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TISSUE DEPENDENCE OF IMMUNITY TO *STAPHYLOCOCCUS AUREUS* INFECTION

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ABSTRACT

Staphylococcus aureus causes severe disease with multiple tissue tropisms. Clinical data suggests that chronic and recurrent disease occurs exclusively in skin and soft tissue sites. Yet, immunity to pathogens can be induced in the skin. This implies that induction of protective, adaptive immunity is critically dependent on the tissue site of initial infection. Furthermore, *S. aureus* may utilize tissue-specific mechanisms to suppress the development of protective immunity. How immunity to *S. aureus* is induced is poorly understood due to the lack of tractable, in vivo antigen-specific models to which *S. aureus*-specific adaptive immunity can be analyzed.

Therefore, we generated a novel chicken egg ovalbumin (OVA) secreting *S. aureus* strain to follow OVA-specific $CD4^+$ T cells in recipient murine hosts. Here, we demonstrate that bacteremia induces protection against secondary skin challenge dermonecrosis and control of pathogen load. Bacteremia induced Th1 OT-II subset differentiation, generating memory cells which responded to skin re-challenge. Conversely, skin infection offered no detectable protection against secondary challenge, and failed to induce both canonical T helper cytokines and responsive, long-lasting memory cells among donor OT-IIs. Loss of Hla expression by *S. aureus* resulted in the rescue of OT-II Th1 responses after skin infection and enhanced memory cell induction.

Intradermal *S. aureus* infection resulted in the loss of $CD11b^+$ dermal dendritic cells and Langerhans cells in the skin and reduced both migration and accumulation of DCs to the skin draining lymph node. Infection with LAC *hla::erm* mutant strain restored DC accumulation in both the skin draining lymph nodes and the skin site. Immune sera protected DC loss in naïve animals during *S. aureus* skin infection. Furthermore, active

immunization against inactive mutant of Hla_{H35L} protected DC populations in the skin and draining lymph nodes after *S. aureus* infection. Taken together, these data implicate Hla as a novel suppressor of antigen-specific $CD4^+$ T cell priming and long-lasting memory formation against *S. aureus*. We hypothesize that DC targeting by Hla prevents efficient priming of OT-II memory and cytokine responses. We propose that successful vaccination trials should target pediatric populations, as they likely have functional *S. aureus*-specific CD4 T cells, and immunize against Hla to protect DCs at barrier sites frequently breached by *S. aureus*. Such strategies will convert normally immunosuppressive encounters with *S. aureus* into immune boosting events.

Introduction

Staphylococcus aureus is a commensal, native to the human gut and skin, and a leading cause of severe invasive disease as well as recurrent, skin and soft tissue infections (SSTIs). Though *S. aureus* asymptomatically colonizes >20% of the population worldwide, it causes significant disease upon epithelial barrier breach(H. F. Wertheim et al. 2004). *S. aureus* SSTIs vary from impetigo to life-threatening necrotizing fasciitis, with abscess and cellulitis as the most common lesions(Stryjewski and Chambers 2008; Klevens R et al. 2007). Severe cases of *S. aureus* infection manifest as life-threatening pneumonia and bacteremia, accounting for >40% of culture-positive health care associated pneumonia (HCAP) and 20 per 100,000 person-years, respectively(Tong et al. 2015). Overall, the public health burden of *S. aureus* is severe, affecting more than 11 million people annually, resulting in roughly 500,000 hospitalizations(McCaig et al. 2006). The rise of antibiotic-resistant strains, such as USA300, the most prevalent methicillin-resistant *S. aureus* (MRSA) variant, have further exacerbated the clinical toll(Tong et al. 2015). Approximately 50% of *S. aureus* infections in the United States were caused by MRSA. Furthermore, infection with antibiotic resistant *S. aureus* is associated with increased mortality rate. Thus, the demand for novel preventative and therapeutic strategies to combat *S. aureus* infection is high, and continues to rise each year.

Despite this, all vaccine trials have failed in human populations, and correlates of immunity to *S. aureus* remain elusive(V. G. Fowler and Proctor 2014). The reasons for these failures are unclear, as even trials which induced high titers of antibodies with in

vitro opsonic activity did not protect vaccinated subjects from *S. aureus* infection(V. G. Fowler and Proctor 2014). Intriguingly, one clinical study was halted prematurely, as vaccine recipients who developed *S. aureus* infections were five times more likely to die than control recipients who acquire infection(V. G. Fowler and Proctor 2014). In addition, though high titers of systemic, *S. aureus*-specific antibodies are present in colonized individuals, these antibodies do not appear to protect them from infection(Goldmann and Medina 2017). This suggests that *S. aureus* may have developed means of perturbing immune responses against itself, perhaps as part of its natural co-evolution with human hosts. As *S. aureus* colonizes barrier tissue sites, one would hypothesize that any mechanisms designed for immune evasion would be tailored to its natural colonization sites. While some evidence suggests that a variety of virulence factors may thwart host immunity, whether *S. aureus* utilizes differential mechanisms that are tissue dependent remains unclear.

Epidemiology of *S. aureus* infection: Tissue Specificity

S. aureus causes disease in multitude of tissues, but the mechanisms by which *S. aureus* modulates immunity is poorly understood. Clinical evidence suggests that recurrence rates of infection with *S. aureus* may depend on the initial route of infect. Curiously, patients who have experienced at least one *S. aureus* SSTI frequently experience recurrent *S. aureus* infections compared to previously uninfected household contacts, with recurrence rates exceeding 50% in some populations(Tong et al. 2015; L. G. Miller et al. 2015). Larger scale studies supported these findings, such that otherwise healthy patients with *S. aureus* infections had higher rates of acquiring another soft

tissue infection cause by *S. aureus* compared to patients who did not report *S. aureus* infections, with an odds ratio of 2.5(Bouvet et al. 2017). Taken together, the clinical data indicate that despite similar environmental exposures, a patient's susceptibility to infection may depend on whether they were previously infected.

However, some studies have provided evidence that environmental exposure and patient colonization with *S. aureus* are predictive of future infections. For example, household contact infection appeared to correlate with *S. aureus* infection in other household members, suggesting that colonized individuals are also important reservoirs for transmission(Fritz et al. 2009; Ellis et al. 2004; Kluytmans, van Belkum, and Verbrugh 1997; von Eiff et al. 2001; Toshkova et al. 2001; H. F. L. Wertheim et al. 2005). In addition, previous colonization with *S. aureus* seems to increase the risk of both SSTIs in otherwise normal, healthy adults, and nosocomial invasive infections following surgical procedures. Several studies demonstrate a 2 to 10 fold increased risk of developing a *S. aureus* surgical site infection, with the majority of infections resulting from the colonizing strain(von Eiff et al. 2001; Butterly, Schmidt, and Wiener-Kronish 2010; Davis et al. 2004; Kao et al. 2015; Gorwitz et al. 2008; Safdar and Bradley 2008). Furthermore, MRSA colonization resulted in 10 times more nosocomial infections when compared to the number of individuals with MSSA colonization who acquired infection(Butterly, Schmidt, and Wiener-Kronish 2010). Thus, barrier tissue exposure, whether through colonization or SSTI, appears to correlate with dramatically increased risks or infection. While the probability of acquiring an initial infection with *S. aureus* may depend on the colonization status of the patient, the acquisition of an infection appears to beget further infections.

Studies which have focused on the recurrence rate of infection in patients who recovered from invasive disease have been either retrospective in design or small in sample size. Such studies analyzed the relapse of bacteremia within a short period of time after recovery, and recurrence was found to be linked to endocarditis, distal septic complications, antibiotic type and frequent vascular manipulation or frequent epidermal breaks for medical procedures(Chambers, Miller, and Newman 1988; Korzeniowski and Sande 1982; Chang et al. 2003; Small and Chambers 1990). Therefore, clinical evidence which specifically addresses whether recurrence of *S. aureus* infection may depend on the type of initial *S. aureus* infection are few. Interestingly, one study demonstrated that patients with invasive infections displayed higher convalescent titers against Hla and Panton-Valentine Leukocidin (PVL) than patients who recovered form cutaneous infection(Fritz et al. 2013). Subsequent infection risk was comparable between primary and recurrent cutaneous infection groups. Cumulative recurrent infection over a 12 month follow up demonstrated that while recurrences rates of infection was >60% in primary and recurrent SSTI cohorts, colonization only and invasive infection groups were approximately 20%(Fritz et al. 2013). This suggests that while cutaneous infection is a risk factor for further infection, this is not true for patients which recovered from invasive disease. Thus, the development of protective immunity to *S. aureus* may depend on the tissue site of initial exposure. For this to be possible, *S. aureus* would require currently unknown, tissue-specific mechanisms by which it could perturb immunity. Such investigation has been hindered by the absence of in vivo methods to track antigen-specific responses to *S. aureus*.

Tissue specific immunity

The skin is a vital physical barrier against pathogen invasion, and is colonized by the normal microflora. Microbial species segregate anatomically based on a variety of determinants, such as temperature, pH, moisture content, sebum, salt and fatty acids. At homeostasis, commensals are thought to live symbiotically with the host, and act as inhibitors to colonization by pathogenic microbes(Proksch, Brandner, and Jensen 2008). This generally accepted view of host-commensal interaction is complicated by the observation that *S. aureus* acts as both a native commensal of the skin and pathogen. The change from commensal to pathogenic is likely dictated by both changes to the barrier environment and the state of the host's immune system at the time of infection(Safdar and Bradley 2008; Proksch, Brandner, and Jensen 2008; L. S. Miller 2008).

The first line of skin immunity is the plethora of microbial communities that reside on the surface of the barrier tissue. These communities act as a living, first response barrier to new environmental factors and pathogenic would-be colonizers. Several studies have addressed how commensals within these community control these pathogens. For example, *Staphylococcus epidermidis* inhibits *S. aureus* colonization through the secretion of serine protease Esp, which blocks the formation of biofilms by the invader(Iwase et al. 2010). Furthermore, *S. epidermidis* orchestrates the expression of antimicrobial peptides by human keratinocytes, resulting in the inhibition of *S. aureus* growth on the surface of the skin(Wanke et al. 2011). The ability of commensal *S. epidermidis* and pathogenic *S. aureus* to induce expression of antimicrobial peptides (AMPs) and activate NF-κB differed, and appeared to oppose each other, suggesting

that commensals utilized unique pathways to alert the innate immune system of the presence of pathogenic colonizers.

The foundation on which the microbial barrier resides is the epidermis of the skin: the top most layer is the stratum corneum. It is composed of a network of cross-linked cells (corneocytes) embedded in an intercellular matrix enriched in lipids, which create the layered brick-like structure of the epidermis(Wanke et al. 2011; Sanford and Gallo 2013a). This network is consistently maintained by the continuous differentiation of nucleated keratinocytes into the flat, anucleated corneocytes of the stratum corneum of the epidermis. To maintain the integrity of the barrier, a system of tight junctions anchor these cells together, making a water proof, impenetrable barrier sufficient to separate deep tissue from the outside environment(Eyerich et al. 2018). The highly organized layer serves as the primary barrier against mechanical, microbial and chemical assaults to the host. Residing with this structure is the “chemical barrier” of the skin, comprising the factors which constitute and maintain the acidic surface pH and the components which naturally moisturize the skin(Eyerich et al. 2018). Critical to this chemical balance is the production of secretions from eccrine, apocrine and sebaceous glands which sit in invaginations along the skin surface. Eccrine sweat glands, which are distributed across the entire skin surface are key in thermoregulation of the host body, as well as salt and electrolytes which work to acidify the skin. In combination with constitutive expression of AMPs, eccrine glands create an environment which limits the composition of microbes that can establish, survive and proliferate on the skin surface(Murakami et al. 2002; S. Rieg et al. 2004; Siegbert Rieg et al. 2006). Sebaceous glands secrete sebum, a lipid-rich substance which works to lubricate the hair and skin. Seeded in the hair follicles,

these glands facilitate distinct microbial communities which are capable of thriving in near anaerobic, lipid-rich environments. The breakdown of these lipids into free fatty acids, too, contribute to the control of microbial colonization, along with unique, sebocyte-derived AMPs, such as cathelicidins, β-defensins, and antimicrobial histones(Nagy et al. 2006; Nakatsuji et al. 2010; D.-Y. Lee et al. 2009). One of the most populous bacteria in the hair follicles is *Propionibacterium acnes*. Specifically, *P. acnes* lipopolysaccharide (LPS) types IA and IB have been found to induce unique, and differential, pro-inflamatory cytokines and chemokines, as well as AMPs(Nagy et al. 2006). Thus, the microbial community that colonizes the skin is uniquely tailored based on the biochemical restrictions imposed by the chemical environment. Moreover, microbes which colonize the skin can then utilize innate host signaling to further alter the environment, potentially increasing the barrier to colonization against foreign pathogens.

Keratinocytes warrant special attention, as they produce and deliver cholesterols and triglycerides to the surface(Eyerich et al. 2018). In coordination with sebaceous glands, which secrete triglycerides, esters and sebum towards the upper hair follicles and onto the skin, the keratinocytes contribute to the acidification of the skin. However, this acidification process is dependent on the bacterial microflora and yeast communities on the skin, which hydrolyze these triglycerides into free fatty acids. The coordinated acidification of the skin illustrates the symbiotic effort of both the host and commensal microbiota to prevent pathogen colonization. Beyond this, keratinocytes are also known to contribute directly towards the elimination of pathogens, and are thought to directly modulate adaptive immunity in the skin. To sense their invaders,

keratinocytes express different pattern recognition receptors (PRRs) such as the Toll-like receptors 2 and 4, the non-classical MHC CD1d and a keratinocyte mannose-binding receptor (KcMR)(Pivarcsi, Kemény, and Dobozy 2004). Thus, keratinocytes are capable of detecting conserved molecular entities expressed by the microflora, such as nucleic acids, cell wall components, flagella and lipoproteins(Sanford and Gallo 2013b). In addition, a multitude of effector cytokines, chemokines and costimulatory molecules have been found to upregulate depending on the nature of an environmental insult. Ultraviolet radiation and physical trauma has been shown to induce several immune resposnes in keratinocytes, from NF-κB upregulation and the induction of IL-1, IL-6, TNFα and vascular endothelial growth factor, to the phosphorylation of epidermal growth factor receptor (EGF-R), activation of c-Jun kinase and autophosphorylation of IL-1 receptor associated kinases(Pfundt et al. 2001; Wan et al. 2001; Abeyama et al. 2000). In the context of microbial interactions, various infection models have demonstrated that keratinocytes can induce an array of factors which dictate the host immune response upon infection. During HPV infection, IL-8, RANTES/CCL5, and macrophage inflammatory protein MIP1α, and IFNγ-inducible protein expression were observed, suggesting that keratinocytes may orchestrate the initial innate immune responses through the recruitment of neutrophils and macrophages(Steinhoff, Brzoska, and Luger 2001). In the case of *Schistosoma mansoni* parasitic infection, prostaglandin E2 and IL-10 production appears to downregulate host immune responses, demonstrating a means by which invaders can take advantage of the immune modulatory function of keratinocytes(Ramaswamy, Kumar, and He 2000).

Connected to a vast network of host immune cells, keratinocytes also coordinate both the innate and adaptive immune responses. In mice, there is a resident population of dendritic epidermal T cells (DETCs) that express an invariant Vγ3Vδ1 T cell receptor and are tightly anchored to the keratinocytes meshwork of the skin(Nielsen, Witherden, and Havran 2017). Producers of IL-17, these cells are thought to recruit neutrophils upon barrier injury, as damaged or stressed keratinocytes express TCR ligands that can activate these DETCs through non-MHC-mediated mechanisms(Nielsen, Witherden, and Havran 2017). Cross talk between keratinocytes and adaptive immunity was also demonstrated to be critical in preventing inflammatory disease in a IκBα dependent manner(Rebholz et al. 2007). Furthermore, keratinocytes act as one of the initial innate immune sensors of microbial invasion, as antigens presented by keratinocytes through major histocompatibility complex I (MHCI) can cross prime CD8 T cell responses in a CD4 T cell dependent manner(Gudjonsson et al. 2004). Taken together, keratinocytes contribute not only to the generation of both the physical and chemical barriers on the skin, but also modulate host adaptive immune responses to maintain tissue homeostasis.

Beneath the cutaneous layers of the skin, subsets of skin-specific dendritic cells act as the main instructors and sentinels to the adaptive immune system. Currently, there are 3 general DC subsets in the healthy mouse skin, all of which express CD11c and MHC class II; epidermal Langerhans cells (LCs), $CD11b^{+}$ dermal dendritic cells (DDCs) and $CD207^{+}$(Langerin) DDCs(C. C. Chu, Di, and Nestle 2011). Improvements in phenotypic analysis of these subsets have unveiled that the $CD207^{+}$DDCs can be further subdivided into $CD103^{high}$ and $CD103^{low}$ subsets, and that a fifth, smaller

population of dermal Langerin^{-}XCR1^{-}CD11b^{low}Sirpα^{high}CX$_3$CR1^{high} cells exists. The human and murine skin DC network appears to be highly conserved, as both species harbor analogous populations(Clausen and Stoitzner 2015). Specifically, epidermal Langerhans cells are shared between both species, while in the dermis three subsets of DCs can be identified(Clausen and Stoitzner 2015). As in mice, the largest DC population in the dermis is represented by the CD1c^{+}CD1a^{+} dermal DC, which corresponds to the murine CD11b^{+} dermal DC: it composes over 65% of the dermal DC population(Henri et al. 2010). The smallest subset of DCs in the human dermis highly express CD141 and XCR1, and have been characterized as homologous to murine Langerin^{+}CD103^{+} dermal DCs, which also express XCR1(Haniffa et al. 2012; C.-C. Chu et al. 2012). Beyond these homologous subsets, the remaining DCs in murine skin have yet to be matched with their human counterpart. Recent evidence has identified a small subset of DDCs which weakly express Langerin and CD1c, which emphasize that Langerin expression may not be restricted to Langerhans cell alone in either species(Bigley et al. 2015). Taken together, DC subsets between humans and mice appear homologous, which indicate that *in vivo* studies addressing DC function during skin infection may be highly translatable to human disease.

Langerhans cells are derived at birth from the primitive yolk sac and the fetal liver(Collin and Milne 2016). Historically, LCs were viewed as constitutive immune-activating cells due to their ability to efficiently present antigen. However, recent evidence promotes the idea that LCs also play a key role in inducing tolerance to both self-antigens and commensal microbes through the induction of regulatory T cells during homeostasis(Seneschal et al. 2012; Igyártó and Kaplan 2013; Romani, Brunner,

and Stingl 2012a). Currently, LCs are thought to have dual functions which are context dependent. Due to their ability to efficiently present self-antigen, LCs have been recently determined to induce T regulatory populations after ionizing radiation treatment on the skin, and negatively regulate inflammatory responses against pathogens, presumably as a check on self-inflicted damage(Kautz-Neu et al. 2011; Price et al. 2015a). Recently, it was demonstrated that intradermal immunization with chicken egg ovalbumin (OVA)-coupled anti-Langerin antibodies (Langerin/OVA) induced CD8 T cell memory and cytotoxic responses when co-injected with Poly(I:C) and anti-CD40 agonist antibody. However, exclusive targeting Langerin/OVA to LCs failed to prime cytotoxicity, despite initial antigen cross-presentation to CD8 T cells in the presence of strong adjuvants, and deletion of OT-IIs, CD4 T cells specific to an OVA epitope presented on MHC class II, was observed(Flacher et al. 2014). This suggests that CD8 peripheral tolerance could be orchestrated by LCs in an antigen-specific manner. Conversely, LCs have been shown to necessary and sufficient for the generation of antigen-specific T helper-17 (Th17) subset differentiation *in vivo* using an OT-II system, but not for the generation of cytotoxic lymphocytes (CTLs)(Igyártó et al. 2011a). Taken together, Langerhans cells are dual purposed to initiate antigen-specific tolerance of Th17 immunity in a context specific manner which is dependent on the microbe and/or type of trauma induced at the skin site.

In coordination with Langerhans cells, Langerin^{+} dDCs modulate immunity at the skin site, and prime T cell immunity in the skin draining lymph nodes. Langerin^{+} dDCs are classically identified based on their expression of Langerin and CD103, and the absence of CD11b, CD8 and EpCam surface markers(Kaplan 2010). As

aforementioned, a recent population of Langerin^{+} CD103^{-} dDCs has been identified in the dermis, which is functionally and phenotypically distinct from LCs, but little is known about the activity of this population. A substantial body of literature has supported the idea that Langerin^{+} dDCs, but not LCs, cross present antigen to CTL to induce cytotoxic responses(Igyártó et al. 2011a; Kaplan 2010; Idoyaga et al. 2013; Bedoui et al. 2009; Bobr et al. 2010). An elegant demonstration of this utilized OT-I (CD8 T cells specific to OVA) and OT-II systems, which recognize chicken egg ovalbumin sequences specific for MHCI and MHCII epitopes. They demonstrated that Langerin^{+} dDCs are required for not only the generation of antigen-specific Th1 cells, but also induce CTL cytotoxic activity through cross presentation of antigen in *S. aureus* and *Candida albicans* models of infection(Igyártó et al. 2011a). Furthermore, Langerin^{+} dDCs were found to inhibit the development of antigen-specific Th17 cell responses, moderately counteracting the action of LCs. Thus, Langerin^{+} dDCs not only are required for antigen-specific induction of Th1 and CTL responses, but also counteract the induction of Th17 responses by LCs during skin infection.

Similarly to Langerin^{+} dDCs, CD11b^{+} Langerin^{-} dDCs mediate the priming of Th1 immunity in the context of parasitic *Leishmania major* infection. Originally, Langerin^{+} dDCs and LCs were thought to be critical for the induction of protective immunity in this disease model, but this view was challenged when it was discovered that Langerin^{-} CD8α^{-} DCs acted as the principal antigen-presenting cells(Ritter et al. 2004). It was known that Th1 and CD8 CTL responses were critical to both resistance against *L. major* infection and the skin healing process(Kautz-Neu et al. 2010, 2012). However, the use of Langerin-DTR (diphtheria toxin receptor) mice demonstrated that while CD8

T cell responses were significantly reduced during L. major infection, priming of $CD4^+$ T cells to Th1 subtype remained intact(Brewig et al. 2009). Thus, $CD11b^+$ Langerin- dDCs appear to dictate $CD4^+$ T cell Th1 priming in the context of parasitic infections.

