration of CD8 T_{eff} cells requires signaling through heterotrimeric G-proteins. Effector CD8 T cells isolated 6 dpi with VSV were pre-incubated with pertussis toxin (black bars) or vehicle (white bars) for 90 minutes prior to migration to 100ng/mL IL-15 for 90 minutes. CCL5 was used as a positive control for pertussis toxin blockade of heterotrimeric G-proteins. Migrated cells were stained for CD44 expression and tetramer reactivity. Data are expressed as frequency of migrated cells of total input ± SEM (*$p < 0.05$, NS=not significant), and is representative of two independent experiments (n= 3 or 4 mice/group).

Figure 2.7

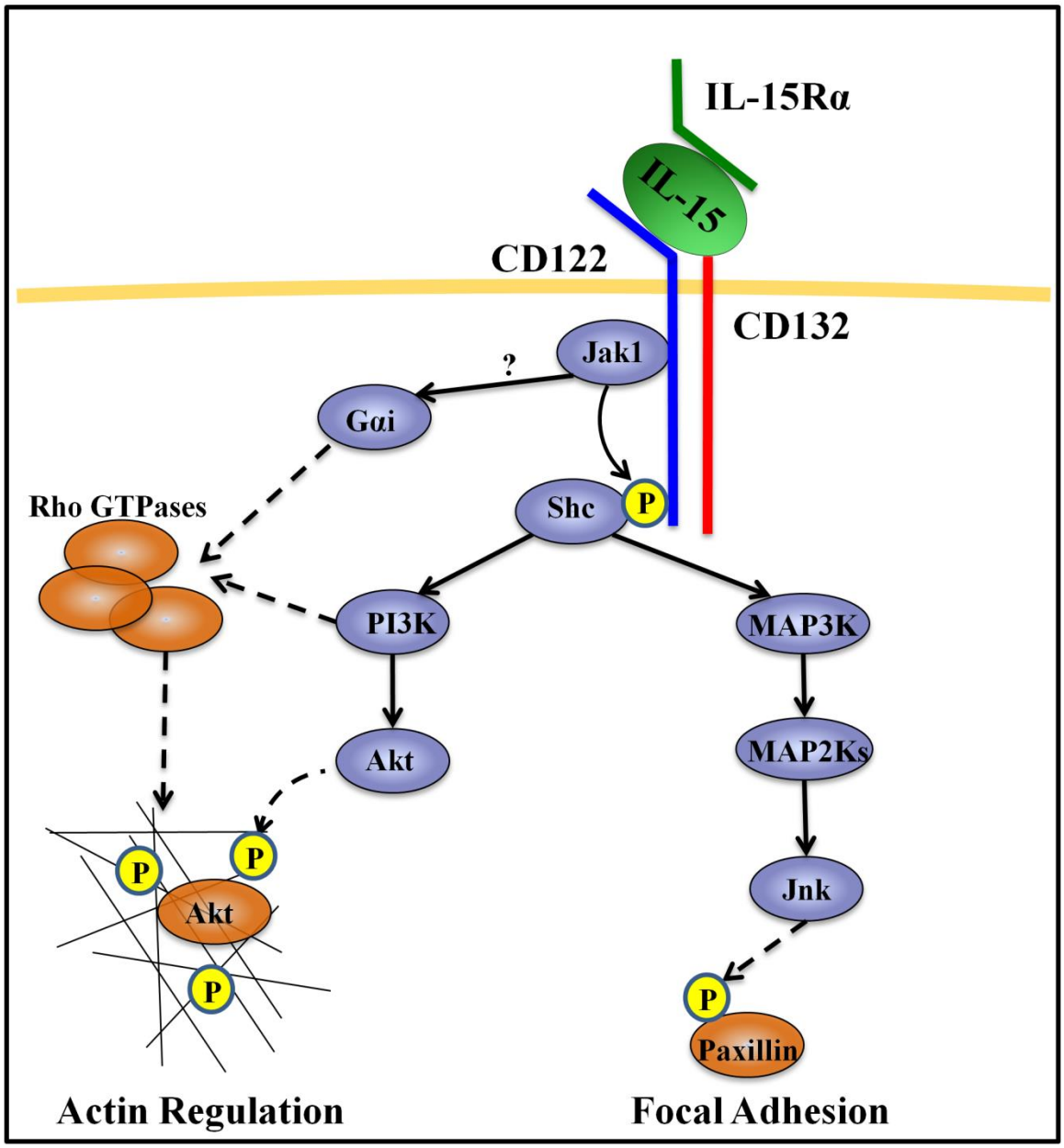
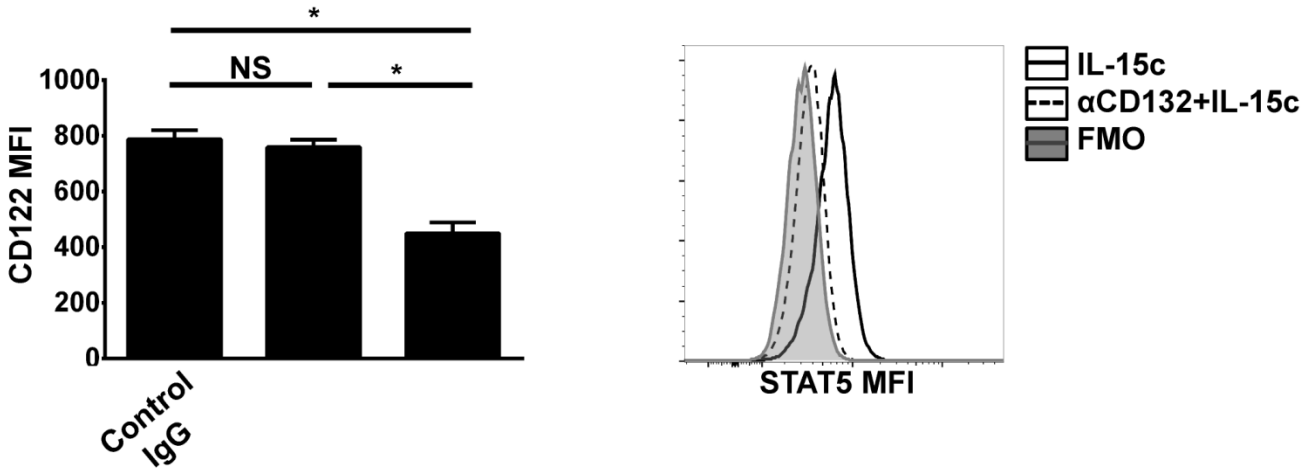