Skin-resident dendritic cells migrate from the skin site of infection to the draining lymph nodes, and prime the adaptive immune system. Depending on the inflammatory environment, the aforementioned DCs can induce tolerance or immunity. The unique instructions provided by DCs can determine whether the development of protective antibody or T cell memory responses are generated(Pasparakis, Haase, and Nestle 2014).

Efficient generation of T effector cells and T follicular helper (Tfh) cells dictate the development of high affinity antibody production by B cells, which is key to neutralizing toxins, opsonization of bacterial wall targets for phagocytosis by innate immune cells, and the activation of complement pathways(Charles A Janeway et al. 2001; Crotty 2014; Ma et al. 2012). In particular, Tfh cell express unique combination of effector molecules that dictate their control of adaptive immunity and antibody responses, such as IL-21, ICOS, and CD40 ligand (CD40L)(Ma et al. 2012). These molecules are critical for controlling the differentiation, activation, proliferation and survival of B cells during a germinal center reaction post infection. For example, CD40L prevents apoptosis in B cells and promotes their proliferation, while IL-21 is crucial for differentiation of CD40L-stimulated B cells and inducing Ig isotype class switching and affinity maturation(Crotty 2014; Ma et al. 2012). In addition to Tfh, naïve T cells can be programmed into T helper 1, 2 17 (Th1, Th2, Th17) and regulatory subsets (Treg), each with unique functions against specific classes of microbial pathogens. Th1 cells secrete IFNγ, which activate

macrophages, induce MHC I expression and direct defenses against intracellular pathogens. Th2 cells secrete IL-4, IL-5 and IL-13, are key to the activation of eosinophils and dictate the control of extracellular parasites. Furthermore, Th2 cells are indicated in allergy. Th17 cells secrete IL-17 and IL-22, which activate and recruit neutrophils to the site of infection, stimulate the expression of antimicrobial peptides at barrier tissues, promote epithelial proliferation and primarily dictate control of fungal and extracellular bacterial infections. As a check on active immunity, Tregs regulate T cell priming and function by dampening the activation of T cells during an immune response to prevent self-tissue damage. Furthermore, Tregs are critical to peripheral tolerance to self-antigen, as newly derived CD4 and CD8 T cells that escape negative selection in the thymus, or central tolerance, may be reactive to self-antigen. To suppress self-reactivity and autoimmune disease, Treg cell utilize multiple mechanisms: suppression by inhibitory cytokines, cytolysis, inhibition through metabolic disruption and direct modulation of DC maturation and function(Vignali, Collison, and Workman 2008). Of the suppressive cytokines, IL-10, TGFβ and IL-35 are crucial mediators of Treg function, though the settings in which they are utilized differs, suggesting that they are non-overlapping in their function(Vignali, Collison, and Workman 2008). Taken together, naïve T cells can be differentiated into multiple subsets with unique functions in different context. These T cells then instruct B cell memory formation and high affinity, neutralizing antibody production which is essential for protective immunity to pathogenic re-challenge.

S. aureus establishes infection both systemically and at mucosal barrier sites. In contrast to the skin, the systemic immune system is a fluid entity which is linked to

mucosal sites through the bloodstream. Against blood-borne pathogens and foreign antigens, the spleen is the main filter and behaves as a secondary lymphoid organ for the generation of protective immune responses. The organ receives blood through the afferent arterial blood which ends in the marginal zone. This zone monitors the blood and contains a host of tissue-resident phagocytes, as well as divides the red pulp of the spleen from the white pulp. Generally, the red pulp also contributes towards filtering the blood while the white pulp generates active immunity in the case of a blood-borne infection(Bronte and Pittet 2013). Hosting all major types of mononuclear phagocytes, these cells identify pathogens and cellular stress while also removing dying cells and foreign material(Bronte and Pittet 2013). Circulating T and B cells, along with resident lymphocytes, compose the adaptive immune elements of the spleen. As the systemic circulation is thought to be sterile, live microbial entry in the spleen is thought to generate protective immunity. Circulating lymphocytes scan the splenic microenvironment for antigen presented by splenic APCs, and their trafficking is guided by stromal cell networks, integrins, and chemokines, among other factors(Bronte and Pittet 2013). Furthermore, various infection models have pointed to the spleen as prime site for the development of protective immunity during tissue and systemic infection. For instance, recent data has demonstrated that Th1 and Th17 cells can be induced at both the intestine and spleen following Salmonella infection, and the cells induced at each site were specific to different subsets of antigens(S.-J. Lee et al. 2012). During bacterial infection, unique lymphocyte populations can also be recruited which modulate the immune response. For example, unique populations of B cells are recruited to the spleen during bacterial invasion, which accumulate in the red pulp and are the primary

producers of granulocyte-macrophage-colony-stimulating factor (GM-CSF). This population appears to be critical for bacterial control, as mice which lack GM-CSF in these specific populations of B cells undergo an IL-6, TNF-α and lL-1β cytokine storm, which appears to impair bacterial control and results in higher mortality(Rauch et al. 2012).

The spleen is also a prime site in which peripheral tolerance is mediated. Splenic macrophages are thought to be major contributors to the induction of tolerance. Characterized as unresponsiveness to antigen, tolerance can be induced through high, innocuous antigen exposure or mucosal contact with antigen, as in oral tolerance(Bronte and Pittet 2013). Both splenectomized and F4/80-deficient mice, which are devoid of macrophages, do not develop tolerance to innocuous injection in the eye, another immune privileged site in which tolerance is induced(H.-H. Lin et al. 2005). It is thought that once intraocular macrophages acquire antigen, they traffic to the marginal zone of the spleen, and present antigen while establishing an immunosuppressive environment through TGF-β and IL-10 release("Expression of Thrombospondin in TGFβ-Treated APCs and Its Relevance to Their Immune Deviation-Promoting Properties | The Journal of Immunology" n.d.). These events result in the induction of CD4 Tregs and tolerogenic CD8 T cells, which are thought to maintain long-lasting tolerance against the presented antigens. Collectively, systemic and mucosal immunity are overlapping systems which facilitate the identification and clearance of invading pathogens. Though these two systems are differentially regulated, they both function to protect the host against pathogenic encounters and develop long-lasting immunity to foreign invaders.

Immunity to *S. aureus*: A clinical quandary

The correlates of immunity to *S. aureus* remain elusive. While it is thought that protective immunity to pathogen re-encounter depends on the secretion of antibodies that both neutralize toxins and opsonize pathogens, the role of antibodies in *S. aureus* immunity is unclear. For example, X-linked agammaglobunlinemia (XLA), or patients who lack mature B cells, are typically susceptible to infections from a variety of bacteria and viruses. This susceptibility is normally reversed through administration of donor antibodies. Interestingly, XLA patients do not display increased susceptibility to infection by *S. aureus*(Smith and Berglöf 1993; Bruton 1952). Furthermore, clinical reports have conflicted on whether susceptibility to staphylococcal infection correlates with preexisting antibodies towards *S. aureus* virulence factors. Several studies observed that serum antibodies against a multitude of exotoxins found that low antibody titers correlated with increased risk for the development of sepsis(Stentzel et al. 2015; Adhikari et al. 2012). In support, a recent study demonstrated that elevated serum titers against *S. aureus* α-toxin (Hla) correlated with protection against subsequent infection over a 12 month follow up, and invasive infection resulted in higher antibody titers compared to the convalescent titers of patients who reported skin infections(Fritz et al. 2013). Curiously, colonized patients without a history of previous *S. aureus* infection, or carriers, appeared to have high titers of anti-staphylococcal antibodies. This observation complements previous findings, which showed that carriers had improved recovery from invasive infection despite an increased risk of developing infection compared to non-carriers(von Eiff et al. 2001; H. F. L. Wertheim et al. 2005). However, antibodies against

S. aureus through harmless or minor infection exposure seems to do little to perturb skin and soft tissue disease, as up to a third of patients with high antibody titers suffer from recurrent infections(H. F. Wertheim et al. 2004; Nguyen, Mascola, and Brancoft 2005; L. G. Miller et al. 2007). This seemingly contradictory observation suggests that while antibodies may abrogate severity of disease, prevention strategies may require input from other aspects of the adaptive immune system. Alternatively, the quality of antibody responses to *S. aureus* virulence factors may play an unappreciated role in infection control, as antibody isotype is crucial in dictating immune responses in other infection models; this is currently understudied in the field(Bournazos et al. 2016, 2014).

Despite evidence that antibodies can modulate the severity of disease, attempts to vaccinate against *S. aureus* have been unsuccessful in preventing infection. Most vaccine candidates against *S. aureus* to date were selected and assessed based on their ability to generate opsonizing and neutralizing antibodies in vitro, after confirmatory testing in vivo(Pozzi et al. 2012; A. Fattom et al. 1990; Ohlsen and Lorenz 2010). Yet, despite confirmed antibody titers against their proposed targets, all vaccine trials have failed to prevent infection.

Various vaccines have entered phase III clinical trials with no success. The first of these conjugate vaccinates against *S. aureus* was StaphVax, which targeted the capsular polysaccharide (CP) serotypes 5 and 8. This was not a unique approach to *S. aureus*, as the same strategy had been successful in the prevention of *Streptococcus pneumonia* and *Haemophilus influenzae* infection(Pozzi et al. 2012; A. I. Fattom et al. 1996; Shinefield et al. 2002). While mouse models demonstrated that CP-specific antibodies protected mice from lethal *S. aureus* challenge, and phase III clinical trials in

hemodialysis patients suggested a modest reduction of bactermia cases early after vaccination, subsequent boosters failed to prevent bacteremia despite increased antibody titers against CP5 and 8(A. I. Fattom et al. 1996; Shinefield et al. 2002; Schaffer and Lee 2008; Ali Fattom et al. 2004). Though the main reasons for this failure are poorly understood, these studies emphasize the multi-factorial virulence mechanisms *S. aureus* utilizes to induce infection, as some strains, such as USA300, lack capsule production(Karauzum and Datta 2016).

A more recent attempt to vaccinate patients against *S. aureus* iron-binding surface determinant B (IsdB) was met with similar preclinical promise before failure in phase III. This candidate target was identifed through an initial screen of patients with high antibody titers against *S. aureus* surface antigens(Etz et al. 2002). Immunization of this protein demonstrated remarkable protection against lethal *S. aureus* sepsis in mice, and high antibody titers were observed in mice, macaques and humans(Etz et al. 2002; Stranger-Jones, Bae, and Schneewind 2006; Kim, DeDent, et al. 2010; Harro et al. 2012; Kuklin et al. 2006). Despite its promise, phase IIB/III clinical trials with the IsdB vaccine in cardiothoracic surgery patients was halted prematurely when excessive deaths were found in the vaccine group among patients who developed post-operative *S. aureus* infections(Vance G. Fowler et al. 2013). To investigate the cause, subsequent analysis of serum cytokines found that low IL-2 and IL-17 levels post-vaccination corresponded with mortality in these patients, suggesting that a T cell dependent mechanism was at play. However, the directionality was not established, as no follow up studies were performed(McNeely et al. 2014).

Considering the multitude of virulence strategies employed by *S. aureus*, it may be the case that single antigen vaccine strategies may not be successful. New vaccine initiatives have focused on targeting superantigen toxins, and secreted toxins such as Hla. Similar to their predecessors, these targets have demonstrated preclinical promise in mouse models of soft tissue and invasive disease(Bubeck Wardenburg and Schneewind 2008; Boles et al. 2003; Kennedy et al. 2010). However, others are now attempting multi-antigenic vaccine strategies. For example, one group has demonstrated that vaccination against 7 secreted virulence factors can induce antibody-mediate protection against lethal pneumonia in rabbit models of infection. However, this same cocktail vaccination strategy was also found to enhance lethality in rabbit models of infective endocarditis(Bubeck Wardenburg and Schneewind 2008). The suggested mechanism behind this surprising result was antibody-mediated bacterial aggregation, highlighting the potential danger in eliciting antibody responses without careful investigation behind antibody-pathogen interactions. Taken together, *S. aureus* specific antibody responses may limit disease severity, though the efficacy of vaccine induced antibody responses have been remarkable in animal models with little success in humans.

The role of T cell immunity in *S. aureus* infection has become a primary focus in recent years. Unlike in the case of B cell deficiencies, patient with notable T cell deficiencies, as in the case of HIV, or partial disorders of T cell function are susceptible to *S. aureus* infection(Cook and Tangye 2009; Hidron et al. 2010). However, separate studies have found that *S. aureus* is only an occasional cause of infection in humans with genetic T cell deficiencies, though a clear confounder is the susceptibility of such

patients to a host of other organisms(Stephan et al. 1993). Thus, the requirement of T cells in controlling *S. aureus* infection in humans is still under investigation, and warrants further investigation. Detectable “memory” T cell responses can be observed in human PBMCs through re-stimulation with *S. aureus* antigen in vitro(J. B. Kolata et al. 2015; Zielinski et al. 2012). Interestingly, one study demonstrated that while *Candida albicans* induced a canonical Th17 response in PBMCs from humans, *S. aureus* Th17 cells also produced the suppressive cytokine, IL-10(Zielinski et al. 2012). This suggests that while memory cells may be induced upon exposure to *S. aureus* in humans, such T cells may suppress the development of protective immunity and subvert the host response. The mechanism by which *S. aureus* may induce tolerance in T cells is currently unknown, though recent studies have suggested that commensal colonizers of the skin and gut may induce commensal-specific Tregs soon after birth in neonatal mice and in PBMCs isolated from human neonates(Scharschmidt et al. 2015; Rabe et al. 2014; Li et al. 2015). The study of *S. aureus*-specific T cell responses in both an infection and colonization models have been hampered by the lack of antigen-specific models in which they can be studied. Thus, animal studies to date have focused on in vitro assessment of T cell responses and re-stimulation using specific *S. aureus* antigens or whole, killed bacteria. Indeed, in vivo models in which *S. aureus* priming of the T cell response will be imperative towards advancing the field’s current understanding of adaptive immune modulation by the pathogen.

Immunity to *S. aureus* in animal models

Immunity to pathogens is driven by both the innate and adaptive immune systems. Through recognition of conserved pathogen associated molecular patterns (PAMPs), innate immune cells are activated to respond appropriately. Presentation of antigenic epitopes to T and B cells, and provision of costimulatory and cytokine signals then drives the adaptive immune response towards the development of memory against the invader. Differences in tissue specific environments and innate immune cells may drive unique responses against the same pathogen. *S. aureus* infection elicits differential responses depending on the nature of the infection.

The innate immune system's initial responses are dictated by the recognition of conserved, pathogen associated molecular patterns (PAMPs), signatures which *S. aureus* shares with other pathogenic bacteria. Notably, Toll-like receptors (TLRs), peptidoglycan receptor proteins (PGRPs), Nucleotide-binding oligomerization domain containing 2 (NOD2) and TNFR1, which curiously binds *S. aureus* Protein A, are the relevant pattern recognition receptors which respond to *S. aureus*. Of the TLRs, TLR2 is primarily implicated in host responses to *S. aureus*(Krishna and Miller 2012). With expression on numerous cell types in the skin, such as keratinocytes, Langerhans cells, dendritic cells, and monocytes, TLR2 recognizes various *S. aureus* components due to its ability to form heterodimers with TLR1 or TLR6(Hoebe et al. 2005; Hashimoto et al. 2006; L. S. Miller 2008). TLR2 heterodimers recognize tri- and di-acyl lipopeptides, which include the Gram positive cell wall component lipoteichoic acid. In addition TLR also recognizes *S. aureus* peptidoglycan, and deficiency of TLR in mice demonstrate larger skin lesions with increased bacterial burden compared to WT mice during *S. aureus* infection(Ahmad-Nejad et al. 2004). This complements clinical data well, as

TLR2 polymorphisms have been associated with an increased severity of atopic dermatitis, which correlates with high susceptibility to *S. aureus* infections in these patient populations(L. S. Miller et al. 2006).

In contrast to cell membrane based PRRs, NOD receptors are found free in the cytosol. Relevant to *S. aureus* infection, NOD2 recognizes muramyl dipeptide, which is a catabolic product of peptidoglycan in both gram-positive and gram-negative bacteria(Elinav et al. 2011). Though *S. aureus* is conventionally considered an extracellular pathogen, recent evidence supports the notion that *S. aureus* can invade and persist within the cytoplasm of many cellular targets(Garzoni and Kelley 2009). In cutaneous infection models, mice deficient in NOD2 had impaired bacterial clearance and larger overall skin lesions compared to wildtype controls. Interestingly, the NOD2 host response was dependent on *S. aureus* α-toxin, which was demonstrated to facilitate entry of *S. aureus* into the cytoplasm via pore formation in cellular membranes(Frodermann et al. 2011).

Once innate immune cells have encountered the invading pathogen, antigen presenting cells activate the adaptive immune system through priming of T and B cells. As aforementioned, the role of B cells and antibodies in protection against *S. aureus* infection is controversial in humans, as XLA patients do not appear to have an increased susceptibility to *S. aureus* infection. Mouse models of infection support this observation, as neither B cell nor antibody-deficient mice display worsened phenotypes in various models of *S. aureus* infection(Gjertsson et al. 2000; Schmaler et al. 2011a; Gaidamakova et al. 2012). However, vaccine candidates have had remarkable success in protection against *S. aureus* disease in animal models, though this does not translate

to human trials. While antibodies may play a role, it is likely that successful immunization strategies will require the incorporation of other immune cells.

Recent work comparing the susceptibility of C57BL/6 mice and Balb/C mice after primary skin infection demonstrated that *S. aureus* cutaneous infection could induce antibody-mediated protection that was CD4 T cell dependent: only Balb/C mice generated protective antibodies(Adhikari et al. 2012). Furthermore, when CD4 T cells from skin challenged Balb/C mice were transferred to naïve hosts, a partial reduction in lesion size was observed during skin challenge, suggesting that memory CD4 T cells could be generated after primary skin infection. Why such drastic differences were observed between these two genetic backgrounds is unclear, though the authors proposed a difference in skewing of T helper responses: Balb/C mice produced Th17 biased responses, while C57BL/6 mice appeared to produce higher Th1 responses during skin infection.

CD4 T cell responses to *S. aureus* have been found to be both protective against infection and deleterious to the host. Each of the canonical T helper subsets have been implicated in *S. aureus* disease, with various implications and cautionary lessons for future vaccine initiatives which aim to utilize T cell memory responses. Interestingly, the context in which Th1, Th2, Th17 and Treg responses are elicited is dependent on the tissue site of exposure.

S. aureus infection can elicit Th1 responses, which have the potential to be either beneficial or detrimental. Initially, Th1 responses were found to improve control of septicemia in murine models, with enhanced IFNγ and TNFα production correlating with improved bacterial clearance and reduced mortality(Guillén et al. 2002). Indeed, Th1

immunity seems to be protective in vaccine-induced Th1 responses against *S. aureus* antigen. CD4 T cell IFNγ production was required for protection in a systemic infection model post vaccination(L. Lin et al. 2009). Vaccination with *S. aureus* extracellular vesicles also induced Th1 responses, which were sufficient for protection in a pneumonia model(Choi et al. 2015). Finally, a recent study demonstrated that adoptive transfer of *S. aureus*-specific Th1 cells was protective in naïve mice in a murine model of recurrent *S. aureus* peritonitis(Brown et al. 2015). However, the protective efficacy of Th1 responses should be met with caution, as other studies have hinted at the potential detriments of vaccine-induced Th1 responses. Mice which were vaccinated with heat-killed *S. aureus* had worsened disease phenotypes following intravenous infection, despite the presence of IFNγ production by CD4 T cells(Schmaler et al. 2011b). Corroborating these results, increased mortality was observed in mice vaccinated with lethally irradiated, whole-cell vaccines after intravenous challenge in CD4 T cell IFNγ dependent manner(Karauzum and Datta 2016). Taken together, Th1 responses can be protective or detrimental, which may depend on a multitude of factors that must be considered prior to implementation in a human vaccination setting.

Primarily implicated in parasite control, allergy, autoimmunity and atopic diseases, Th2 immunity has been associated with *S. aureus* disease through colonization on patients with prevalent inflammatory skin disorders. Skin colonization and infection by *S. aureus* is a virtually universal morbidity of atopic dermatitis (AD), a Th2 driven autoimmune disease(Hamid, Boguniewicz, and Leung 1994; Boguniewicz and Leung 2011). Due to the ability of Th2 cytokines to dampen AMP expression, gross over-colonization with *S. aureus* is thought to be due to, in part, the lack of antimicrobial

activity at the skin surface(Howell et al. 2006; Nomura et al. 2003; Kisich et al. 2008). In addition, *S. aureus* colonization may also perpetuate AMP suppression, as staphylococcal cell wall components, such as LTA and peptidoglycan have been shown to induce Th2 responses, creating a potentially vicious cycle of Th2 permanence at atopic dermatitis lesion sites(Schlievert et al. 2010; Matsui and Nishikawa 2012, 2002). Thus, Th2 responses may be deleterious in the context of *S. aureus* related diseases.

The field at large has only begun to dive into the relevance of Th17 cells in *S. aureus* disease, especially in the context of skin and respiratory infection. Th17 cytokines IL-17A, IL-17F and Il-22 promote AMP production, neutrophil recruitment and enhance epithelial cell function during infection. The role of Th17 cells was first suggested through the use of IL-17A and F deficient mice, which were highly susceptible to *S. aureus* mucocutaneous infections(Ishigame et al. 2009). Follow up studies demonstrated that IL-17A from $\gamma\delta$ T cells were crucial in controlling *S. aureus* burden and lesion size during primary, subcutaneous infection, solidifying their role as critical innate immune cells in barrier tissue defenses. Importantly, IL-17A deficiency does not appear to increase susceptibility of hosts to *S. aureus* systemic challenge, highlighting a role for IL-17A action in barrier and mucosal sites(Ishigame et al. 2009; Henningsson et al. 2010; Narita et al. 2010). The importance of IL-17A in adaptive immune responses is apparent due to protective function of from vaccine-induced, *S. aureus* specific Th17 cells in both skin and systemic models of infection(L. Lin et al. 2009; Narita et al. 2010; Joshi et al. 2012). Indeed, antibody-mediated immunity was significantly enhanced through the incorporation of a TLR7 agonist which induced Th1 and Th17 cells against *S. aureus*(Bagnoli et al. 2015). Furthermore, Hla-dependent

induction of Th17 responses indicates a role for Th17 cells in *S. aureus* pneumonia, though depletion of Th17 cells appears to improve outcomes. As is the case with Th1 immunity, control of IL-17 production by $CD4^+$ T cell is important to prevent protective immunity from becoming pathological in nature. However, the implication for Th17 immunity in *S. aureus* infection is strong, considering that Th17 depletion in HIV-infected patients early during the course of disease dramatically increased the incidence rate of *S. aureus* SSTI(Hidron et al. 2010; Prendergast et al. 2010). In support of this, AD patients also exhibit decreased IL-17 responses and associated cytokines, and decreased antimicrobial peptides at lesions sites, which may facilitate *S. aureus* colonization(Guttman-Yassky et al. 2008). Furthermore, psoriasis patients appear to harbor improved resistance to *S. aureus* infection, potentially due to the heightened Th17 responses displayed in these patients(Guttman-Yassky et al. 2008). Taken together, IL-17 responses from both the innate and adaptive players are critical in *S. aureus* control at skin and mucosal sites. Their role in control of *S. aureus* infection in human disease, albeit through indirect clinical evidence, suggests that vaccine trials which target the Th17 pathway may hold great promise. Still, the exact contribution of IL-17 mediate immunity in human *S. aureus* disease remains to be elucidated.

Despite a strong understanding of basic adaptive immunity, the antigen-specific *S. aureus* responses, especially in the realm of T cell immunity, have been difficult to address. Though various T cell subsets have been implicated in protection to *S. aureus* infection, the means by which *S. aureus* may induce or suppress these responses remains elusive. Studies which can demonstrate the generation or perturbation of immunity to *S. aureus* in a variety of tissue contexts will allow the field to understand

how the pathogen alters immunity in physiologically relevant manners. Such investigation may poise one to design rationale vaccines that account for the full extent to which *S. aureus* perturbs tissue specific immunity.

Mechanisms of immune evasion

S. aureus employs multiple mechanisms by which immunity may be suppressed. Of them, the most well studied toxins which compromise T cell function are the superantigens, which have been the classic example of the *S. aureus*-T cell interaction. Genome-wide analysis of *S. aureus* variants reveals 23 superantigen genes, which encode enterotoxins (SEs), enterotoxin-like proteins (SEls) and the toxic shock syndrome toxin-1 (TSST-1). Superantigens act through binding of MHCII molecules to the Vβ elements of αβ T cell receptors, stimulating an aberrant T cell response. The action of any given superantigen can affect up to 20% of the whole T cell repertoire(Bröker, Mrochen, and Péton 2016). In rare cases of toxic shock, this simultaneous activation of the t cell repertoire can result in a life-threatening cytokine storm, which is thought to suppress the activation of antigen-specific T cell responses. These superantigens are ubiquitous, as approximately 80% of *S. aureus* strains, including commensal and pathogenic isolates, harbor the genes encoding them(Bröker, Mrochen, and Péton 2016). Despite this, toxic shock syndrome is a rare condition, with a yearly incidence approximately 3 to 7 individuals per million people in the USA(DeVries et al. 2011). Superantigens also appears to be immunogenic in studies observing the antibody responses in mildly infected and colonized patients, with the latter having improved outcomes in sepsis infection(J. Kolata et al. 2011; Holtfreter et al.

2006). This raises the possibility that superantigens may promote *S. aureus* colonization and minor infections in order to facilitate bacterial persistence, colonization and continued transmission from host to host.

The production of suppressive cytokines and the induction of Tregs are another means by which *S. aureus* may perturb the development of protective immunity and dampen overall immune responses. Peptidoglycans have been demonstrated to elicit the release of IL-10 in monocytes and monocyte-derived macrophages, while IL-12 and IL-23 are induced in monocyte-derived dendritic cells in vitro(Frodermann et al. 2011; Belkaid and Tarbell 2009). Depending on the APC encountered, the immune response may differ as tissues will be composed of local, unique APCs. Furthermore, it was recently shown that phenol-soluble modulins, appear to favor the differentiation of Tregs *in vitro*(Schreiner et al. 2013). Taken together, *S. aureus* appears to utilize multiple mechanisms by which T cell function can be suppressed or altered for the pathogen's advantage.

Adaptive immunity may also be altered through the manipulation of B cells responses. Antibodies play a crucial role in opsonizing bacteria, neutralizing toxins and enhancing or suppressing innate immune function. Though many antibodies are produced against a variety of virulence factors, surface anchored proteins, and non-protein antigens expressed by *S. aureus*, the ability of *S. aureus* to evade them is best illustrated through the existence of staphylococcal protein A (SpA), which binds immunoglobulins on the F_C and F_{ab} portion of antibodies, preventing the antibody from targeting *S. aureus* in the correct orientation and utilizing their F_c mediated effects (Foster 2005). Recent studies have also demonstrated that SpA engages B cell

receptors and initiates activation induced apoptotic death of V_H3^+ B cells in murine models of infection(Goodyear and Silverman 2004). Mutation of SpA active regions display virulence defects in invasive models of disease, arthritis and renal abscess formation, indicating a major role in virulence for SpA(Palmqvist et al. 2002; Kim, Cheng, et al. 2010; Pauli et al. 2014). Immunization against mutant, non-toxigenic SpA allows for the production of broadly neutralizing antibodies against the pathogen, overriding the effect of SpA produced by *S. aureus*. Finally, recent work in human patient sera has demonstrated that previous exposure to *S. aureus* overwhelmingly skews B cell responses towards the production of antibodies against SpA and limited production against other antigens(Pauli et al. 2014). The immunodominance of SpA as a B cell superantigen restricts the host response against other *S. aureus* virulence factors, suggesting that antibodies detected against other antigens may be low in affinity or function, which would explain the lack of protection against infection in patients previously exposed. Taken together, *S. aureus* utilizes multiple methods by which adaptive immunity can be directly perturbed or altered.

Hla: a modulator of immunity and disease

S. aureus utilizes a multitude of virulence factors that facilitate tissue adhesion, immune cell evasion and modulation, and host cell injury. Among hemolysin α, or Hla, is a 33 kDA secreted toxin which is found in virtually all clinical isolates(Berube and Bubeck Wardenburg 2013). As a single copy on the staphylococcal chromosome, the *hla* locus is conserved across *S. aureus* strains, and appears invariant in its primary amino acid sequence(Tobkes, Wallace, and Bayley 1985; Tweten, Christianson, and

Iandolo 1983). Utilizing chemical cross-linking and circular dichroism, studies have revealed that Hla is composed almost entirely of β-strands with almost no α-helical structure. Secreted as a water soluble monomer, Hla oligomerizes into a heptameric, β barrel upon binding its receptor, A Disintegrin And Metalloprotease 10 (ADAM10)(Wilke and Bubeck Wardenburg 2010a).

Electron micrograph studies revealed that the Hla oligomer is a ring-like structure, approximately 10 nm in diameter with approximately 7 subunits and a central pore which is 2-3 nm wide(Freer, Arbuthnott, and Bernheimer 1968; Arbuthnott, Freer, and Bernheimer 1967; Freer, Arbuthnott, and Billcliffe 1973; Arbuthnott, Freer, and McNiven 1973). The fully assembled Hla pore constitutes three general domains: (1) the cap domain on the extracellular face of the toxin, which composes the pore entry site and is exposed to the extracellular environment; (2) the rim domain which is attached to the outer leaflet of the host cell membrane; and (3) the stem region which forms the β-barrel pore that penetrates the membrane(Song et al. 1996).

Expression of Hla is controlled by several global regulatory systems employed by *S. aureus*(Berube and Bubeck Wardenburg 2013). Primarily, the accessory gene regulator (*agr*) locus is a quorum-senseing system that controls Hla production through the regulatory RNA molecule, RNAIII(Peng et al. 1988; Novick et al. 1993). Briefly, the Agr system produces a secreted autoinducer peptide (AIP), which binds its cell surface receptor, AgrC, and then activates its response regulator AgrA. AgrA then binds to the P3 promoter of the *agr* locus and activates the production of the RNAIII molecule, and results in the increased expression and secretion of Hla(Peng et al. 1988; Novick et al. 1993; Koenig et al. 2004; Lyon et al. 2002). Beyond the Agr system, expression levels

can also be modulated by both the Sae and Sar regulatory systems(Xiong et al. 2006; Reyes et al. 2011; Cheung, Chien, and Bayer 1999; Cheung et al. 1994). The complex interplay among these various systems appears to tightly regulate the expression of Hla, altering Hla production in response to changing environmental conditions in a timely manner.

The molecular mechanism by which Hla bound to the surface of host cell membranes had been controversial, owing to experimental evidence supporting either that the toxin bound to membrane lipids or with proteinaceous receptors(Valeva et al. 2006a; Hildebrand, Pohl, and Bhakdi 1991; Cassidy and Harshman 1976). While substantial evidence appeared to support the notion that Hla could bind lipids directly, such results could not explain the cell type and species specific susceptibility to Hla binding and intoxication(Cassidy and Harshman 1976; Weissmann, Sessa, and Bernheimer 1966; Watanabe, Tomita, and Yasuda 1987; Valeva et al. 2006b). Specifically, Hla-mediated lysis is substantially different between rabbit and human erythrocytes, in which the former exhibits lysis at low concentrations of Hla(Berube and Bubeck Wardenburg 2013). Utilizing the difference in susceptibility of rabbit and human erythrocytes to lysis, ADAM10 was determined to be the proteinaceous receptor of Hla based on the following key observations: (1) ADAM10 is precipitated by Hla from the membrane of host cells; (2) Toxin binding and oligomerization requires ADAM10 expression; and (3) the observed species specificity of Hla-mediated lysis correlated ADAM10 expression, such that rabbit erythrocytes expressed high levels of ADAM10, in contrast to the absence of ADAM10 on human erythrocytes(Wilke and Bubeck

Wardenburg 2010a). Taken together, ADAM10 acts as the proteinaceous receptor for Hla by mediating toxin binding, assembly and function.

Hla is required in multiple murine models of lethal *S. aureus* disease, and necessary for skin dermonecrosis(Sampedro et al. 2014; Wardenburg and Schneewind 2008). Genetic knockout of Hla, antibody neutralization and biochemical inhibition of metalloproteinase activity of ADAM10 were sufficient to prevent dermonecrosis and lethality, demonstrating Hla's importance in *S. aureus* disease pathogenesis(Sampedro et al. 2014; Wardenburg and Schneewind 2008; Powers et al. 2015). Hla intoxication of monocytes, neutrophils and T cells induce a multitude of effects, ranging from lysis at the highest concentrations of toxin to aberrant cytokine secretion, inflammasome activity and apoptosis(Berube and Bubeck Wardenburg 2013). This suggests potential mechanisms by which *S. aureus* evades adaptive immunity through Hla activity.

Of note, ADAM10 is expressed on a wide variety of host cells, with particularly high levels of mRNA expression in immune cell compartments(Berube and Bubeck Wardenburg 2013; "Cell Atlas - ADAM10 - The Human Protein Atlas" n.d.; Ezekwe, Weng, and Duncan 2016), and the highest expression is found in dendritic cells, NK cells and myeloid cells. Its importance cannot be understated, as ADAM10$^{-/-}$ mice die at embryonic day 9.5 due to major defects in the development and vasculogenesis(Jorissen et al. 2010; Glomski et al. 2011). Furthermore, cell-type specific knockouts highlight the importance of ADAM10 in maintaining tissue homeostasis. Keratinocyte targeted deletion of ADAM10 leads to perinatal lethality, barrier impairment and the absence of sebaceous glands; the few surviving mice exhibit hair loss, epidermal hyperproliferation and immune dysfunction(Weber et al. 2011). With

the ubiquitous expression of ADAM10 and importance in regulating tissue homeostasis, it perhaps is no surprise that successful commensals such as *S. aureus* may alter the host environment through modulation of ADAM10 activity.

Upon binding of ADAM10, Hla induces signaling events in target cells which result in cell-specific effects that are deleterious to the host. The small pore formed by Hla permits the rapid release of ATP, K^+ ions and restricts the movement of macromolecules across the cell membrane(Cassidy and Harshman 1976; Lizak and Yarovinsky 2012; Bhakdi et al. 1989). One of the initial, and likely most important events to follow toxin pore formation is the influx of extracellular calcium into the target cell. Crucial to a multitude of cell signaling pathways, increased intracellular calcium stimulates hydrolysis of membrane phospholipids, generation of nitric oxide in endothelial and epithelial cells, activation of protein kinase C, and induction of NF-κB nuclear translocation(Rose et al. 2002; Grimminger et al. 1997; Suttorp et al. 1993). These events culminate in the production of innate inflammatory cytokines such as IL-1β, IL-6 and IL-8(Bhakdi et al. 1989; Rose et al. 2002). In combination with cell death via pyroptosis and necrosis, these inflammatory stimuli may exert an impact on the local tissue environment, stimulating immune cell recruitment and activation, and modulate host immunity in ways that may be deleterious or beneficial to the host(Buerke et al. 2002; Craven et al. 2009).

Hla is known to induce inflammatory pathways and cell death, presumably through surface pore formation and when expressed within the intracellular location in a variety of cells, from mononuclear cells to epithelial and endothelial targets(Garzoni and Kelley 2009; Schnaith et al. 2007; Menzies and Kourteva 2000, 2000). Recently, it has

been shown that expression of α-toxin is required for *S. aureus* phagosomal escape in a variety of in vitro cell lines, suggesting a means by why *S. aureus* may evade immunity(Jarry, Memmi, and Cheung 2008; Hruz et al. 2009a; Löffler et al. 2014). However, it appears the mammalian immune system has developed means by which to detect this escape, as NOD2 and NOD1 mediated detection in the cytosol appears to be dependent on the expression of α-toxin and bacterial escape from the phagosome(Hruz et al. 2009b). Stimulation of NOD2 leads to the direction activation of NF-κB, with downstream activation of caspase-1 and promotion of IL-1β release. This appears to be important for downstream IL-6 production, which can in turn activate neutrophils for bacterial killing. Despite this, *S. aureus* has been found to persist in innate immune cells after phagocytosis(Gresham et al. 2000; O'Keeffe et al. 2015; Lacoma et al. 2017). Specifically, *S. aureus* was shown to not only persist within neutrophils, but that isolation of *S. aureus*-infected neutrophils was sufficient to establish infection in naïve animals(Gresham et al. 2000). Thus, in vitro demonstration of Hla activity may not be reflective of its physiologic activity, raising the question as to what effector function Hla may have in suppressing innate or adaptive immunity. Recent studies have suggested that Hla may modulate the development of protective immunity in subcutaneous infection models. Primary infection with $Hla^{-/-}$ or inactive Hla mutant (H35L) producing strains protected against secondary, skin challenge compared to hosts which received primary WT infection(Sampedro et al. 2014). The same effect was achieved also through subcutaneous administration of an ADAM10 inhibitor to the site of primary infection, demonstrating that Hla modulates the host's ability to develop protective immunity after skin infection. However, the mechanism by which this occurs is unknown.

To our knowledge, the importance of Hla in suppressing immunity is poorly understood, and only hints of its immune modulating function have been suggested.

Beyond the horizon: Insights that may change *S. aureus* vaccination strategies

The devastating mortality that is inflicted by *S. aureus* on the world population at large demands new strategies which can prevent or treat *S. aureus* infection, as current clinical approaches are limited. Failure to develop an effective vaccine is a testament to our limited understanding of host-commensal interaction which occurs at both barrier tissues and in the blood stream. The ability of *S. aureus* to recurrently inflict skin and soft tissue infections suggests that the pathogen utilizes still unrecognized mechanisms to suppress immunity after the first infectious encounter.

We report that skin specific dendritic cells are depleted during subcutaneous challenge with *S. aureus*, which suppresses proper activation and function of antigen-specific CD4 T cell responses. To investigate antigen-specific CD4 T cell responses to *S. aureus*, we utilized the OT-II transgenic T cell system in combination with a USA300 strain that secretes chicken egg ovalbumin (OVA). Given differential recurrence rates between patients who experience SSTIs and invasive disease, we hypothesized that antigen-specific CD4 T cell responses would be suppressed during skin infection. We observed Th1 differentiation following invasive challenge to USA300, with memory cell persistence and active re-call responses to subsequent infections. Primary skin infection failed to induce T helper subset cytokines. Furthermore, while OT-IIs did express CD44 early on, we did not observe an active re-call response during re-challenge, suggesting that skin infection failed to induce a functional memory response. The failure of skin

infections to induce OT-II responses correlated with the loss of skin-resident, migratory dendritic cells (mDCs): Langerhans cells (LCs) and $CD11b^+$ dermal dendritic cells were the most affected subsets. Infection with an Hla- mutant, and antibody blockade of Hla rescued DC populations in both the skin and skin draining lymph nodes. Furthermore, skin infection with an Hla- mutant that expressed OVA resulted in the enhanced formation of effector and central memory cells during primary infection, which we hypothesize will be responsive to re-challenge infection. These data demonstrate that Hla targets skin-resident DC populations, and suppresses the priming of antigen-specific CD4 T cell responses following skin infection. Furthermore, *S. aureus*-specific, $CD44^+$ memory T cells were severely reduced in number following re-challenge, suggesting that they were either deleted or rendered anergic. To our knowledge, this work is the first demonstration of an antigen-specific system by which adaptive immune responses can be studied in vivo with cellular resolution. We demonstrate that *S. aureus* provokes differential responses that are tissue specific, and observe a failure to induce antigen-specific CD4 T cell memory and subset differentiation following skin infection. This corresponds with a lack of antibody titers against Hla and a failure to protect the host during secondary skin infection. Furthermore, these findings are corroborated by previous studies which demonstrate minimal antibody responses following *S. aureus* infection in both animal models and human sera. As CD4 T cells are required for the production of high affinity antibody, the failure to induce a response following skin infection reveal a complementary mechanism by which protective antibody responses are perturbed, in conjunction with SpA activity.

Finally, the loss of antigen-specific CD4 T cells following primary skin infection suggests that such cells were either rendered anergic or deleted. This implies that skin infection can alter the adaptive immune landscape of the host, rendering the host almost absent of antigen-specific CD4 T cells by which immunity can be imprinted. Thus, continual exposure to *S. aureus* may guarantee a suppressed host response, rendering vaccination attempts ineffective at producing high-affinity antibody due to a lack of essential immune players in *S. aureus* exposed hosts. We believe these observations begin to elucidate the mystery of recurrent *S. aureus* infections, as skin and soft tissue infection paradoxically does not allow for the development of protective immunity. Taken together, we propose that childhood immunization against Hla will not only protect hosts from the clinical outcomes of *S. aureus* infection, but also allow for skin-resident DCs to prime effective, adaptive immune responses against *S. aureus* specific antigens.

Chapter I

PROTECTIVE IMMUNITY TO *STAPHYLOCOCCUS AUREUS* RE-CHALLENGE IS TISSUE DEPENDENT

Introduction

Clinical evidence suggests that recurrence rates of infection with *S. aureus* may depend on the initial route of infect. Curiously, patients who have experienced at least one *S. aureus* SSTI frequently experience recurrent *S. aureus* infections compared to previously uninfected household contacts, with recurrence rates exceeding 50% in some populations(Tong et al. 2015; L. G. Miller et al. 2015). Larger scale studies support these findings, as healthy patients with *S. aureus* infections had higher rates of acquiring another soft tissue infection cause by *S. aureus* than patients who did not report *S. aureus* infections(Bouvet et al. 2017). Collectively, clinical studies point to currently undefined mechanisms by which the adaptive immune system is suppressed during skin and soft tissue infection, as patients are unable to generate protective immunity. In contrast, the re-infection outcomes following invasive disease have observed a lower incidence of recurrence. Of note, patients with invasive infections displayed higher convalescent titers against Hla and PVL than patients who recovered form cutaneous infection(Fritz et al. 2013). Subsequent infection risk was comparable between primary and recurrent cutaneous infection groups. Cumulative recurrent infection over a 12 month follow up demonstrated that while recurrences rates of

infection was >60% in primary and recurrent SSTI cohorts, colonization only and invasive infection groups were approximately 20%(Fritz et al. 2013). This suggests that while cutaneous infection begets further infection, this is not true for patients which were recovered invasive disease.

The correlates of immunity to *S. aureus* infection are poorly understood. Recently, it was demonstrated that protective immunity to *S. aureus* skin infection could be generated in BALB/C but not C57BL/6 mice(Montgomery et al. 2014). Though the reason for this dichotomy was not explored in depth, it was shown that protection required neutralizing antibody and IL-17A function. Corroborating these findings, skin infection with a Hla deficient strain of USA300 *S. aureus* was found to allow for the generation of protective immunity against WT *S. aureus* secondary, skin challenge, while primary WT infection did not afford the same protection to hsots(Sampedro et al. 2014). The observation that C57BL/6 mice are able to generate protective, Th1 immunity following *S. aureus* peritonitis, suggests that the host does not lack the elements needed for immunity(Brown et al. 2015). Rather, it is likely that *S. aureus* employs yet unrecognized mechanisms which are more effective in the context of a C57BL/6 skin infection. Therefore, we hypothesized that immunity to *S. aureus* re-challenge is tissue-dependent, and that the potential mechanisms by which the commensal would suppress immunity are specific to the skin.