IL-15Rα
IL-15
CD122
CD132
Jak1
?
Gαi
Rho GTPases
Shc
P
PI3K
MAP3K
Akt
MAP2Ks
P
P
Akt
P
Jnk
P
Paxillin
Actin Regulation
Focal Adhesion

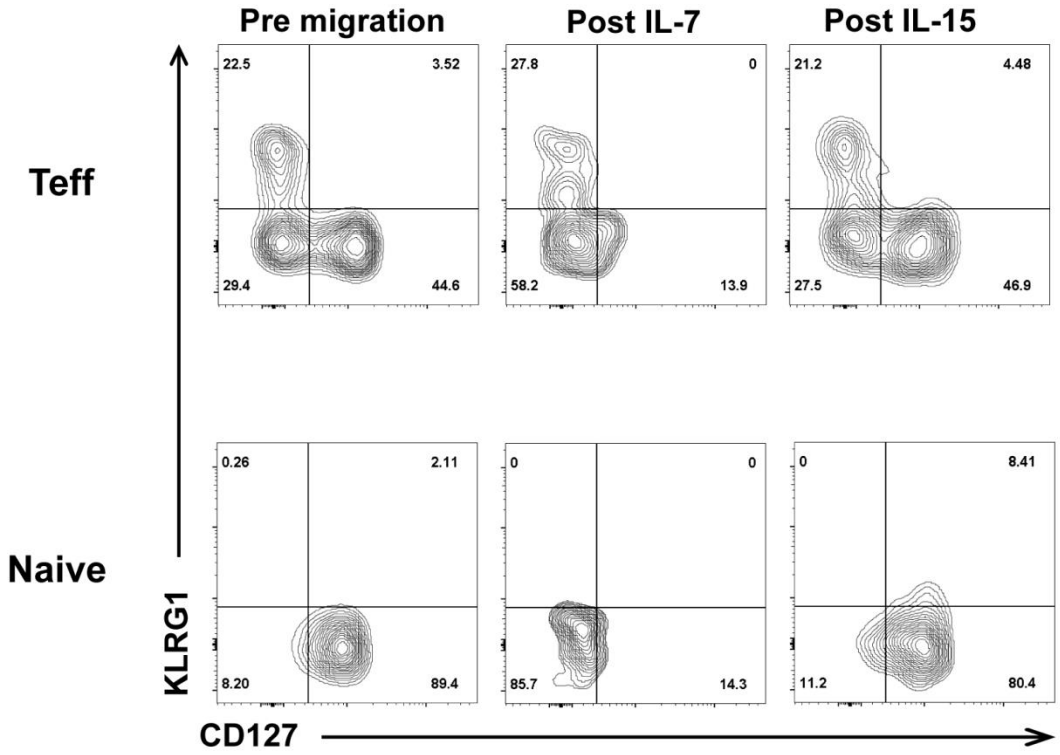
Figure 2.7: Proposed model IL-15 effectors required for mediating T_{eff} migration. IL-15 engagement of CD122 triggers the activation of Jak1. Jak1 phosphorylates tyrosine 338 of CD122, which allows for the recruitment of the adaptor molecule Shc. Shc in turn recruits PI3K and the adaptor Grb2 (not shown), which initiates MAP3K signaling. PI3K activation results in subsequent Akt activation, as well as Rho GTPase regulation. In turn, activated Akt localizes and targets actin for phosphorylation, which influences filament stability and rearrangement. Additionally, Rho GTPases triggered by PI3K help influence cell polarity, as well as formation of the uropod and cell protrusions. MAP3K signaling leads to the eventual activation of the MAPK Jnk. One target of Jnk phosphorylation in T cells Paxillin, which helps regulate focal adhesion and early migration events. Blue circles are components critical for migration to IL-15. Orange circles represent potential candidates necessary migration to IL-15. Dotted lines indicate the transition between established pathways and hypothetical targets.