Results

Intravenous Exposure Protects Against Secondary Challenge

Clinical evidence suggests that *S. aureus* recurrence rates depend on the initial tissue site of infection. We investigated whether immunity to *S. aureus* re-challenge was dependent on the infection site(Sampedro et al. 2014). To this end, we challenged mice intravenously or subcutaneously with USA300\LAC strains of *S. aureus* and allowed them to recover (Fig 1a). Mice were then re-challenged subcutaneously and their control of the infection was assessed 4 days later. Mice which were given primary, sub-lethal bacteremia had smaller dermonecrotic lesions during secondary skin challenge (Fig 1c). In many cases, dermonecrosis was completely prevented. Furthermore, infection site bacterial burden was markedly reduced in bacteremia mice compared to those which were skin challenged with *LAC*, or mock inoculated with PBS (Fig 1d). Interestingly, primary skin challenge did not offer protection compared to PBS control, which is consistent with previous observations(Sampedro et al. 2014; Montgomery et al. 2014). This suggests that immunity to *S. aureus* failed to generate during primary skin infection, but was successfully induced following *S. aureus* bacteremia.

Protection against secondary infection and Anti-Hla IgG production requires CD4 T cells early during *S. aureus* bacteremia

We have previously shown that Hla (Hla) secretion by *S. aureus* is required for dermonecrosis during skin infection(Sampedro et al. 2014). Indeed, primary intravenous challenge produced significantly higher levels of anti-Hla IgG compared to mice that were subcutaneously infected (Fig 1b). High affinity antibody production requires CD4 T cell help during primary infection by providing necessary cytokine signals and co-stimulation(Charles A Janeway et al. 2001; Crotty 2014; Ma et al. 2012). Furthermore,

recent evidence suggests that CD4 and CD8 T cells play a critical role in early pathogen control upon re-challenge in mucosal tissue sites(Glennie, Volk, and Scott 2017; Schenkel and Masopust 2014; Naik et al. 2012, 2015). Thus, we hypothesized that T cells were required for the observed protection against dermonecrosis upon secondary, skin challenge. Therefore, we depleted hosts of CD4 T cells during primary infection (Fig 2a). As expected, CD4 T cell depletion resulted in the absence of anti-Hla IgG in mice that underwent intravenous infection (Fig 2b). Furthermore, early depletion of CD4 T cells resulted in the loss of protection, as these mice developed lesions comparable in size to hosts that underwent primary skin infection (Fig 2c). Thus, tissue specific differences in immunity to *S. aureus* were lost upon CD4 depletion in intravenously challenged hosts. Together, this data demonstrates that T cells play a central role in the development of protective immunity against *S. aureus*. Furthermore, generation of immunity is tissue restricted, such that only intravenous challenge promotes protective immune responses.

B cells are required for immunity to *S. aureus* re-challenge

We and others have observed that protection against *S. aureus* re-challenge requires functional, neutralizing antibody against *S. aureus* virulence factors in a variety of animal models[25,89,98,147,154,175]. Indeed, patients with increased convalescent titers against *S. aureus* virulence factors have improved mortality outcomes during invasive disease, and appear to have lower incidence of re-infection in the case of anti-Hla and PVL antibodies(Fritz et al. 2013). Therefore, we hypothesized that the protective immunity generated by *S. aureus* bacteremia was B cell dependent. To assess this, we

intravenously challenged either WT or μMT hosts, as μMT mice lack mature B cells. 40 days post infection, mice were given an intradermal re-challenge with *S. aureus*, and lesion phenotype and bacterial CFU were assessed 4 days post infection. As expected, no anti-Hla IgG antibodies were detected in either μMT mice or WT mice which were primary, intradermal infection (Fig 3a). μMT mice developed large, dermonecrotic lesions upon secondary infection, in contrast to WT hosts, suggesting that B cells were required for the development of protective immunity following *S. aureus* bacteremia. (Fig 3b, c). Bacterial burden correlated with protection against dermonecrosis, as only WT hosts that were given primary intravenous challenge experienced a 10-fold decrease in *S. aureus* CFU (Fig 3C). The data suggest that B cells are required for control of bacterial burden and dermonecrosis during secondary, intradermal challenge with *S. aureus*.

Discussion

Clinical studies suggest that the recurrent *S. aureus* infections is a hallmark of skin and soft tissue infection. Patients that report to the hospital with *S. aureus* SSTIs have dramatically increased incidence of re-infection(Stryjewski and Chambers 2008; Fritz et al. 2013). This is in contrast to patients who have recovered from invasive disease, as they have incidence rates comparable to colonized patients in lieu of re-infection due to vascular manipulation and other invasive procedures(Ellis et al. 2004; Korzeniowski and Sande 1982; Chang et al. 2003; Fritz et al. 2013). However, the mechanism dictating *S. aureus* SSTI recurrence are poorly understood. Furthermore, a comprehensive analysis of tissue-specific differences in the immune system's ability to

generate protective immunity against *S. aureus* is absent in the field. We provide new evidence that immunity to *S. aureus* is tissue specific, and that only invasive disease allows for the generation of protective immunity.

Recent evidence has suggested that various invasive models of *S. aureus* disease may afford protection to the host. For example, Th1 memory cells have been isolated from human PBMCs, and IFNγ responses were enhanced in recurrent S aureus peritonitis models(Brown et al. 2015). Transfer of *S. aureus* specific Th1 cells isolated from previously infected mice, and then expanded in vitro, afforded naïve hosts protection against *S. aureus* peritonitis. Our work corroborates the notion that invasive disease primes the immune system against secondary infection at barrier tissues. As *S. aureus* colonizes the respiratory tract, skin and gut, breaches in barrier tissue may be the first event preceding more severe disease. We demonstrate that previous invasive exposure prevents the development of dermonecrosis upon secondary challenge. Analysis of the CFU at the lesion site indicated improved control of the pathogen in mice which were intravenously challenged with *S. aureus*. Furthermore, a highly organized abscess is formed by which cellular infiltrate is contained and the skin architecture is maintained, suggesting that the pathogen is spatially contained. This is in contrast to mice which were given primary WT skin infection or PBS intradermal injection, as the epidermis is completely destroyed and cellular infiltrate is disorganized across the lesion site. These marked differences strongly suggest that adaptive immunity is primed during the course of *S. aureus* bacteremia in C57BL/6 hosts.

Here, we demonstrate that both CD4 T cells and B cells are required for protection against secondary infection dermonecrosis. Furthermore, CD4 T cells were

required for the generation of anti-Hla IgG, suggesting that the presumably protective humoral response generated after intravenous infection is CD4 T cell dependent. These data support the classic understanding that high affinity antibody responses require CD4 T cell help. It remains a mystery why there exists a dichotomy between the systemic and skin response to *S. aureus* infection. As the loss of CD4 T cells during *S. aureus* bacteremia obliterates the generation of protective immunity, our data suggests that perhaps CD4 T cell or B cell responses may be impaired during skin challenge.

Previous studies have demonstrated that Balb/C mice and C57BL/6 hosts differed in their ability to generate protective immunity to *S. aureus* after skin infection, with the suggestion that C57BL/6 hosts may have been unable to do so due to a skew towards Th1 responses(Montgomery et al. 2014). While this remains a possibility, our studies demonstrate that immunity to *S. aureus* may also depend on tissue-specific differences that have been previously unappreciated. The ability of Balb/c mice to generate immunity after skin infection is at odds with what is known about human responses to *S. aureus*, as patients who report skin and soft tissue infection with *S. aureus* suffer recurrent skin infections. While studies in Balb/c mice may reveal new, still unknown, mechanisms that allow it to generate protective immunity post skin infection, this difference in skin response may be a physiologic divergence that cannot be reconciled between the mice and men. In contrast, C57BL/6 mice, like humans, appear unable to generate protective immunity to *S. aureus* after skin infection, but do upon post intravenous challenge. Like humans, C57BL/6 mice displayed high titers of anti-Hla IgG only after intravenous infection, while SSTIs did not induce detectable ant-Hla IgG titers(Fritz et al. 2013). This suggests that the adaptive immune response during skin

infection is somehow impaired. These tissue-specific differences in response to infection allow for in-depth exploration into potential *S. aureus*-driven mechanisms of immune suppression that have yet to be discovered. Further studies analyzing these differences may likely reveal why humans suffer recurrent infections after primary, SSTIs, and not after invasive disease.

Chapter II

T CELL PRIMING AND MEMORY INDUCTION IS IMPAIRED DURING *STAPHYLOCOCCUS AUREUS* SKIN INFECTION

Introduction

Efficient generation of T effector cells and T follicular helper (Tfh) cells dictate the development of high affinity antibody production by B cells, which is key to neutralizing toxins, opsonization of bacterial wall targets for phagocytosis by innate immune cells, and the activation of complement pathways(Charles A Janeway et al. 2001; Crotty 2014; Ma et al. 2012). The role of Th17 cells have been of particular interest to the field, after the discovery that IL-17A and F deficient mice were highly susceptible to *S. aureus* mucocutaneous infections(Ishigame et al. 2009). Follow up studies demonstrated that IL-17A from γδ T cells were crucial in controlling *S. aureus* burden and lesions during subcutaneous infection, solidifying their role as critical innate immune cells in barrier tissue defenses. Importantly, IL-17A deficiency does not appear to increase susceptibility of hosts to *S. aureus* systemic challenge, highlighting a role for IL-17A action in barrier and mucosal sites(Ishigame et al. 2009; Henningsson et al. 2010; Narita et al. 2010). *S. aureus* infection can also elicit Th1 responses, which have the potential to be either beneficial or detrimental. Initially, Th1 responses were found to improve control of septicemia in murine models, with enhanced IFNγ and TNFα production correlating with improved bacterial clearance and reduced mortality(Guillén et al. 2002). Indeed, Th1 immunity seems to be protective in vaccine-induced Th1 responses against *S. aureus* antigen.

Currently, no studies in the *S. aureus* field have been able to observe the in vivo priming, development and formation of memory T cells. Exploration of memory cells is restricted to *in vitro* T cell proliferation and cytokine responses after antigenic pulse. Such systems do not allow for molecular and cellular analysis of memory cells during and after infection in vivo, and permit only superficial analyses of general populations of T cells. For responses to be detected, the T cell population analyzed must respond in bulk, and implicit averaging of many measurements may mask subtle phenotypic changes which can have dramatic, physiologic impact on disease. For any given pathogen epitope, approximately 100 CD4 T cells in the entire CD4 T cell repertoire may recognize it, resulting in a $1:10^4$ – $1:10^6$ precursor frequency(Ford et al. 2007; Blattman et al. 2002; Whitmire, Benning, and Whitton 2006). This provides an extremely narrow window for observation and kinetic analysis of the priming and memory response *in vivo*.

To circumvent this problem, several methods have been developed to track antigen-specific CD4 T cell responses. Perhaps the most well-known system for C57BL/6 mice is the OT-II system, which utilizes CD4 T cells that have been genetically engineered to overexpress fixed TCR-α and β chains specific for the chicken egg ovalbumin residue 323-339 in the context of MHC class II I-A^b molecule; this MHCII molecule is expressed in C57BL/6 mice(Barnden et al. 1998). Since its genesis, the OT-II system, as well as other antigen-specific systems, have been used to assess in vivo responses to pathogens, host-commensal interactions, tumor responses and peripheral tolerance(Igyártó et al. 2011a; Idoyaga et al. 2013; Ford et al. 2007; Broom et al. 2010; Bertholet et al. 2005; Shaulov and Murali-Krishna 2008). As we have demonstrated that

CD4 T cell responses are required for the development of protective immunity, analysis of the CD4 T cell responses may reveal tissue specific differences in T cell priming that have yet to be appreciated. We hypothesized that CD4 T cell priming and memory responses would be impaired following skin infection. As anti-Hla IgG titers were absent in mice that underwent intradermal *S. aureus* infection, we hypothesized that CD4 T helper cell cytokine production would be impaired, as effector CD4 T cells provide cytokine and co-stimulatory help to B cells prior to the production of high affinity antibody. Thus, we generated a strain of *S. aureus* which constitutively expressed chicken egg ovalbumin, and utilizing the OT-II system to track antigen-specific CD4 T cell responses to *S. aureus* infection during skin and intravenous challenge.

Results

Antigen-specific CD4 T cell priming and memory formation against *S. aureus* is tissue restricted

Given that *S. aureus* infection induced CD4 T cell dependent IgG responses after intravenous challenge, we reasoned that *S. aureus* suppressed the development of skin-specific adaptive immunity. Mechanistic understanding of how *S. aureus* interferes with skin immunity is poorly understood. Moreover, research to date fails to observe in vivo antigen-specific responses to *S. aureus*, and thus lacks the resolution necessary to determine how *S. aureus* modulates immunity at a cellular level in specific tissues.

To overcome this, we decided utilized the OT-II system in combination with a chicken egg ovalbumin (OVA) secreting strain of *S. aureus*. First, we modified the pOS1 plasmid to include an enhanced translational enhancer and modified shine dalgarno sequence, as previously described, and called it pMagic (Fig 4a)(W. G. Miller and Lindow 1997). We then inserted our target locus in pMagic, which included the Hla secretion signal upstream a truncated OVA gene, OVA(138-386), to create pMagic-OVA (Fig 4a)(W. G. Miller and Lindow 1997). As a negative control, we transformed pMagic into *LAC*. Utilizing these strain, we verified OVA production through western blot, and confirmed that OVA generation did not negatively affect growth kinetics in culture (Fig 4b, c).

In order to track antigen-specific CD4 T cell responses to *S. aureus* infection, we transferred 100,000 CD45.1 OT-II cells into mice one day prior to intravenous or subcutaneous challenge with pMagic or pMagic-OVA containing strains of *LAC* (Fig 5a). Most OT-IIs accumulated in their respective draining sites, with OT-IIs accumulating in the skin dLNs (dLN) of mice which were challenged in the skin, and high OT-II accumulation in the spleen after intravenous infection (Fig 6a, b; Fig 7a). At baseline, approximately 250 cells (95% CI: 260.2 ± 132.6) were recovered from hosts infected with *S. aureus* pMagic, in which OVA antigen was not present. While *S. aureus* pMagic-OVA skin infection induced a 10 fold expansion compared to baseline (95% CI; pMagic-OVA: 2054 ± 1142) on day 7, cell numbers decreased to approximately baseline 14 days post challenge (95% CI; pMagic: 199.5 ± 43.55; pMagic-OVA: 329.5 ± 162.5) (Fig 6a,b; Fig 7a). In contrast, intravenous infection with *S. aureus* pMagic-OVA resulted in significantly higher OT-II numbers on both Day 7 (95% CI: 5648 ± 3005) and Day 14

(95% CI: 1875 ± 1344). We next analyzed the induction of central and effector memory cells to infection. Memory cells can be identified by the expression of CD44 and CD62L; $CD44^{high}CD62L^{low}$ as effector memory and $CD44^{high}$ $CD62L^{high}$ as central memory [ref]. Both skin and bacteremia infections induced OT-IIs to become effector and central memory, though invasive infection resulted in approximately 60-80% of OT-IIs harboring an effector memory phenotype (Fig 6a, b). In contrast, OT-IIs primed during skin infection split evenly into effector and central memory compartments. Of note, approximately 20% (95% CI: 23.68% ± 13.16) of OT-IIs on day 14 post skin infection remained naïve, in contrast to 6% (95% CI: 6.038% ± 3.002) of OT-IIs after bacteremia infection. Total numbers of OT-IIs recovered Taken together, this data demonstrate that *S. aureus*-specific priming of T cells is tissue dependent, such that invasive infection induces marked T cell expansion and higher frequencies of effector memory T cells.

S. aureus **bacteremia induces Th1 programming**

Recent literature has emphasized a role for Th17 cells in the control of bacterial infections across a broad range of mucosal sites (Montgomery et al. 2014; Patel and Kuchroo 2015; Khader, Gaffen, and Kolls 2009). Furthermore, Th1 cells were implicated in host protection against *S. aureus* invasive disease (Brown et al. 2015).Thus, we assayed our OT-IIs for T helper subset differentiation by cytokine expression. OT-IIs from bacteremia challenged hosts expressed high levels of IFNγ, characteristic of previously reported Th1 responses to invasive *S. aureus* infection. In contrast, infection failed to induce IL-10, IL-17A or IFNγ responses above background (Fig 8a). This indicates that OT-IIs were unable to produce canonical Th cytokine

responses to *S. aureus* skin infection despite antigen detection. Tfh cells can be surface stained through expression of PD-1 and CXCR5(Baumjohann and Ansel 2013). Our preliminary data suggests that bacteremia induced greater numbers of Tfh cells on day 7 compared to skin challenge, which warrants further investigation (Fig 8b). This data would suggest that skin infection is inefficient in its induction of Tfh cells, which are required for the development of high affinity and class switched antibody by antigen-specific B cells during primary infection.

Primary skin infection fails to induce a long-lasting memory CD4 T cell response that responds to re-challenge

The failure to produce significant cytokine responses, coupled with a significant reduction in OT-II numbers after infection resolution suggests that the initial priming of antigen-specific T cells was impaired. Therefore, we hypothesized that OT-II re-call responses would be observed in hosts that recovered from intravenous infection but would not be elicited in primary skin infected mice. To assess this, we analyzed re-call responses following skin re-challenge. Circulating CD4 T memory cells expand 3 days post re-challenge, while the expansion of naïve T cells takes 4 days or longer(Merica et al. 2000). Knowing this, we intravenously or subcutaneously infected mice with pMagic-OVA or pMagic containing strains of *S. aureus*. On day 40 post infection, mice were re-challenged subcutaneously with pMagic-OVA *S. aureus*. Mice that recovered from bacteremia produced a ~3 fold increase in OT-II accumulation in skin dLNs 3 days post re-challenge (Fig 9a). Consistent with this, virtually all OT-IIs were $CD44^{high}$. In contrast, hosts which were skin challenged with pMagic-OVA containing *S. aureus* had the same

numbers of OT-IIs in the skin dLNs as mice which were initially infected with pMagic, empty vector harboring *S. aureus*. Furthermore, the majority of OT-IIs in skin pMagic-OVA *S. aureus* primary challenged mice were naïve ($CD44^{low}$ $CD62L^{high}$). As mice were pooled for each memory experiment, further repetitions will be needed. Our preliminary data suggests that memory cells were decreased in skin dLNs after secondary challenge in primary skin challenged hosts. Taken together, this data suggests that T cell priming and memory formation during *S. aureus* infection is dependent on the tissue site of infection.

Discussion

Exploration of the T cell response to *S. aureus*, in both animal and human studies have been restricted to analyzing *in vitro* T cell proliferation and cytokine responses after antigenic pulse. For animal studies, such systems do not allow for molecular and cellular analysis of memory cells during and after infection in vivo, and permit only superficial analyses of general populations of T cells. The threshold of detection is extremely high, as changes must be visible among the bulk CD4 or CD8 T cell population. This renders the observation of antigen-specific responses, which may be restricted to only several hundred cells in the entire population, virtually impossible if such changes are subtle despite producing dramatic, physiologic effects on the immune system. To circumvent this challenge, we developed the pMagic plasmid to constitutively overexpress a truncated form of chicken egg ovalbumin. Utilizing the OT-II

system, we tracked antigen-specific CD4 T cell responses in the context of *S. aureus* skin and invasive disease.

We demonstrate that antigen-specific CD4 T cell responses to *S. aureus* infection are tissue specific. Interestingly, OT-II numbers differed significantly at all point after primary infection. Notably, bacteremia induced higher numbers of OT-IIs compared to skin infection. This correlated with a dramatic increase in both the proportion of effector memory cells, and numbers of effector and central memory cells (Fig 5c). In contrast, *S. aureus* skin infection induced fewer numbers of OT-II memory cells at all time points, and had a higher number of naïve OT-IIs, suggesting a weaker priming of the CD4 T cell response after infection. The data indicate that *S. aureus* infection in the skin somehow fails to induce the same degree of response as invasive exposure, despite obliterating the skin site of infection. Other infection models have demonstrated the formation of memory CD8 or CD4 T cells post primary skin infection, thus the skin itself does not appear to be a privileged site(Igyártó et al. 2011a; Jiang et al. 2012; Iborra et al. 2016). However, models of commensal colonization on the skin have demonstrated that T regulatory cells can be induced in an antigen specific manner. Our data do not appear to suggest the formation of tolerogenic T cells, as IL-10 production (Figure 6a) and Foxp3 expression (Figure 7) are not induced after skin infection, though other means of suppression have not been excluded. Indeed, *S. aureus* bacteremia induces Th1 programming as IFNγ expression was detected in OT-IIs. However, canonical Th1, Th2 nor Th17 cytokines could not be detected after skin infection above background levels. Our preliminary assessment of Tfh differentiation also implies that *S. aureus* bacteremia induces higher numbers of Tfh cells, which are required for the

production of high affinity antibody, as signals IL-21 and CD40L are all provided by Tfh cells to B cells during the affinity maturation and isotype class switching stages of B cell germinal center responses(Ma et al. 2012). Collectively, this demonstrates that while the immunity at the skin site can be induced in other infection models, *S. aureus* is an exception, and functional T cell immunity does not appear to be generated.

In addition to Tfh, effector T cell subsets are implicated in the control a variety of pathogens, and cytokines produced by them are critical in inducing proper antibody class switching in B cells(Mongini, Paul, and Metcalf 1982; Lange et al. 2012). The absence of effector T cell subsets imply that a lack of high affinity, class switched antibody would be produced against *S. aureus* antigens during skin infection. This mirrors what we and others have observed, as skin infection does not appear to produce robust antibody responses in C57BL/6 hosts and do not develop protective immunity. The same appears to be true regarding T cell memory. While memory cells do appear to be formed to some degree during skin infection, they do not accumulate in the lymph node upon re-challenge compared to background levels. Interestingly, majority of OT-IIs recovered from the lymph nodes of primary skin infected mice after skin re-challenge were naïve. This was in stark contrast to OT-IIs recovered from the draining lymph nodes of intravenously infected mice which were re-challenged on the skin, as there was an approximately 3 fold increase in recovery and the majority were $CD44^+$ memory cells. Therefore, while antigen is clearly observed by OT-II cells following skin infection, the induced $CD44^+$ cells appear to be transient in existence, as they do not respond to re-call. This has interesting implications in vaccine strategy, as most vaccine trials to date have been attempted in adults, or have used passive transfer

of antibodies isolated from adults with high titers against *S. aureus* antigens(V. G. Fowler and Proctor 2014). Based on our observations, we hypothesize that patients that have been infected with *S. aureus*, perhaps repeatedly, may have a low frequency of *S. aureus*-specific, naïve CD4 T cells that vaccines can utilize. In support of this theory, recent evidence suggests that *S. aureus* specific CD4 T cells appear to have plasticity in their functionality, as they can produce both immune activating and suppressive cytokines(Zielinski et al. 2012; Alegre et al. 2016; Leech et al. 2017). Thus, *S. aureus*-specific CD4 T cells may be anergic or altered in function from previous encounters with the pathogen, preventing proper development of a protective immune response during vaccination. Indeed, the one trial which seemed to suggest some promise was a trial in which humanized, mouse chimeric monoclonal antibody against LTA was passively infused into low birthweight infants, and tested for the prevention of late on-set sepsis(Weisman et al. 2011). In this case, antibody production against *S. aureus* was not done in an environment by which the pathogen could manipulate the immune response. Taken together, our insights point to the importance of targeting future vaccine trials at the pediatric population, as they are less likely to have been exposed to *S. aureus* in an infectious context compared to adults.

Chapter III

T CELL PRIMING AND SKIN-SPECIFIC DENDRITIC CELLS ARE MODULATED BY *STAPHYLOCOCCUS AUREUS* HLA

Introduction

The skin is a tightly regulated, dynamic barrier which interacts with both pathogenic and commensal microbes. Dendritic cells of the skin constantly survey the environment, and are specialized in their ability to induce tolerance or T helper subset differentiation. There are 3 general DC subsets in the healthy mouse skin, all of which express CD11c and MHC class II; epidermal Langerhans cells (LCs), $CD11b^{+}$ dermal dendritic cells (DDCs) and $CD207^{+}$(Langerin) DDCs(C. C. Chu, Di, and Nestle 2011). The human and murine skin DC network appears to be highly conserved, as both species harbor analogous populations(Clausen and Stoitzner 2015).

Functionally, antigen-specific CD4 and CD8 T cell studies, in combination with specific DC subset ablation, have demonstrated that each skin DC subset has a multitude of roles in inducing T cell immunity and tolerance. LCs are necessary and sufficient for the induction of Th17 but not for the priming of CD8 cytotoxic lymphocytes (CTLs) in a *Candida albicans* and *S. aureus* infection model by which antigen was exogenously provided prior to infection(Igyártó et al. 2011a). However, the idea that LCs are dedicated to priming T cell immunity is challenge by the observation that LCs have been implicated in a plethora of studies observing LC dependence of immune suppression in the skin(Romani, Brunner, and Stingl 2012b; West and Bennett 2018;

Price et al. 2015b; Gomez de Agüero et al. 2012; Igyarto et al. 2009). In the absence of Langerin$^+$ dDCs, “classical” DCs, which consist of the Langerin$^-$ CD11b dDCs and Langerin$^-$ CD103$^-$ CD11b$^-$ dDCs, are sufficient to induce Th17 programming, though CD11b dDCs have also been noted to induce Th1 immunity in viral infection models(Zhao et al. 2003a). Furthermore, Langerin$^+$ dDCs were required for the generation of antigen specific CTL and Th1 responses. Langerin$^+$ dDCs appear to also inhibit the ability of LCs and, from inducing Th17 programming in antigen specific CD4 T cells(Igyártó et al. 2011b). Generally, Langerin$^+$ dDCs have been implicated in instructing Th1 immunity in mucosal sites and cross presenting antigen to CD8 T cells(Igyártó et al. 2011a; Kaplan 2010; Liang et al. 2016).

Despite their designation as “classic” dDCs, this population of Langerin$^-$ dDCs is heterogenous, composed of CD11b$^-$ and CD11b$^+$ dDCs, and their functions are poorly understood. In addition to their aforementioned roles in Th17 and Th1 immunity, skin and lung CD11b$^+$ CD103$^-$ dDCs were found to have the ability to produce retinoic acid (RA), which was previously thought to be produced by CD103$^+$ dDCs in order to induce peripheral T regulatory cells (iTreg) (Guilliams et al. 2010; Igyártó et al. 2011b). These RA-producing DCs were found to be capable of generating iTregs, and provides a mechanistic explanation for the large numbers of Foxp3$^+$ Tregs which are present in the dermis during both steady-state and inflammatory environments(McLachlan et al. 2009; Dudda et al. 2008). This suggests that classical DCs not only have tolerogenic potential but may also play a role in priming protective immunity under certain inflammatory conditions.

The mechanisms by which *S. aureus* may manipulate DC function are unclear. Purified staphylococcal enterotoxin A or exfoliative toxin lead to the significant depletion of LCs, though genetic knockout of either of these genes was not tested in a *S. aureus* infection(Pickard, Shankar, and Burnham 1994). Furthermore, staphylococcal enterotoxin B was demonstrated to increase the expression of T cell immunoglobulin mucin domain 4 on dendritic cells, which ligates to TIM1 on CD4 T cells and drives Th2 subset differentiation(al n.d.). However, as in previous studies demonstrating the effects of superantigens on DC function, the investigators co-cultured purified toxin with human DCs, and then assessed their ability to prime naïve CD4 T cells. Thus, the in vivo and physiologic relevance of these studies is difficult to assess without the use of genetic knockouts of *S. aureus* in live infection models.

In vitro analyses have also suggested that (PSM) peptide toxins, which are highly expressed in MRSA, bind formyl peptide receptor (FPR) 2 on DCs. PSM treated bone marrow derived DCs (BMDCs) showed reduced secretion of TNF, IL-12, and IL-6 while IL-10 expression was enhanced. Consequently, IL-10 producing DCs were demonstrated to inhibit Th1 priming in vitro and instead skew priming towards Treg induction(Schreiner et al. 2013). However, in vivo testing was not performed in order to verify whether the proposed mechanisms occurred during live infection.

Recently, *S. aureus* was shown to utilize leukocidins as a means of directly killing dendritic cells in order to avoid immune clearance. Initially, Leukocidin A/B (LukAB) was demonstrated to be responsible for the intoxication of monocyte-derived DCs, and that both LukA and B components were required for potent killing of DCs. Notably, *S. aureus* strains lacking lukAB were severely impaired in their ability to kill

phagocytes during a murine infection model, and is upregulated in the abscess during skin infection. Indeed, genetic knockout of lukAB severely inhibitied bacterial survival at the site of infection, which correlated with higher numbers of PMNs and macropahges(Dumont et al. 2011). Leukocidin ED was recently confirmed to bind to CCR5 and mediate the killing of $CCR5^+$ T cells and myeloid cells in vivo(Iii et al. 2013). Impressively, CCR5-deficient mice were resistant to lethal *S. aureus* infection, demonstrating the importance of CCR5 in S aureus lethal disease. Furthermore, these studies marked some of the first in vivo evidence of DC targeting by *S. aureus*.

Hla, which is highly expressed in the most common clinical strain USA300, has been shown to mediate its lytic and sublytic effects through binding of its receptor, ADAM10. As ADAM10 is almost ubiquitously expressed, the receptor is poised as a prime target for *S. aureus* to manipulate its function. Few studies have observed the effect of ADAM10 on dendritic cells in disease models. A recent study demonstrated that loss of ADAM10 signaling in DCs dampens Th2 responses in an allergic asthma murine model, suggesting that inhibition of ADAM10 activity can suppress allergic responses in susceptible patients(S. Damle, Martin, and Conrad 2016). However, a plethora of in vitro investigation points to the potential for Hla in directly lysing or modulating APC function at mucosal sites of infection, where tissue-specific DCs are localized. Indeed, depending on the concentration of toxin, monocytes and neutrophils have been reported to undergo aberrant inflammasome activation, cytokine and chemokine secretion, decrease phagocytosis and lysis(Berube and Bubeck Wardenburg 2013). As dermonecrosis and keratinocyte death are hallmarks of *S.*

aureus skin infection with Hla producing strains of *S. aureus*, lysis of DCs is a likely outcome, though studies to date have not investigated this potential phenomena.

Collectively, these studies suggest that *S. aureus* may modulate antigen presentation by skin dendritic cells during infection. However, the lack of antigen specific models by which the activity of DC-T cell interactions can be tracked has prevented the field from determining which activities are physiologically relevant during infection. Given that antigen-specific CD4 T cell responses were impaired during skin infection, we hypothesized that Hla inhibited DC presentation of antigen or co-stimulation to T cells. We propose that Hla lyses DCs at the skin site, preventing mature DCs from migrating to the skin and efficiently presenting antigen.

Results

Skin-resident Dendritic Cells are Targeted by Hla

We have previously shown that primary skin infection with either isogenic USA300/LAC *hla::erm* (Δ*hla*) or pH35L(Δ*hla*/p*hla*$_{H35L}$*)* harboring LAC strains of *S. aureus*, which produce an inactive form of the toxin, protect hosts against secondary skin challenge(Sampedro et al. 2014). This suggests that Hla may modulate the development of protective immunity. Given that failure to induce protection and CD4 T cell memory was restricted to the skin, we hypothesized that Hla inhibited dendritic cell (DC) cross talk with CD4 T cells during primary infection. This could be through loss of DC populations, absence of co-stimulation, or failure of DCs to produce necessary cytokine signaling for T helper cell differentiation(Naik et al. 2015; Manicassamy and

Pulendran 2011; Belz 2008; Lu, Wang, and Linsley 1997; Idoyaga et al. 2013). In the skin, DC populations can be segregated into dermal $CD11b^+$ and $CD103^+$ DCs, and epidermal Langerhans cells(Platt et al. 2013; Vermaelen et al. 2001). To assess, we subcutaneously infected hosts with either phosphate buffered saline (PBS), *LAC* or *LAC hla::erm* mutant strains, and assessed both skin site and draining lymph node DC populations 4 days post infection. Compared to PBS control, $CD11b^+$ DDCs and LCs were significantly reduced at both the site of infection and skin dLNs, while $CD103^+$ DDCs were unaffected after *LAC* infection (Fig 11). $CD11b^+$ DDCs and LCs were rescued during infection with *LAC hla::erm* mutant strains, suggesting that these were targets of Hla-mediated destruction. Application of FITC dye to the skin of mice allows tracking of skin-specific dendritic cells to the dLNs(Platt et al. 2013; Vermaelen et al. 2001). If Hla directly targeted DCs, we would expect that skin-resident, $FITC^+$ DCs would fail to accumulate in the skin dLNs during WT LAC infection. To assess, FITC dye was then painted onto the skin of mice immediately before intradermal infection with either WT or *hla::erm* mutants. DC migration was analyzed 4 days later in the skin dLNs. A trend towards increased $FITC^+$ Langerhans cell accumulation was observed in *LAC hla::erm* infections (Fig 12b) compared to WT. In addition, a higher accumulation of CD11b dDCs was observed during *LAC hla::erm* infections compared to WT. FITC dye did not appear to accumulate in $CD103^+$ dDCs. Taken together, these data suggest that impaired DC migration from the skin site of infection is Hla dependent.

Passive and Active Immunization against Hla protects skin DCs

Previously, we demonstrated that the Hla receptor, ADAM10, is required for toxin function and lysis(Wilke and Bubeck Wardenburg 2010a; Inoshima et al. 2011). To determine whether blocking of Hla action would rescue these DC populations, we passively immunized hosts with rabbit polysera specific for the inactive mutant of Hla, H35L. Currently, our data demonstrate that there is a trend towards higher accumulation of Langerhans cells in the skin in mice which received rabbit polysera specific for the inactive mutant of Hla, though these pilots were underpowered. On-going investigation will demonstrate whether serum of immune sera will protect DC populations in the skin. Furthermore, immune sera from mice which received either skin or intravenous *S. aureus* infection will be passively transferred to naïve hosts in order to determine whether endogenously generated antibody can protect skin DCs. These experiments may demonstrate that anti-Hla antibody can mediate DC protection.

Our data demonstrate that *S. aureus* infection in naïve hosts will deplete skin specific DCs and potentially prevent efficient priming of the adaptive immune system. Thus, vaccination against Hla may not only prevent dermoncrosis as previously reported, but also protect DCs and allow for the restoration of adaptive immune priming during *S. aureus* SSTI(Kennedy et al. 2010; Sampedro et al. 2014). In vaccinated hosts naive to *S. aureus*, subsequent challenge with the pathogen may serve as an immunogenic boost against *S. aureus* antigens, rather than a suppressive encounter. To demonstrate that active immunization against Hla would induce sufficient antibody response to protect skin DCs, mice were immunized with alum alone or suspended with H35L (20 μg per mouse) and boosted 14 days later. 7 days after the boost, mice were subcutaneously infected with WT *S. aureus*. Vaccinated hosts exhibited increased

accumulation of CD11b$^+$ dDCs and Langerhans cells in the skin draining lymph nodes (Fig 14b). Furthermore, vaccinated hosts displayed increased accumulation in all DC subsets at the skin site (Fig 14a). This data demonstrate that vaccination against Hla alone can protect DC populations in the skin and allow for the subsequent accumulation of DCs into the skin draining lymph nodes during *S. aureus* infection.

Hla suppresses T cell priming, site accumulation and memory induction during skin infection

Both LCs and CD11b$^+$ dDCs have been shown to be sufficient to induce Th17 responses(Sampedro et al. 2014). Furthermore, CD11b$^+$ dDCs play role in the induction of Th1 immunity in other mucosal infection models(Zhao et al. 2003b). Thus, we hypothesized that Hla may directly suppress antigen presentation, T helper subset differentiation and memory induction through the destruction of these skin resident dDCs, as they appeared the most affected. Therefore, we hypothesized that skin infection with a LAC *hla::erm* mutant harboring pMagic-OVA should rescue T helper cell differentiation in the absence of Hla, as skin DCs will then be able to carry out their normal function. OT-II cells were transferred into naïve hosts prior to infection with either WT or LAC *hla::erm* mutant harboring pMagic-OVA. Preliminary data demonstrate that a high accumulation of effector and central memory OT-IIs were observed day 7 post LAC *hla::erm* infection, but not WT infection (Fig 15a, b). This suggests that the initial priming of memory OT-IIs is restored in the absence of Hla. If this is true, we should also observe a restoration of T helper subset differentiation following skin infection and long-last memory T cells which can respond to re-challenge

infection. Furthermore, OT-IIs were observed to accumulate at the skin site of infection during LAC *hla::erm* infection but not after WT infection, suggesting that Hla may affect migration or accumulation of OT-IIs at the site of infection (Fig 16a, b). Finally, preliminary analysis of cytokine production on day 14 post infection revealed that Th1 subset differentiation was rescued during LAC *hla::erm* pMagic-OVA infection, as an increase in IFNγ induction was observed in OT-IIs compared to OT-IIs isolated from WT infection (Fig 17a). A trend towards IL-17A induction was also observed, though further investigation with larger sample sizes is needed. Taken together, our preliminary data suggests that Hla suppresses the priming of T cell memory, accumulation of antigen-specific CD4 T cells at the site of infection and Th1 subset differentiation during skin infection.

Discussion

Our lab has previously described that skin infection with LAC *hla::erm* mutant protected hosts against secondary skin challenge with WT *S. aureus*(Sampedro et al. 2014). Knowing that Hla is a known mediator of cellular lysis among a diversity of host cell types, we demonstrate that migratory dendritic cell accumulation in the skin draining lymph nodes after WT *S. aureus* infection was reduced in an Hla dependent manner. Both the skin site and draining lymph nodes demonstrated total migratory DC reduction following skin infection with WT *S. aureus*. The most affected subsets were $CD11b^+$ dDCs and Langerhans cells, both of which have been implicated in Th1 and Th17 immunity, respectively. In contrast, $CD103^+$ dDCs did not appear to be infected in the skin nor draining lymph nodes, though function of skin DCs was not performed.

Significant loss of these populations was also observed at the site of infection, which suggested that DCs may be lysed by Hla during infection. Indeed, infection with LAC *hla::erm* mutant demonstrated an accumulation of DCs in the skin draining lymph nodes and rescue of the DC population at the skin site. In order to demonstrate that DC migration from the skin to the draining lymph node was impaired, $FITC^+$ DCs were tracked after infection with either WT or LAC *hla::erm* mutant *S. aureus*. We observed that $CD11b^+$ dDC trafficking was impaired following WT infection, but rescued during LAC *hla::erm* infection. A trend towards higher accumulation of $FITC^+$ LCs was observed following LAC *hla::erm* infection as well, suggesting that the migration of both populations may be rescued in the absence of Hla. Taken together, this data demonstrate that Hla impairs the migration of DCs to the skin draining lymph nodes, and depletes DCs at the skin site of infection. The loss of LCs and $CD11b^+$ dDCs may result in the downregulation of either Th17 induction or Th1 priming of *S. aureus*-specific CD4 T cells, which would impair the development of both a protective antibody response and functional T cell memory. Indeed, infection in the skin with an LAC *hla::erm* mutant allows or the development of protective immunity against re-challenge, suggesting that Hla affects adaptive immunity. Thus, the lysis of *S. aureus* “educated” DCs may prevent proper antigen presentation and impair the delivery of necessary costimulatory factors and cytokines to *S. aureus*-specific T cells.

As DCs are impaired in an Hla-specific manner, we investigated whether passive transfer of immune sera or active immunization against Hla_{H35L} would protect DC populations during WT *S. aureus* infection. A trend towards DC protection in the skin has been observed in small pilots in which immune sera from Hla_{H35L} immunized rabbits

was passively transferred to naïve mice, followed by skin infection with WT *S. aureus*. Active vaccination against HlaH35L significantly enhanced the accumulation of all DC subsets at the skin, and in particular increased the accumulation of $CD11b^+$ dDCs and Langerhans cells in the draining lymph nodes during WT *S. aureus* infection. Taken together, these results demonstrate that vaccination against Hla can bolster DC accumulation in the lymph nodes during *S. aureus* infection and protect DCs in the skin from Hla mediated depletion. This implies that vaccination against Hla may then allow for DCs to properly prime the adaptive immune system to develop protective immunity. The development of a successful vaccine has evaded the *S. aureus* field, perhaps due to the multitude of virulence factors employed by *S. aureus* to evade adaptive immunity. An appreciation for the tissue specific mechanisms employed by *S. aureus* to evade immunity may inform more rationale vaccine designs that can prime the immune system to generate complete immunity upon subsequent exposure. Our data suggest a strategy which utilizes pre-emptive immunization to protect DC populations, which would then allow for the priming of immunity against other critical *S. aureus* antigens and virulence factors during the course of minor infection.

We observed that T cell priming during primary skin infection was significantly impaired in its ability to generate cytokine responses and long last memory. Hla is known to harbor multiple functions, from lysis of cellular targets to activation of inflammasome activity through pore formation(Berube and Bubeck Wardenburg 2013). Thus, we hypothesized that Hla manipulates this process, and generated a LAC *hla::erm* mutant that expressed OVA (LAC *hla::erm*-pMagic-OVA)to probe the effect of Hla on T cell induction. Thus, we investigated whether Hla could suppress T cell

priming. Of note, OT-IIs expressed IFNγ after skin challenge with LAC *hla::erm*-pMagic-OVA. This confirmed that Hla modulated T cell cytokine responses during skin infection. Furthermore, memory T cell accumulation was enhanced following LAC *hla::erm*-pMagic-OVA infection, similar to our initial findings during *S. aureus* bacteremia (Fig 15a, b). Taken together, our preliminary data suggests that Hla suppresses Th1 differentiation and effector memory cell induction during primary skin infection. The implication that Th1 responses may drive protective immunity to *S. aureus* skin infection, contrary to current evidence which suggests that IL-17A and Th17 responses are critical for control of *S. aureus* infection during primary and secondary immune responses in the skin(Montgomery et al. 2014; Cho et al. 2010). However, Th1 immunity has been implicated in protection in systemic models of *S. aureus* disease, suggesting that either response can induce protection in mice(Guillén et al. 2002; L. Lin et al. 2009; Brown et al. 2015). Thus, neutralization of Hla appears to be critical in protecting the induction of T cell memory and cytokine responses during skin infection. This implies that immunization against Hla may convert a would-be immune suppressive, *S. aureus* SSTI into a non-symptomatic, immune bolstering encounter, as neutralization of Hla prevents skin dermonecrosis.

Chapter IV

Conclusion

The ability of *S. aureus* to recurrently infect humans at a population level suggests that the adaptive immune system fails to mount protective immunity after the first exposure. While a multitude of virulence factors have been characterized, little attention has been paid to the tissue-specific elements which drive immunity to *S. aureus*. Despite this, clinical evidence suggests that the initial site of infection may predict re-infection outcomes(Fritz et al. 2013). Notably, patients which had first time or recurrent *S. aureus* SSTIs were highly likely to report subsequent infections within the same year(Klevens R et al. 2007; Tong et al. 2015; L. G. Miller et al. 2015). In contrast, patients that underwent invasive disease had re-infection rates comparable to the incidence rate of patients who did not report *S. aureus* infection(Fritz et al. 2013). A previous study also found that patients who reported *S. aureus* SSTIs had dramatically higher re-infection rates than household contacts(L. G. Miller et al. 2015). The clinical data indicate that *S. aureus* SSTIs beget further infections, while invasive disease does not. This suggests that *S. aureus* may have tissue specific mechanisms by which it suppresses adaptive immunity. Our studies have uncovered a novel mechanism of Hla, in which it suppresses T cell responses and the development of protective immunity, likely through the killing of skin resident dendritic cells.