Supplemental Figure 2.1



Supplemental Figure 2.1: Validation of CD132 blocking on CD8 T cells. Enriched splenic CD8 T cells were harvested 6 dpi with VSV. Cells were blocked with anti-CD132 or control IgG for 20 minutes, followed by assessment of CD122 expression (left) and pSTAT5 activation (right). Loss of expression of CD122 after IL-15 treatment indicates functional CD122 activity after antibody treatment (left). Lack of pSTAT5 in response toIL-15 indicates blockade of CD132 (right). (NS=not significant, *$p < 0.05$). Data are representative of 2 independent experiments (n=3 mice/group)

Supplemental Figure 2.2



Supplemental Figure 2.2: IL-7 responsiveness of CD8 T cell subsets. Enriched splenic CD8 T cells were harvested 6 dpi with VSV. Cells were allowed to migrate towards IL-7 or IL-15c for 90 minutes in Transwell chemotaxis chambers. Post migration, naïve (bottom) and tetramer positive (top) cells were stained for CD127 and compared between input cells (left) and IL-7 (middle) or IL-15c (right) migrated cells. Loss of CD127 expression indicates IL-7 responsiveness. Representative staining is shown from 2 independent experiments (n=3-4 mice/group)

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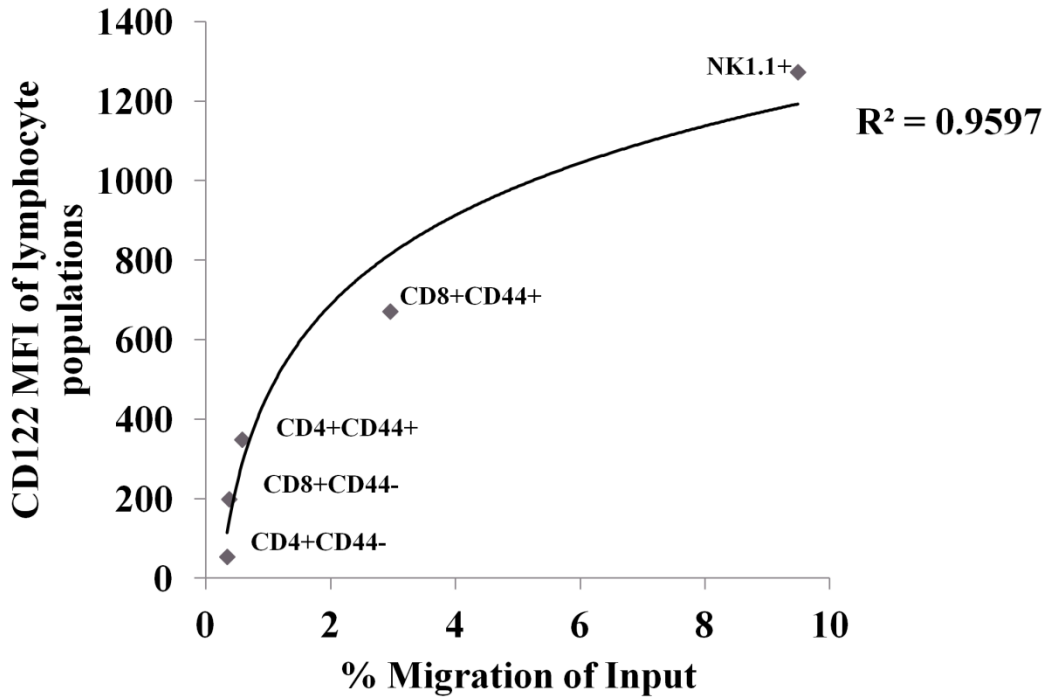
APPENDIX A: Migration to IL-15 is relative to lymphocyte expression of CD122



Migration to IL-15 is relative to lymphocyte expression of CD122. Bulk lymphocytes harvested from spleens of mice infected with VSV 6 dpi were assayed for their ability to migrate to 100ng/mL IL-15c in 5μM pore Transwell chambers. Rate of migration was plotted against CD122 expression of lymphocytes prior to IL-15c exposure. NK1.1+ (NK cells); CD8+CD44+ (antigen experienced CD8 T cells); CD8+CD44- (antigen inexperienced CD8 T cells); CD4+CD44+ (antigen experienced CD4 T cells); CD4+CD44- (antigen inexperienced CD4 T cells).