Development of protective immunity is tissue dependent and modulated by Hla

The skin barrier site is tightly regulated, physical barrier that is constantly surveyed by multiple immune cells. Despite this, we and others have reported that primary skin infection with *S. aureus* does not protect C57BL/6 hosts from secondary challenge, a finding which mirrors human patients(Sampedro et al. 2014; Montgomery et al. 2014). Our work demonstrates that primary intravenous, but not skin, infection allows for the development of protective immunity against *S. aureus* skin re-challenge. Protected hosts prevented dermonecrosis and had reduced *S. aureus* CFU at the site of infection. Immunity generated by intravenous challenge was found to be B cell and CD4 T cell dependent. The fact that skin infection does not induce the same protection suggested that *S. aureus* utilized tissue specific mechanisms to impair adaptive immunity. There are few known mechanisms by which *S. aureus* manipulates adaptive immunity: one is through protein A manipulation of the B cell response(Pauli et al. 2014). T cell superantigens are also produced by *S. aureus*, though their relevance in human disease is less clear other than in the extremely rare cases of toxic shock syndrome(Bröker, Mrochen, and Péton 2016; DeVries et al. 2011; Holtfreter et al. 2006). Thus, whether *S. aureus* manipulates CD4 T cell responses via other means remains elusive. Furthermore, the assessment of antigen-specific CD4 T cell responses *in vivo* have been difficult without the availability of a suitable model system. This has remained an outstanding issue in the field despite an increasing demand towards understanding how *S. aureus* may alter adaptive immunity. We circumvent this barrier through the generation of an OVA expressing strain of *S. aureus*, and demonstrate heightened accumulation of OT-IIs in the secondary lymphoid organs at all time points

after *S. aureus* bacteremia compared to skin infection. Furthermore, we show that Th1 priming is not only a feature of intravenous *S. aureus* infection, but that suppression of IFNγ expression is Hla mediated during skin infection. These observations suggest that Hla mediates the suppression of Th1 subset differentiation in antigen-specific T cells during skin infection. As this only occurs during skin infection, Hla must exert its action in a skin tissue specific manner, perhaps by targeting skin specific subsets of immune cells.

Our lab has previously demonstrated that Hla modulates the development of adaptive immunity after primary skin infection(Sampedro et al. 2014). Re-challenge with LAC WT strain of *S. aureus* in mice which were given primary LAC *hla::erm* mutant infection exhibited smaller lesions compared to mice which were administered primary WT infection. This effect was enhanced when the LAC *hla::erm* mutant was complemented with a plasmid that induced expression of an inactive variant of the toxin: Hla_{H35L}. Further studies dissecting the mechanism behind this protection have not been performed. Given the now recognized tissue dependence of immunity, we believe that Hla modulates the development of protective immunity at the skin site of infection, presumably through alteration of the adaptive immune response. As *S. aureus* immunity generated via intravenous infection was CD4 T cell dependent, we hypothesize that the protection afforded by infection with LAC *hla::erm* mutant also requires CD4 T cell help. We will address this through administration of CD4 T cell depleting antibody or isotype control to mice prior to and during skin infection with LAC *hla::erm*. We will then re-challenge them with WT LAC on the skin 30 days post infection. Dissecting the

importance of CD4 T cell help in skin specific immunity to *S. aureus* will further elucidate Hla's role as a suppressor of adaptive immunity.

Memory T cells are required for protection against pathogenic re-challenge in various infection models, and can bolster B cell responses upon re-challenge to produce improved antibody responses(Hale and Ahmed 2015; MacLeod, Kappler, and Marrack 2010). We show that efficient priming of memory OT-IIs occurred only during intravenous infection, despite the initial formation of $CD44^{high}$ cells during primary skin infection. These data indicate that the magnitude of OT-II proliferation and memory induction are tissue dependent. Interestingly, skin infection with LAC *hla::erm*-pMagic-OVA efficiently induced high numbers of effector and central memory T cells: the majority of OT-IIs were effector memory, as also seen in the case of intravenous infection with WT pMagic-OVA. This shows that both T helper subset differentiation and induction of memory T cell responses are impaired by Hla during *S. aureus* skin infection. Though the OT-IIs clearly see antigen, as evidenced by CD44 expression and proliferation, they do not appear to successfully produce pro-inflammatory cytokine profiles. This indicates that Hla suppresses T cell priming during skin infection.

Memory cells induced during intravenous infection were able to accumulate in the skin dLNs. Indeed, during re-infection, a high number of OT-IIs were recovered from the skin draining lymph nodes of mice that were given primary intravenous infection. However, this was not the case with mice that underwent primary skin infection, and these hosts had OT-II accumulation similar to mice that were skin or intravenously infected with *S. aureus* harboring the empty vector pMagic. Interestingly, the few OT-IIs that accumulated in the skin draining lymph node were naïve based on their

$CD44^{low}CD62L^{high}$ phenotype. These data suggest that skin infection failed to induce long lasting memory T cells that could respond to re-call. This observation aligns with both clinical and animal data regarding *S. aureus* SSTIs, as primary SSTI does not protect against secondary challenge. Our data suggest that part of the reason for this failure is a tissue-specific failure of the host to generate protective T cell memory responses after skin infection. Furthermore, the lack of memory cells on secondary skin challenge suggests that OT-IIs which initially responded to the primary skin infection may either have been deleted or rendered anergic. Taken together, our observations highlight a novel function for Hla as a key suppressor of Th1 induction and memory cell formation during skin infection.

Hla manipulates skin specific dendritic cells

Dendritic cells are tasked with relaying critical information from the infection site to the adaptive immune system, including antigen presentation, co-stimulation help and cytokine production. In the skin, the DC compartment is composed of multiple subsets, each with different, though potentially overlapping, function in priming T cell immunity. Our work demonstrates that upon *S. aureus* infection, $CD11b^+$ dDCs and Langerhans cells are reduced in both the skin and skin draining lymph nodes in an Hla dependent manner. Furthermore, we show that migration of skin resident Langerhans cells and $CD11b^+$ dDCs was impaired, and reduced accumulation of $FITC^+$ DCs was drive by Hla. These data demonstrate that Hla impairs trafficking of mature DCs which have been exposed to *S. aureus* antigen. Given that Hla can induce lysis among a variety of host cells, including monocytes and neutrophils, we believe that the loss of skin DCs and lack

of accumulation of specific DC subsets in the sLNs are due to Hla mediated lysis. Hypothetically, this could result in the impaired presentation of *S. aureus* antigen, as skin resident DCs would be unable to traffic antigen from the site of infection to the draining lymph nodes. Though we do not currently demonstrate this, future work will utilize a CD11c-Cre system to specifically delete ADAM10 expression on dendritic cells ($DC^{\Delta ADAM10}$), as previously described(Ezekwe, Weng, and Duncan 2016; S. R. Damle et al. 2018). WT LAC pMagic-OVA infection in $DC^{\Delta ADAM10}$ should prevent Hla mediated damage on dendritic cells, and restore OT-II priming during *S. aureus* skin infection. Combined with the observation that LAC *hla::erm*-pMagic-OVA induced Th1 priming and robust memory induction, DC protection should restore the priming defect observed.

Given our observations, vaccination against Hla should mediate the protection of DC subsets in the skin and draining lymph nodes. Indeed, vaccinated hosts retained all DC subsets in the skin, and improved accumulation of $CD11b^{+}$ dDCs and Langerhans cells in the skin draining lymph nodes, which suggests that they were able to migrate from the skin. We also show that polyclonal immune sera against Hla, and immune murine sera, is sufficient to protect skin DCs, demonstrating that protection of these cells is likely through antibody mediated toxin neutralization. This work illustrates that vaccinating naïve hosts against a single toxin can protect skin dendritic cells from *S. aureus* mediated deletion. As these DCs serve as the first line of defense and messengers of the immune system, it stands to reason that protection of DCs should allow for the efficient priming of T cell immunity during skin infection.

Recent work in the field has also unveiled other toxins which manipulate dendritic cells. For example, Leukocidin A/B (LukAB) was demonstrated to be responsible for the

intoxication of monocyte-derived DCs, and *S. aureus* strains lacking lukAB were severely impaired in their ability to kill phagocytes during a murine infection model. Genetic knockout of lukAB severely inhibited bacterial survival at the site of infection, which correlated with higher numbers of PMNs and macropahges(Dumont et al. 2011). Leukocidin ED was recently confirmed to bind to CCR5 and mediate the killing of $CCR5^+$ T cells and myeloid cells in vivo(Iii et al. 2013). Collectively, *S. aureus* may have more than one means in which to suppress APC function, as multiple toxins have been implicated in DC killing. Other toxins, such as PSM have been implicated in inducing suppressive cytokines in dendritic cells, though this remains to be demonstrated *in vivo*. Given that each encounter with *S. aureus* may be a immune suppressive event, protection of barrier tissue DCs can be key in changing the immune environment to favor the development of protective immunity. Strategies which guarantee symptomatic protection during skin encounter with *S. aureus*, while also protecting the dendritic cell network, may be successful in turning natural encounters with *S. aureus* into immune boosting events.

Implications for vaccines: a naïve approach

Our findings have interesting implications in vaccine strategy, as most vaccine trials to date have been attempted in adults, or have used passive transfer of antibodies isolated from adults with high titers against *S. aureus* antigens(V. G. Fowler and Proctor 2014). Given that *S. aureus* is the leading cause of SSTIs and colonizes over >30% of the human population, it is likely that a substantial portion of previous vaccine trials, which mostly targeted adults, were conducted on exposed hosts(H. F. Wertheim et al. 2004). Based on our observations, we propose that patients that have been infected

with *S. aureus*, perhaps repeatedly, may have a low frequency of *S. aureus*-specific, naïve CD4 T cells that vaccines can utilize to produce high affinity antibody responses. Given the absence of memory T cells upon re-call in hosts that underwent primary skin infection, *S. aureus*-specific CD4 T cells may be rendered anergic or deleted from previous encounters with the pathogen, preventing proper development of a protective immune response during vaccination. Indeed, the one vaccine trial which seemed to suggest some promise was a trial in which humanized, mouse chimeric monoclonal antibody against LTA was passively infused into low birthweight infants, and tested for the prevention of late on-set sepsis(Weisman et al. 2011). In this case, antibody production against *S. aureus* was not done in an environment by which the pathogen could manipulate the immune response. These data suggest that *S. aureus* employs Hla to either delete *S. aureus*-specific CD4 T cells or render them anergic to re-call. This highlights a new *in vivo* mechanism by which *S. aureus* suppresses T cell function and the development of protective immunity. Furthermore, our data describe a previously unrecognized role for Hla as a critical suppressor of adaptive immunity, which may explain its ubiquity, as most clinical strains express Hla(Berube and Bubeck Wardenburg 2013; Sharma-Kuinkel et al. 2015).

Recent work has demonstrated findings in human populations which have not been described in animal models of *S. aureus* infection. Notably, Th1/Th17 cell plasticity has not been fully appreciated in current *S. aureus* studies, but recent human studies of T cell responses to *S. aureus* have observed the presence of IL-17$^+$ IFNγ^+ and IL-17$^+$IL-10$^+$ antigen-specific CD4 T cells in response to skin pathogens, with the latter induced during only during *S. aureus* infection(Zielinski et al. 2012). As both are commensals

which colonizes mucosal barriers, the differences in suppressive cytokine induction point to pathogen specific modulation of the host T cell response. The effect of *S. aureus* colonization on adaptive immunity is poorly understood, and requires new tools to study their responses. Utilizing our pMagic-OVA system, our work provides the groundwork for future studies which can follow antigen specific T cell and B cell responses, through serum anti-OVA responses, during *S. aureus* colonization. Such cellular resolution would then allow one to characterize how colonization shapes adaptive immunity prior to the first infectious or antigenic encounter with *S. aureus*.

Our studies did not observe the induction of IL-10 in OT-IIs in either intravenous or skin infection contexts. This could be due to the absence of prior colonization in our murine hosts, or a physiologic difference between human and murine T cell responses to *S. aureus* infection. However, we did not assess for memory T cell cytokine responses after primary infection. It is possible that our “naïve” T cells after skin infection can produce IL-10 when stimulated with antigen. Alternatively, the memory T cells from primary intravenously challenge mice may produce IL-10 upon re-call. Another distinct possibility is that while murine hosts may respond to weak antigenic stimulus with the deletion of OT-IIs, human T cells may impart regulatory function in otherwise inflammatory Th17 cells, as the factors which regulate the plasticity of T helper subsets is poorly understood. However, what is clear from our studies, and previous human and animal data, is that *S. aureus* utilizes distinct virulence factors to perturb the development of productive T and B cell immunity.

A key consideration that has not been addressed in our studies is the extent to which the effects we observe are exclusive to Hla. Substantial evidence in the field has

demonstrated that Hla, along with other *S. aureus* toxins, are both cell-type and species specific(Wilke and Bubeck Wardenburg 2010b; Tromp et al. 2018; B. Lee and Wardenburg 2018; III et al. 2013a). As the role of Hla in murine skin infection is to induce massive inflammation and dermonecrosis, one should wonder whether the findings we observe are unique to Hla, or can be recapitulated with any virulence factor which can induce massive inflammatory damage or lyse immune cells. As aforementioned, CCR5 was recently identified as the receptor for *S. aureus*, and mediates the killing of myeloid cell subsets and T lymphocytes in mice bearing humanized CCR5(III et al. 2013b). Furthermore, recent work has demonstrated that CD45 is an F-component receptor for PVL, suggesting that general immune cells are also targeted for cell lysis, though the nature in which lymphocytes are affected remains unclear(Tromp et al. 2018; B. Lee and Wardenburg 2018). Given the lytic activity of other virulence factors on immune cells, one must then wonder whether our observations are unique to Hla's lytic effect. Interestingly, gross dermonecrosis and lysis of specific immune cell populations have not been separated in our studies thus far, though current work in underway to specifically delete ADAM10 from dendritic cells. Though we hypothesize that protection of DC lysis will re-capitulate the restoration of T cell responses seen in LAC *hla::erm* pMagicOVA infection, we may find that T cell responses are still suppressed. In this case, we would hypothesize that massive inflammation may suppress the priming of adaptive immunity through local hyper-inflammation or cytokine-storm like responses. Indeed, cytokine storms and other examples of over activation of immunity, such as superantigen activity on T and B cells, have been found to retard immune responses(Goldmann and Medina 2017; Bröker,

Mrochen, and Péton 2016; Holtfreter et al. 2006; Liu, Zhou, and Yang 2016; D'Elia et al. 2013). This possibility leads to the intriguing hypothesis that local tissue tolerance to commensal virulence factors or antigens may be key towards eliciting protective immunity during skin infection. While traditional vaccine approaches have focused on eliciting protective immunity, pathogens which have been targeted do not colonize the human host. However, commensals are thought to be in continuous contact with the host during homeostasis, and can stimulate immunity in lieu of infection(Scharschmidt et al. 2015; Naik et al. 2015; Manicassamy and Pulendran 2011). Thus, purely pro-inflammatory strategies against *S. aureus* may result in aberrant inflammation at any barrier tissue sites where *S. aureus* is present. This outcome may be conceptually similar to what we observe in psoriasis patients, such that *S. aureus* is often found on lesional skin sites(Balci et al. 2009; Hasse-Cieślińska 2007). Perhaps the appropriate vaccine strategy would not only induce systemic pro-inflammatory, adaptive immunity to the invading commensal, but also elicit tissue specific tolerance to commensal antigens. This strategy allows for the induction of sterilizing immunity in systemic regions, where the commensal should not be present, while also imparting tolerance to the commensal at native colonization sites. Rational vaccine design should consider how to maintain host homeostasis with the commensal in question.

Taken together, our insights point to the importance of targeting future vaccine trials at patient populations, which are the least likely to have had infectious encounter with *S. aureus*. In such patients, *S. aureus*-specific CD4 T cells will be naïve to pathogenic encounter and will be readily utilized by vaccine candidates. By protecting hosts against dermonecrosis and DC loss, subsequent subclinical encounters with *S.*

aureus in vaccinated hosts will no longer suppress adaptive immunity. Instead, such challenges may serve as boosters to a prepared host, further priming the adaptive immune response to the plethora of *S. aureus* virulence factors.

CHAPTER V

Experimental Methods

Ethics Statement

Animal studies were conducted in accord with protocols approved by the Institutional Animal Care and Use Committees at both the University of Chicago and the Washington University in St. Louis.

Mice.

All animal experiments were approved by the University of Chicago Institutional Animal Care and Use Committee (IACUC) or Washington University in St. Louis IACUC. Mice were housed in specific-pathogen-free animal facilities at University of Chicago or Washington University in St. Louis (WashU). C57BL/6J mice were purchased from Jackson Laboratories. μMT mice were generously provided by Michael Diamond (WashU). OT-II CD45.1 mice were kindly gifted by Anne Sperling (University of Chicago).

Bacterial strains and cultures.

For infections, MRSA strain USA300/LAC was grown overnight (O/N) at 37°C in tryptic soy broth (TSB, Sigma) on a rotator, and was subcultured at 1:100 dilution for 1.5 hours in TSB to mid-log phase. USA300/LAC *hla::erm* was grown O/N at 37°C in TSB supplemented with 40 μg/ml erythromycin on rotation, and subcultured with TSB

supplemented with erythromycin for 2.5 hours. USA300/LAC pMagic, USA300/LAC pMagicOVA, and USA300/LAC *hla::erm* pMagicOVA were grown overnight in TSB supplemented with 20 μg/ml chloramphenicol on rotation, and subcultured at 1:100 dilution in 20 μg/ml chloramphenicol for 2 hours. All subcultures were performed with shaking at 200 r.p.m at 37°C. For all strains, OD600 was measured to estimate bacterial density, and verified by serial plating on tryptic soy agar (TSA) plates to quantify CFU values.

Bacterial bacteremia and skin infections.

For all infections, age-matched 4-6 week-old males and females were studied. For subcutaneous infections, 50 μl containing 1.0 x 10^8 CFU USA300/LAC or LAC *hla::erm* in PBS was inoculated per mouse. For intravenous infections, 100 ul containing 5.0 x 10^6 CFU USA300/LAC in PBS was retro-orbitally infected per mouse. Control animals were injected with equivalent volumes of PBS only. In experiments utilizing *S. aureus* strains harboring pMagic or pMagicOVA, 0.5 mg/ml chloramphenicol and 1% sucrose solution drinking water was given to mice one day prior to subcutaneous or intravenous infection. 100,000 CD45.1 OT-II cells were also adoptively transferred, via retro-orbital injection, one day prior to infection. On designated days post re-challenge, skin lesions were punch biopsied with an 8 mm punch biopsy (Integra Miltex), and placed in 1 ml of 0.1% Triton-X PBS solution in cryogenic tubes. All lesions were then immediately processed using the Bio-Gen Pro200 Homogenizer (Pro Scientific). Homogenates were plated on TSA plates to quantify CFU values.

Skin histology.

8 mm punch biopsies of skin lesions were extracted from mice after euthanasia, then fixed and stored in 10% formalin (VWR, Avantor). Saples were embedded, sectioned and stained with H&E by Nationwide Histology LLC.

Immune cell depletion.

For CD4 T cell depletion, 100 μg of anti-CD4 antibody (clone GK1.5, University of Chicago) were intraperitoneally (i.p) injected 6 and 3 days prior to infection, and every 3 days post infection for 15 days. Control mice received 100 μg of rat IgG2b isotype control (University of Chicago) on the same timeline.

Tissue Isolation and Flow Cytometric Staining

Skin lesions were punch biopsied, and blunt removal of fat tissue was done with forceps. Then, tissue was minced and digested in RPMI (Gibco) containing 0.25 mg/ml Liberase TL (Roche Diagnostic Corp), 100 μM β-mercaptoethanol, 20 μM HEPES (Hyclone), 100 U/ml penicillin and 100 μg/ml streptomycin, and incubated for 2-3 hours at 37°C and 5% CO_2. For dendritic cell studies, draining axillary, brachial and subinguinal lymph nodes were extracted and digested with 0.1 mg/ml Liberase TL (Roche), 100 μM β-mercaptoethanol, 20 μM HEPES (Hyclone), 100 U/ml penicillin and 100 μg/ml streptomycin, and incubated for 30 minutes at 37°C and 5% CO_2. Tissue and

lymph nodes were gently strained through a 40 μm cell strainer in single cell suspensions, and then incubate with FC block (anti-CD16/32) in FACs buffer (1% BSA, 0.1% NaN3, 5 mM EDTA) for 15 minutes on ice. Cells were stained in FACs buffer on ice for 30-45 minutes and then analyzed on the cytometer, or fixed in 1% paraformaldehyde solution. For tissue, live-dead staining was performed before FC block. Briefly, cells were incubated with Live Dead Fixable Aqua (Thermo Fischer) at 1:1000 in PBS for 30 minutes at 4°C in the dark. Cells were washed twice in FACs buffer before incubation with FC block.

Flow cytometric analysis was performed on the BD LSR Fortessa II. Murine cell suspensions were incubated with fluorochrome conjugated antibodies against the following surface markers: CD45 (30-F11), CD45.1 (A20), CD62L (MEL-14), CD4 (RM4-4, GK1.5), TCRβ(H57-597), CD44 (IM7), CD8b (Ly-3), MHCII I-A/I-E (M5/114.15.2), CD103 (2E7), CD207/Langerin (4C7), CD11c (N418), CD11b (M1/70), anti-CD16/32 (2.4G2). Cell counts were enumerated using AccuCount beads (Spherotech) according to manufacturer instructions. Live dead analysis was performed using LIVE/DEAD Fixable Aqua (Life Technologies).

For intracellular cytokine staining, stimulated cells were fixed and permeabilized using BD cytoperm/fix kit reagents according to manufacturer instructions. Cells were then incubated with fluorochrome conjugated antibodies against IL-4 (11B11), IL-17A (TC11-18H10.1) , IFNγ (XMG1.2), and IL-10 (JES5-16E3) in BD Perm Buffer for 1 hour at 4°C.

In vitro Re-stimulation

Murine skin draining lymph nodes and spleens were gently homogenized into single cell suspensions. To positively select donor OT-II cells, CD45.1-biotin antibodies (eBioscience) were incubated for 30 minutes on ice. Streptavidin microbeads at 1:100 dilution (Miltenyi) were subsequently incubated in cell suspensions for 30 minutes on ice prior to LS column sorting (Miltenyi). The positive fraction was cultured in complete RPMI (100 μM β-mercaptoethanol, 20 μM HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin, 1X GlutaMAX (Gibco)) supplemented with phobol 12-myristate-13-acetate (PMA; 50ng/ml) and ionomycin (500 ng/ml) for 4-5 hours at 37°C, 5% CO_2. To detect intracellular cytokine production, Golgi Stop and Plug (BD Bioscience) at a final concentration of 0.7 μl/ml and 1 μl/ml, respectively.

Adoptive Transfer and *S. aureus* pMagic-OVA infections

One day prior to infection, lymph nodes and spleens from OT-II CD45.1 males were harvested for CD4 T cell isolation. To extract OT-IIs, the $CD4^+$ T cell Isolation Kit (Miltenyi) was used according to manufacturer instructions. Approximately 100,000 OT-II cells were suspended in PBS and then retro-orbitally transferred into recipient, 5 week old C57BL/6 males (CD45.2). Drinking water was supplemented with chloramphenicol (250 mg/bottle). The next day, recipients were infected either subcutaneously or retro-orbitally with 1×10^8 or 5×10^6 *S. aureus* pMagic-OVA, respectively. For memory experiments, water was switched back to normal drinking on day 14 of the experiment.

On day 40 post primary infection, mice were re-challenged subcutaneously with 1 x 10^8 CFU *S. aureus* pMagic-OVA, and both the infection site and draining lymph nodes were harvested 3 days later to assess re-called memory OT-II cells.

In vivo dendritic cell migration

One day prior to infection, mice were shaved, and Nair was applied to the skin for 20-30 seconds. A wet towel was applied to the affected side to remove hair and excess Nair. On the day of infection, 20 μl of 2% FITC solution in acetone was suspended onto to flank skin of mice. The paint solution was allowed to quickly dry prior to intradermal infection with *S. aureus* at the painted site. Four days post infection, the skin draining lymph nodes were harvested and processed as aforementioned, and dendritic cells were enriched through LS column positive selection. Briefly, cells were incubated with anti-CD16/32 antibody prior to incubation with biotinylated anti-CD11c (N418, eBioscience™) antibodies for 30 minutes on ice. Cells were then washed with FACs buffer and resuspended in FACs buffer and streptavidin beads for an additional 30 minutes on ice prior to column sorting. Cells were then stained, and live, $FITC^+$ migratory dendritic cells were analyzed and quantified by flow cytometry. Quantification was performed using AccuCount beads (Spherotech).

Bibliography

Abeyama, K., W. Eng, J. V. Jester, A. A. Vink, D. Edelbaum, C. J. Cockerell, P. R. Bergstresser, and A. Takashima. 2000. “A Role for NF-KappaB-Dependent Gene Transactivation in Sunburn.” *The Journal of Clinical Investigation* 105 (12): 1751–59. https://doi.org/10.1172/JCI9745.

Adhikari, Rajan P., Adebola O. Ajao, M. Javad Aman, Hatice Karauzum, Jawad Sarwar, Alison D. Lydecker, J. Kristie Johnson, Chinh Nguyen, Wilbur H. Chen, and Mary-Claire Roghmann. 2012. “Lower Antibody Levels to Staphylococcus Aureus Exotoxins Are Associated with Sepsis in Hospitalized Adults with Invasive S. Aureus Infections.” *The Journal of Infectious Diseases* 206 (6): 915–23. https://doi.org/10.1093/infdis/jis462.

Ahmad-Nejad, Parviz, Salima Mrabet-Dahbi, Kristine Breuer, Martina Klotz, Thomas Werfel, Udo Herz, Klaus Heeg, Michael Neumaier, and Harald Renz. 2004. “The Toll-like Receptor 2 R753Q Polymorphism Defines a Subgroup of Patients with Atopic Dermatitis Having Severe Phenotype.” *The Journal of Allergy and Clinical Immunology* 113 (3): 565–67.

al, Liu T., et. n.d. “Staphylococcal Enterotoxin B Increases TIM4 Expression in Human Dendritic Cells That Drives Naïve CD4 T Cells to Differentiate into Th2 Cells. - PubMed - NCBI.” Accessed May 21, 2018. https://www.ncbi.nlm.nih.gov/pubmed/17439824/.

Alegre, Maria-Luisa, Luqiu Chen, Michael Z. David, Caroline Bartman, Susan Boyle-Vavra, Neha Kumar, Anita S. Chong, and Robert S. Daum. 2016. “Impact of

Staphylococcus Aureus USA300 Colonization and Skin Infections on Systemic Immune Responses in Humans." *The Journal of Immunology* 197 (4): 1118–26. https://doi.org/10.4049/jimmunol.1600549.

Arbuthnott, J. P., J. H. Freer, and A. W. Bernheimer. 1967. "Physical States of Staphylococcal Alpha-Toxin." *Journal of Bacteriology* 94 (4): 1170–77.

Arbuthnott, J. P., J. H. Freer, and A. C. McNiven. 1973. "Physical Properties of Staphylococcal Alpha-Toxin and Aspects of Alpha-Toxin Membrane Interactions." *Contributions to Microbiology and Immunology* 1: 285–97.

Bagnoli, Fabio, Maria Rita Fontana, Elisabetta Soldaini, Ravi P. N. Mishra, Luigi Fiaschi, Elena Cartocci, Vincenzo Nardi-Dei, et al. 2015. "Vaccine Composition Formulated with a Novel TLR7-Dependent Adjuvant Induces High and Broad Protection against Staphylococcus Aureus." *Proceedings of the National Academy of Sciences of the United States of America* 112 (12): 3680–85. https://doi.org/10.1073/pnas.1424924112.

Balci, Didem Didar, Nizami Duran, Burcin Ozer, Ramazan Gunesacar, Yusuf Onlen, and Julide Zehra Yenin. 2009. "High Prevalence of Staphylococcus Aureus Cultivation and Superantigen Production in Patients with Psoriasis." *European Journal of Dermatology: EJD* 19 (3): 238–42. https://doi.org/10.1684/ejd.2009.0663.

Barnden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. "Defective TCR Expression in Transgenic Mice Constructed Using CDNA-Based Alpha- and Beta-Chain Genes under the Control of Heterologous Regulatory Elements."

Immunology and Cell Biology 76 (1): 34–40. https://doi.org/10.1046/j.1440-1711.1998.00709.x.

Baumjohann, Dirk, and K. Mark Ansel. 2013. “Identification of T Follicular Helper (Tfh) Cells by Flow Cytometry,” June. https://www.nature.com/protocolexchange/protocols/2707.

Bedoui, Sammy, Paul G. Whitney, Jason Waithman, Liv Eidsmo, Linda Wakim, Irina Caminschi, Rhys S. Allan, et al. 2009. “Cross-Presentation of Viral and Self Antigens by Skin-Derived CD103+ Dendritic Cells.” *Nature Immunology* 10 (5): 488–95. https://doi.org/10.1038/ni.1724.

Belkaid, Yasmine, and Kristin Tarbell. 2009. “Regulatory T Cells in the Control of Host-Microorganism Interactions (*).” *Annual Review of Immunology* 27: 551–89. https://doi.org/10.1146/annurev.immunol.021908.132723.

Belz, Gabrielle T. 2008. “Getting Together: Dendritic Cells, T Cells, Collaboration and Fates.” *Immunology and Cell Biology* 86 (4): 310–11. https://doi.org/10.1038/icb.2008.18.

Bertholet, Sylvie, Alain Debrabant, Farhat Afrin, Elisabeth Caler, Susana Mendez, Khaled S. Tabbara, Yasmine Belkaid, and David L. Sacks. 2005. “Antigen Requirements for Efficient Priming of CD8+ T Cells by Leishmania Major-Infected Dendritic Cells.” *Infection and Immunity* 73 (10): 6620–28. https://doi.org/10.1128/IAI.73.10.6620-6628.2005.

Berube, Bryan J., and Juliane Bubeck Wardenburg. 2013. “Staphylococcus Aureus α-Toxin: Nearly a Century of Intrigue.” *Toxins* 5 (6): 1140–66.

Bhakdi, S., M. Muhly, S. Korom, and F. Hugo. 1989. “Release of Interleukin-1 Beta Associated with Potent Cytocidal Action of Staphylococcal Alpha-Toxin on Human Monocytes.” *Infection and Immunity* 57 (11): 3512–19.

Bigley, Venetia, Naomi McGovern, Paul Milne, Rachel Dickinson, Sarah Pagan, Sharon Cookson, Muzlifah Haniffa, and Matthew Collin. 2015. “Langerin-Expressing Dendritic Cells in Human Tissues Are Related to CD1c+ Dendritic Cells and Distinct from Langerhans Cells and CD141high XCR1+ Dendritic Cells.” *Journal of Leukocyte Biology* 97 (4): 627–34. https://doi.org/10.1189/jlb.1HI0714-351R.

Blattman, Joseph N., Rustom Antia, David J. D. Sourdive, Xiaochi Wang, Susan M. Kaech, Kaja Murali-Krishna, John D. Altman, and Rafi Ahmed. 2002. “Estimating the Precursor Frequency of Naive Antigen-Specific CD8 T Cells.” *The Journal of Experimental Medicine* 195 (5): 657–64.

Bobr, Aleh, Irlanda Olvera-Gomez, Botond Z. Igyarto, Krystal M. Haley, Kristin A. Hogquist, and Daniel H. Kaplan. 2010. “Acute Ablation of Langerhans Cells Enhances Skin Immune Responses.” *Journal of Immunology (Baltimore, Md.: 1950)* 185 (8): 4724–28. https://doi.org/10.4049/jimmunol.1001802.

Boguniewicz, Mark, and Donald Y. M. Leung. 2011. “Atopic Dermatitis: A Disease of Altered Skin Barrier and Immune Dysregulation.” *Immunological Reviews* 242 (1): 233–46. https://doi.org/10.1111/j.1600-065X.2011.01027.x.

Boles, James W., M. Louise M. Pitt, Ross D. LeClaire, Paul H. Gibbs, Edna Torres, Beverly Dyas, Robert G. Ulrich, and Sina Bavari. 2003. “Generation of Protective Immunity by Inactivated Recombinant Staphylococcal Enterotoxin B Vaccine in

Nonhuman Primates and Identification of Correlates of Immunity.” *Clinical Immunology (Orlando, Fla.)* 108 (1): 51–59.

Bournazos, Stylianos, Anna Gazumyan, Michael S. Seaman, Michel C. Nussenzweig, and Jeffrey V. Ravetch. 2016. “Bispecific Anti-HIV-1 Antibodies with Enhanced Breadth and Potency.” *Cell* 165 (7): 1609–20. https://doi.org/10.1016/j.cell.2016.04.050.

Bournazos, Stylianos, Florian Klein, John Pietzsch, Michael S. Seaman, Michel C. Nussenzweig, and Jeffrey V. Ravetch. 2014. “Broadly Neutralizing Anti-HIV-1 Antibodies Require Fc Effector Functions for in Vivo Activity.” *Cell* 158 (6): 1243–53. https://doi.org/10.1016/j.cell.2014.08.023.

Bouvet, Cindy, Shpresa Gjoni, Besa Zenelaj, Benjamin A. Lipsky, Elif Hakko, and Ilker Uçkay. 2017. “Staphylococcus Aureus Soft Tissue Infection May Increase the Risk of Subsequent Staphylococcal Soft Tissue Infections.” *International Journal of Infectious Diseases* 60 (July): 44–48. https://doi.org/10.1016/j.ijid.2017.05.002.

Brewig, Nancy, Adrien Kissenpfennig, Bernard Malissen, Alexandra Veit, Thomas Bickert, Bernhard Fleischer, Sven Mostböck, and Uwe Ritter. 2009. “Priming of CD8+ and CD4+ T Cells in Experimental Leishmaniasis Is Initiated by Different Dendritic Cell Subtypes.” *Journal of Immunology (Baltimore, Md.: 1950)* 182 (2): 774–83.

Bröker, Barbara M., Daniel Mrochen, and Vincent Péton. 2016. “The T Cell Response to Staphylococcus Aureus.” *Pathogens* 5 (1). https://doi.org/10.3390/pathogens5010031.

Bronte, Vincenzo, and Mikael J. Pittet. 2013. “The Spleen in Local and Systemic Regulation of Immunity.” *Immunity* 39 (5): 806–18. https://doi.org/10.1016/j.immuni.2013.10.010.

Broom, Jennifer K., Andrew M. Lew, Hiroaki Azukizawa, Tony J. Kenna, Graham R. Leggatt, and Ian H. Frazer. 2010. “Antigen-Specific CD4 Cells Assist CD8 T-Effector Cells in Eliminating Keratinocytes.” *Journal of Investigative Dermatology* 130 (6): 1581–89. https://doi.org/10.1038/jid.2010.17.

Brown, Aisling F., Alison G. Murphy, Stephen J. Lalor, John M. Leech, Kate M. O’Keeffe, Micheál Mac Aogáin, Dara P. O’Halloran, et al. 2015. “Memory Th1 Cells Are Protective in Invasive Staphylococcus Aureus Infection.” *PLoS Pathog* 11 (11): e1005226. https://doi.org/10.1371/journal.ppat.1005226.

Bruton, O. C. 1952. “Agammaglobulinemia.” *Pediatrics* 9 (6): 722–28.

Bubeck Wardenburg, Juliane, and Olaf Schneewind. 2008. “Vaccine Protection against Staphylococcus Aureus Pneumonia.” *The Journal of Experimental Medicine* 205 (2): 287–94. https://doi.org/10.1084/jem.20072208.

Buerke, Michael, Ulf Sibelius, Ulrich Grandel, Ute Buerke, Friedrich Grimminger, Werner Seeger, Jürgen Meyer, and Harald Darius. 2002. “Staphylococcus Aureus Alpha Toxin Mediates Polymorphonuclear Leukocyte-Induced Vasocontraction and Endothelial Dysfunction.” *Shock (Augusta, Ga.)* 17 (1): 30–35.

Butterly, Arielle, Ulrich Schmidt, and Jeanine Wiener-Kronish. 2010. “Methicillin-Resistant Staphylococcus Aureus Colonization, Its Relationship to Nosocomial Infection, and Efficacy of Control Methods.” *Anesthesiology: The Journal of the*

American Society of Anesthesiologists 113 (6): 1453–59. https://doi.org/10.1097/ALN.0b013e3181fcf671.

Cassidy, P., and S. Harshman. 1976. “Studies on the Binding of Staphylococcal 125I-Labeled Alpha-Toxin to Rabbit Erythrocytes.” *Biochemistry* 15 (11): 2348–55.

“Cell Atlas - ADAM10 - The Human Protein Atlas.” n.d. Accessed April 27, 2018. https://www.proteinatlas.org/ENSG00000137845-ADAM10/cell.

Chambers, H. F., R. T. Miller, and M. D. Newman. 1988. “Right-Sided Staphylococcus Aureus Endocarditis in Intravenous Drug Abusers: Two-Week Combination Therapy.” *Annals of Internal Medicine* 109 (8): 619–24.

Chang, Feng-Yee, James E. Peacock, Daniel M. Musher, Patricia Triplett, Brent B. MacDonald, Joseph M. Mylotte, Alice O’Donnell, Marilyn M. Wagener, and Victor L. Yu. 2003. “Staphylococcus Aureus Bacteremia: Recurrence and the Impact of Antibiotic Treatment in a Prospective Multicenter Study.” *Medicine* 82 (5): 333. https://doi.org/10.1097/01.md.0000091184.93122.09.

Charles A Janeway, Jr, Paul Travers, Mark Walport, and Mark J. Shlomchik. 2001. “B-Cell Activation by Armed Helper T Cells.” https://www.ncbi.nlm.nih.gov/books/NBK27142/.

Cheung, A. L., Y. T. Chien, and A. S. Bayer. 1999. “Hyperproduction of Alpha-Hemolysin in a SigB Mutant Is Associated with Elevated SarA Expression in Staphylococcus Aureus.” *Infection and Immunity* 67 (3): 1331–37.

Cheung, A. L., K. J. Eberhardt, E. Chung, M. R. Yeaman, P. M. Sullam, M. Ramos, and A. S. Bayer. 1994. “Diminished Virulence of a Sar-/Agr- Mutant of

Staphylococcus Aureus in the Rabbit Model of Endocarditis." *The Journal of Clinical Investigation* 94 (5): 1815–22. https://doi.org/10.1172/JCI117530.

Cho, John S., Eric M. Pietras, Nairy C. Garcia, Romela Irene Ramos, David M. Farzam, Holly R. Monroe, Julie E. Magorien, et al. 2010. "IL-17 Is Essential for Host Defense against Cutaneous Staphylococcus Aureus Infection in Mice." *The Journal of Clinical Investigation* 120 (5): 1762–73. https://doi.org/10.1172/JCI40891.

Choi, Seng Jin, Min-Hye Kim, Jinseong Jeon, Oh Youn Kim, Youngwoo Choi, Jihye Seo, Sung-Wook Hong, et al. 2015. "Active Immunization with Extracellular Vesicles Derived from Staphylococcus Aureus Effectively Protects against Staphylococcal Lung Infections, Mainly via Th1 Cell-Mediated Immunity." *PloS One* 10 (9): e0136021. https://doi.org/10.1371/journal.pone.0136021.

Chu, C. C., P. Meglio Di, and F. O. Nestle. 2011. "Harnessing Dendritic Cells in Inflammatory Skin Diseases., Harnessing Dendritic Cells in Inflammatory Skin Diseases." *Seminars in Immunology, Seminars in Immunology* 23, 23 (1, 1): 28, 28–41. https://doi.org/10.1016/j.smim.2011.01.006, 10.1016/j.smim.2011.01.006.

Chu, Chung-Ching, Niwa Ali, Panagiotis Karagiannis, Paola Di Meglio, Ania Skowera, Luca Napolitano, Guillermo Barinaga, et al. 2012. "Resident CD141 (BDCA3)+ Dendritic Cells in Human Skin Produce IL-10 and Induce Regulatory T Cells That Suppress Skin Inflammation." *The Journal of Experimental Medicine* 209 (5): 935–45. https://doi.org/10.1084/jem.20112583.

Clausen, Björn E., and Patrizia Stoitzner. 2015. “Functional Specialization of Skin Dendritic Cell Subsets in Regulating T Cell Responses.” *Frontiers in Immunology* 6 (October). https://doi.org/10.3389/fimmu.2015.00534.

Collin, Matthew, and Paul Milne. 2016. “Langerhans Cell Origin and Regulation.” *Current Opinion in Hematology* 23 (1): 28–35. https://doi.org/10.1097/MOH.0000000000000202.

Cook, Matthew C., and Stuart G. Tangye. 2009. “Primary Immune Deficiencies Affecting Lymphocyte Differentiation: Lessons from the Spectrum of Resulting Infections.” *International Immunology* 21 (9): 1003–11. https://doi.org/10.1093/intimm/dxp076.

Craven, Robin R., Xi Gao, Irving C. Allen, Denis Gris, Juliane Bubeck Wardenburg, Erin McElvania-Tekippe, Jenny P. Ting, and Joseph A. Duncan. 2009. “Staphylococcus Aureus Alpha-Hemolysin Activates the NLRP3-Inflammasome in Human and Mouse Monocytic Cells.” *PloS One* 4 (10): e7446. https://doi.org/10.1371/journal.pone.0007446.

Crotty, Shane. 2014. “T Follicular Helper Cell Differentiation, Function, and Roles in Disease.” *Immunity* 41 (4): 529–42. https://doi.org/10.1016/j.immuni.2014.10.004.

Damle, S. R., R. K. Martin, C. L. Cockburn, J. C. Lownik, J. A. Carlyon, A. D. Smith, and D. H. Conrad. 2018. “ADAM10 and Notch1 on Murine Dendritic Cells Control the Development of Type 2 Immunity and IgE Production.” *Allergy* 73 (1): 125–36. https://doi.org/10.1111/all.13261.

Damle, Sheela, Rebecca Martin, and Daniel H. Conrad. 2016. “Loss of A Disintegrin And Metalloproteinase 10 (ADAM10) in Dendritic Cells Dampens Type 2 Immune Responses.” *The Journal of Immunology* 196 (1 Supplement): 191.17-191.17.

Davis, Kepler A., Justin J. Stewart, Helen K. Crouch, Christopher E. Florez, and Duane R. Hospenthal. 2004. “Methicillin-Resistant Staphylococcus Aureus (MRSA) Nares Colonization at Hospital Admission and Its Effect on Subsequent MRSA Infection.” *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 39 (6): 776–82. https://doi.org/10.1086/422997.

D’Elia, Riccardo V., Kate Harrison, Petra C. Oyston, Roman A. Lukaszewski, and Graeme C. Clark. 2013. “Targeting the ‘Cytokine Storm’ for Therapeutic Benefit.” *Clinical and Vaccine Immunology : CVI* 20 (3): 319–27. https://doi.org/10.1128/CVI.00636-12.

DeVries, Aaron S., Lindsey Lesher, Patrick M. Schlievert, Tyson Rogers, Lourdes G. Villaume, Richard Danila, and Ruth Lynfield. 2011. “Staphylococcal Toxic Shock Syndrome 2000-2006: Epidemiology, Clinical Features, and Molecular Characteristics.” *PloS One* 6 (8): e22997. https://doi.org/10.1371/journal.pone.0022997.

Dudda, Jan C., Nikole Perdue, Eva Bachtanian, and Daniel J. Campbell. 2008. “Foxp3+ Regulatory T Cells Maintain Immune Homeostasis in the Skin.” *Journal of Experimental Medicine* 205 (7): 1559–65. https://doi.org/10.1084/jem.20072594.

Dumont, Ashley L., Tyler K. Nygaard, Robert L. Watkins, Amanda Smith, Lina Kozhaya, Barry N. Kreiswirth, Bo Shopsin, Derya Unutmaz, Jovanka M. Voyich, and Victor J. Torres. 2011. “Characterization of a New Cytotoxin That Contributes to

Staphylococcus Aureus Pathogenesis." *Molecular Microbiology* 79 (3): 814–25. https://doi.org/10.1111/j.1365-2958.2010.07490.x.

Eiff, C. von, K. Becker, K. Machka, H. Stammer, and G. Peters. 2001. "Nasal Carriage as a Source of Staphylococcus Aureus Bacteremia. Study Group." *The New England Journal of Medicine* 344 (1): 11–16. https://doi.org/10.1056/NEJM200101043440102.

Elinav, Eran, Till Strowig, Jorge Henao-Mejia, and Richard A. Flavell. 2011. "Regulation of the Antimicrobial Response by NLR Proteins." *Immunity* 34 (5): 665–79. https://doi.org/10.1016/j.immuni.2011.05.007.

Ellis, Michael W., Duane R. Hospenthal, David P. Dooley, Paula J. Gray, and Clinton K. Murray. 2004. "Natural History of Community-Acquired Methicillin-Resistant Staphylococcus Aureus Colonization and Infection in Soldiers." *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 39 (7): 971–79. https://doi.org/10.1086/423965.

Etz, Hildegard, Duc Bui Minh, Tamás Henics, Agnieszka Dryla, Birgit Winkler, Christine Triska, Aoife P. Boyd, et al. 2002. "Identification of in Vivo Expressed Vaccine Candidate Antigens from Staphylococcus Aureus." *Proceedings of the National Academy of Sciences of the United States of America* 99 (10): 6573–78. https://doi.org/10.1073/pnas.092569199.

"Expression of Thrombospondin in TGFβ-Treated APCs and Its Relevance to Their Immune Deviation-Promoting Properties | The Journal of Immunology." n.d. Accessed May 19, 2018. http://www.jimmunol.org.beckerproxy.wustl.edu/content/168/5/2264.

Eyerich, Stefanie, Kilian Eyerich, Claudia Traidl-Hoffmann, and Tilo Biedermann. 2018. "Cutaneous Barriers and Skin Immunity: Differentiating A Connected Network." *Trends in Immunology*, Special Tissues: Local Immune Responses, 39 (4): 315–27. https://doi.org/10.1016/j.it.2018.02.004.

Ezekwe, Ejiofor A.D., Chengyu Weng, and Joseph A. Duncan. 2016. "ADAM10 Cell Surface Expression but Not Activity Is Critical for Staphylococcus Aureus α-Hemolysin-Mediated Activation of the NLRP3 Inflammasome in Human Monocytes." *Toxins* 8 (4). https://doi.org/10.3390/toxins8040095.

Fattom, A. I., J. Sarwar, A. Ortiz, and R. Naso. 1996. "A Staphylococcus Aureus Capsular Polysaccharide (CP) Vaccine and CP-Specific Antibodies Protect Mice against Bacterial Challenge." *Infection and Immunity* 64 (5): 1659–65.

Fattom, A., R. Schneerson, S. C. Szu, W. F. Vann, J. Shiloach, W. W. Karakawa, and J. B. Robbins. 1990. "Synthesis and Immunologic Properties in Mice of Vaccines Composed of Staphylococcus Aureus Type 5 and Type 8 Capsular Polysaccharides Conjugated to Pseudomonas Aeruginosa Exotoxin A." *Infection and Immunity* 58 (7): 2367–74.

Fattom, Ali, Steve Fuller, Myra Propst, Scott Winston, Larry Muenz, David He, Robert Naso, and Gary Horwith. 2004. "Safety and Immunogenicity of a Booster Dose of Staphylococcus Aureus Types 5 and 8 Capsular Polysaccharide Conjugate Vaccine (StaphVAX) in Hemodialysis Patients." *Vaccine* 23 (5): 656–63. https://doi.org/10.1016/j.vaccine.2004.06.043.

Flacher, Vincent, Christoph H. Tripp, David G. Mairhofer, Ralph M. Steinman, Patrizia Stoitzner, Juliana Idoyaga, and Nikolaus Romani. 2014. "Murine Langerin+

Dermal Dendritic Cells Prime CD8+ T Cells While Langerhans Cells Induce Cross-tolerance." *EMBO Molecular Medicine* 6 (9): 1191–1204. https://doi.org/10.15252/emmm.201303283.

Ford, Mandy L., Brent H. Koehn, Maylene E. Wagener, Wanhong Jiang, Shivaprakash Gangappa, Thomas C. Pearson, and Christian P. Larsen. 2007. "Antigen-Specific Precursor Frequency Impacts T Cell Proliferation, Differentiation, and Requirement for Costimulation." *The Journal of Experimental Medicine* 204 (2): 299–309. https://doi.org/10.1084/jem.20062319.

Foster, Timothy J. 2005. "Immune Evasion by Staphylococci." *Nature Reviews. Microbiology* 3 (12): 948–58. https://doi.org/10.1038/nrmicro1289.

Fowler, V. G., and R. A. Proctor. 2014. "Where Does a Staphylococcus Aureus Vaccine Stand?" *Clinical Microbiology and Infection* 20 (May): 66–75. https://doi.org/10.1111/1469-0691.12570.

Fowler, Vance G., Keith B. Allen, Edson D. Moreira, Moustafa Moustafa, Frank Isgro, Helen W. Boucher, G. Ralph Corey, et al. 2013. "Effect of an Investigational Vaccine for Preventing Staphylococcus Aureus Infections after Cardiothoracic Surgery: A Randomized Trial." *JAMA* 309 (13): 1368–78. https://doi.org/10.1001/jama.2013.3010.

Freer, J. H., J. P. Arbuthnott, and A. W. Bernheimer. 1968. "Interaction of Staphylococcal Alpha-Toxin with Artificial and Natural Membranes." *Journal of Bacteriology* 95 (3): 1153–68.

Freer, J. H., J. P. Arbuthnott, and B. Billcliffe. 1973. "Effects of Staphylococcal -Toxin on the Structure of Erythrocyte Membranes: A Biochemical and Freeze-Etching

Study.” *Journal of General Microbiology* 75 (2): 321–32. https://doi.org/10.1099/00221287-75-2-321.

Fritz, Stephanie A., Emma K. Epplin, Jane Garbutt, and Gregory A. Storch. 2009. “SKIN INFECTION IN CHILDREN COLONIZED WITH COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS.” *The Journal of Infection* 59 (6): 394–401. https://doi.org/10.1016/j.jinf.2009.09.001.

Fritz, Stephanie A., Kristin M. Tiemann, Patrick G. Hogan, Emma K. Epplin, Marcela Rodriguez, Duha N. Al-Zubeidi, Juliane Bubeck Wardenburg, and David A. Hunstad. 2013. “A Serologic Correlate of Protective Immunity Against Community-Onset Staphylococcus Aureus Infection.” *Clinical Infectious Diseases* 56 (11): 1554–61. https://doi.org/10.1093/cid/cit123.

Frodermann, Vanessa, Thu A. Chau, Samar Sayedyahossein, Judit M. Toth, David E. Heinrichs, and Joaquín Madrenas. 2011. “A Modulatory Interleukin-10 Response to Staphylococcal Peptidoglycan Prevents Th1/Th17 Adaptive Immunity to Staphylococcus Aureus.” *The Journal of Infectious Diseases* 204 (2): 253–62. https://doi.org/10.1093/infdis/jir276.

Gaidamakova, Elena K., Ian A. Myles, Dennis P. McDaniel, Cedar J. Fowler, Patricia A. Valdez, Shruti Naik, Manoshi Gayen, et al. 2012. “Preserving Immunogenicity of Lethally Irradiated Viral and Bacterial Vaccine Epitopes Using a Radio- Protective Mn2+-Peptide Complex from Deinococcus.” *Cell Host & Microbe* 12 (1): 117–24. https://doi.org/10.1016/j.chom.2012.05.011.

Garzoni, Christian, and William L. Kelley. 2009. “Staphylococcus Aureus: New Evidence for Intracellular Persistence.” *Trends in Microbiology* 17 (2): 59–65. https://doi.org/10.1016/j.tim.2008.11.005.

Gjertsson, I., O. H. Hultgren, M. Stenson, R. Holmdahl, and A. Tarkowski. 2000. “Are B Lymphocytes of Importance in Severe Staphylococcus Aureus Infections?” *Infection and Immunity* 68 (5): 2431–34.

Glennie, Nelson D., Susan W. Volk, and Phillip Scott. 2017. “Skin-Resident CD4+ T Cells Protect against Leishmania Major by Recruiting and Activating Inflammatory Monocytes.” *PLOS Pathogens* 13 (4): e1006349. https://doi.org/10.1371/journal.ppat.1006349.

Glomski, Krzysztof, Sébastien Monette, Katia Manova, Bart De Strooper, Paul Saftig, and Carl P. Blobel. 2011. “Deletion of Adam10 in Endothelial Cells Leads to Defects in Organ-Specific Vascular Structures.” *Blood* 118 (4): 1163–74. https://doi.org/10.1182/blood-2011-04-348557.

Goldmann, Oliver, and Eva Medina. 2017. “Staphylococcus Aureus Strategies to Evade the Host Acquired Immune Response.” *International Journal of Medical Microbiology*, September. https://doi.org/10.1016/j.ijmm.2017.09.013.

Gomez de Agüero, Mercedes, Marc Vocanson, Fériel Hacini-Rachinel, Morgan Taillardet, Tim Sparwasser, Adrien Kissenpfennig, Bernard Malissen, Dominique Kaiserlian, and Bertrand Dubois. 2012. “Langerhans Cells Protect from Allergic Contact Dermatitis in Mice by Tolerizing CD8(+) T Cells and Activating Foxp3(+) Regulatory T Cells.” *The Journal of Clinical Investigation* 122 (5): 1700–1711. https://doi.org/10.1172/JCI59725.

Goodyear, Carl S., and Gregg J. Silverman. 2004. “Staphylococcal Toxin Induced Preferential and Prolonged in Vivo Deletion of Innate-like B Lymphocytes.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (31): 11392–97. https://doi.org/10.1073/pnas.0404382101.

Gorwitz, Rachel J., Deanna Kruszon-Moran, Sigrid K. McAllister, Geraldine McQuillan, Linda K. McDougal, Gregory E. Fosheim, Bette J. Jensen, George Killgore, Fred C. Tenover, and Matthew J. Kuehnert. 2008. “Changes in the Prevalence of Nasal Colonization with Staphylococcus Aureus in the United States, 2001-2004.” *The Journal of Infectious Diseases* 197 (9): 1226–34. https://doi.org/10.1086/533494.

Gresham, Hattie D., Jon H. Lowrance, Tony E. Caver, Bridget S. Wilson, Ambrose L. Cheung, and Frederik P. Lindberg. 2000. “Survival of Staphylococcus Aureus Inside Neutrophils Contributes to Infection.” *The Journal of Immunology* 164 (7): 3713–22. https://doi.org/10.4049/jimmunol.164.7.3713.

Grimminger, F., F. Rose, U. Sibelius, M. Meinhardt, B. Pötzsch, R. Spriestersbach, S. Bhakdi, N. Suttorp, and W. Seeger. 1997. “Human Endothelial Cell Activation and Mediator Release in Response to the Bacterial Exotoxins Escherichia Coli Hemolysin and Staphylococcal Alpha-Toxin.” *Journal of Immunology (Baltimore, Md.: 1950)* 159 (4): 1909–16.

Gudjonsson, J. E., A. Johnston, H. Sigmundsdottir, and H. Valdimarsson. 2004. “Immunopathogenic Mechanisms in Psoriasis.” *Clinical & Experimental Immunology* 135 (1): 1–8. https://doi.org/10.1111/j.1365-2249.2004.02310.x.

Guillén, Cristina, Iain B. McInnes, Diane M. Vaughan, Sharada Kommajosyula, Patrick H. C. Van Berkel, Bernard P. Leung, Antonio Aguila, and Jeremy H. Brock. 2002. "Enhanced Th1 Response to Staphylococcus Aureus Infection in Human Lactoferrin-Transgenic Mice." *Journal of Immunology (Baltimore, Md.: 1950)* 168 (8): 3950–57.

Guilliams, Martin, Karine Crozat, Sandrine Henri, Samira Tamoutounour, Pierre Grenot, Elisabeth Devilard, Béatrice de Bovis, Lena Alexopoulou, Marc Dalod, and Bernard Malissen. 2010. "Skin-Draining Lymph Nodes Contain Dermis-Derived CD103− Dendritic Cells That Constitutively Produce Retinoic Acid and Induce Foxp3+ Regulatory T Cells." *Blood* 115 (10): 1958–68. https://doi.org/10.1182/blood-2009-09-245274.

Guttman-Yassky, Emma, Michelle A. Lowes, Judilyn Fuentes-Duculan, Lisa C. Zaba, Irma Cardinale, Kristine E. Nograles, Artemis Khatcherian, et al. 2008. "Low Expression of the IL-23/Th17 Pathway in Atopic Dermatitis Compared to Psoriasis." *Journal of Immunology (Baltimore, Md.: 1950)* 181 (10): 7420–27.

Hale, J. Scott, and Rafi Ahmed. 2015. "Memory T Follicular Helper CD4 T Cells." *Frontiers in Immunology* 6. https://doi.org/10.3389/fimmu.2015.00016.

Hamid, Q., M. Boguniewicz, and D. Y. Leung. 1994. "Differential in Situ Cytokine Gene Expression in Acute versus Chronic Atopic Dermatitis." *The Journal of Clinical Investigation* 94 (2): 870–76. https://doi.org/10.1172/JCI117408.

Haniffa, Muzlifah, Amanda Shin, Venetia Bigley, Naomi McGovern, Pearline Teo, Peter See, Pavandip Singh Wasan, et al. 2012. "Human Tissues Contain CD141hi Cross-Presenting Dendritic Cells with Functional Homology to Mouse CD103+

Nonlymphoid Dendritic Cells.” *Immunity* 37 (1): 60–73. https://doi.org/10.1016/j.immuni.2012.04.012.

Harro, Clayton D., Robert F. Betts, Jonathan S. Hartzel, Matthew T. Onorato, Joy Lipka, Steven S. Smugar, and Nicholas A. Kartsonis. 2012. “The Immunogenicity and Safety of Different Formulations of a Novel Staphylococcus Aureus Vaccine (V710): Results of Two Phase I Studies.” *Vaccine* 30 (9): 1729–36. https://doi.org/10.1016/j.vaccine.2011.12.045.

Hashimoto, Masahito, Kazuki Tawaratsumida, Hiroyuki Kariya, Ai Kiyohara, Yasuo Suda, Fumiko Krikae, Teruo Kirikae, and Friedrich Götz. 2006. “Not Lipoteichoic Acid but Lipoproteins Appear to Be the Dominant Immunobiologically Active Compounds in Staphylococcus Aureus.” *Journal of Immunology (Baltimore, Md.: 1950)* 177 (5): 3162–69.

Hasse-Cieślińska, Marta. 2007. “<I>Staphylococcus Aureus</I> Skin Colonization in Atopic Dermatitis Patients.” *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii* 24 (3): 107–15.

Henningsson, Louise, Pernilla Jirholt, Catharina Lindholm, Tove Eneljung, Elin Silverpil, Yoichiro Iwakura, Anders Linden, and Inger Gjertsson. 2010. “Interleukin-17A during Local and Systemic Staphylococcus Aureus-Induced Arthritis in Mice.” *Infection and Immunity* 78 (9): 3783–90. https://doi.org/10.1128/IAI.00385-10.

Henri, Sandrine, Lionel Franz Poulin, Samira Tamoutounour, Laurence Ardouin, Martin Guilliams, Béatrice de Bovis, Elisabeth Devilard, et al. 2010. “CD207+ CD103+ Dermal Dendritic Cells Cross-Present Keratinocyte-Derived Antigens Irrespective

of the Presence of Langerhans Cells." *The Journal of Experimental Medicine* 207 (1): 189–206. https://doi.org/10.1084/jem.20091964.

Hidron, Alicia I., Russell Kempker, Abeer Moanna, and David Rimland. 2010. "Methicillin-Resistant Staphylococcus Aureus in HIV-Infected Patients." *Infection and Drug Resistance* 3: 73–86.

Hildebrand, A., M. Pohl, and S. Bhakdi. 1991. "Staphylococcus Aureus Alpha-Toxin. Dual Mechanism of Binding to Target Cells." *The Journal of Biological Chemistry* 266 (26): 17195–200.

Hoebe, Kasper, Philippe Georgel, Sophie Rutschmann, Xin Du, Suzanne Mudd, Karine Crozat, Sosathya Sovath, et al. 2005. "CD36 Is a Sensor of Diacylglycerides." *Nature* 433 (7025): 523–27. https://doi.org/10.1038/nature03253.

Holtfreter, Silva, Katharina Roschack, Petra Eichler, Kristin Eske, Birte Holtfreter, Christian Kohler, Susanne Engelmann, Michael Hecker, Andreas Greinacher, and Barbara M. Broker. 2006. "Staphylococcus Aureus Carriers Neutralize Superantigens by Antibodies Specific for Their Colonizing Strain: A Potential Explanation for Their Improved Prognosis in Severe Sepsis." *The Journal of Infectious Diseases* 193 (9): 1275–78. https://doi.org/10.1086/503048.

Howell, Michael D., Richard L. Gallo, Mark Boguniewicz, James F. Jones, Cathy Wong, Joanne E. Streib, and Donald Y. M. Leung. 2006. "Cytokine Milieu of Atopic Dermatitis Skin Subverts the Innate Immune Response to Vaccinia Virus." *Immunity* 24 (3): 341–48. https://doi.org/10.1016/j.immuni.2006.02.006.

Hruz, Petr, Annelies S. Zinkernagel, Gabriela Jenikova, Gregory J. Botwin, Jean-Pierre Hugot, Michael Karin, Victor Nizet, and Lars Eckmann. 2009a. "NOD2

Contributes to Cutaneous Defense against Staphylococcus Aureus through Alpha-Toxin-Dependent Innate Immune Activation." *Proceedings of the National Academy of Sciences of the United States of America* 106 (31): 12873–78. https://doi.org/10.1073/pnas.0904958106.

Iborra, Salvador, María Martínez-López, Sofía C. Khouili, Michel Enamorado, Francisco J. Cueto, Ruth Conde-Garrosa, Carlos del Fresno, and David Sancho. 2016. "Optimal Generation of Tissue-Resident but Not Circulating Memory T Cells during Viral Infection Requires Crosspriming by DNGR-1+ Dendritic Cells." *Immunity* 45 (4): 847–60. https://doi.org/10.1016/j.immuni.2016.08.019.

Idoyaga, Juliana, Christopher Fiorese, Lori Zbytnuik, Ashira Lubkin, Jennifer Miller, Bernard Malissen, Daniel Mucida, Miriam Merad, and Ralph M. Steinman. 2013. "Specialized Role of Migratory Dendritic Cells in Peripheral Tolerance Induction." *Journal of Clinical Investigation*, January. https://doi.org/10.1172/JCI65260.

Igyártó, Botond Z., Krystal Haley, Daniela Ortner, Aleh Bobr, Maryam Gerami-Nejad, Brian T. Edelson, Sandra M. Zurawski, et al. 2011a. "Skin-Resident Murine Dendritic Cell Subsets Promote Distinct and Opposing Antigen-Specific T Helper Cell Responses." *Immunity* 35 (2): 260–72. https://doi.org/10.1016/j.immuni.2011.06.005.

Igyarto, Botond Z., Matthew C. Jenison, Jan C. Dudda, Axel Roers, Werner Müller, Pandelakis A. Koni, Daniel J. Campbell, Mark J. Shlomchik, and Daniel H. Kaplan. 2009. "Langerhans Cells Suppress Contact Hypersensitivity Responses via Cognate CD4 Interaction and Langerhans Cell-Derived IL-10." *Journal of*

Immunology (Baltimore, Md.: 1950) 183 (8): 5085–93. https://doi.org/10.4049/jimmunol.0901884.

Igyártó, Botond Z., and Daniel H. Kaplan. 2013. “Antigen Presentation by Langerhans Cells.” *Current Opinion in Immunology* 25 (1): 115–19. https://doi.org/10.1016/j.coi.2012.11.007.

Igyártó, Botond Z., Krystal Haley, Daniela Ortner, Aleh Bobr, Maryam Gerami-Nejad, Brian T. Edelson, Sandra M. Zurawski, et al. 2011b. “Skin-Resident Murine Dendritic Cell Subsets Promote Distinct and Opposing Antigen-Specific T Helper Cell Responses.” *Immunity* 35 (2): 260–72. https://doi.org/10.1016/j.immuni.2011.06.005.

Iii, Francis Alonzo, Lina Kozhaya, Stephen A. Rawlings, Tamara Reyes-Robles, Ashley L. DuMont, David G. Myszka, Nathaniel R. Landau, Derya Unutmaz, and Victor J. Torres. 2013. “CCR5 Is a Receptor for *Staphylococcus Aureus* Leukotoxin ED.” *Nature* 493 (7430): 51–55. https://doi.org/10.1038/nature11724.

III, Francis Alonzo, Lina Kozhaya, Stephen A. Rawlings, Tamara Reyes-Robles, Ashley L. DuMont, David G. Myszka, Nathaniel Landau, Derya Unutmaz, and Victor J. Torres. 2013a. “CCR5 Is a Receptor for Staphylococcus Aureus Leukotoxin ED.” *Nature* 493 (7430): 51–55. https://doi.org/10.1038/nature11724.

Inoshima, Ichiro, Naoko Inoshima, Georgia A. Wilke, Michael E. Powers, Karen M. Frank, Yang Wang, and Juliane Bubeck Wardenburg. 2011. “A Staphylococcus Aureus Pore-Forming Toxin Subverts the Activity of ADAM10 to Cause Lethal Infection in Mice.” *Nature Medicine* 17 (10): 1310–14. https://doi.org/10.1038/nm.2451.

Ishigame, Harumichi, Shigeru Kakuta, Takeshi Nagai, Motohiko Kadoki, Aya Nambu, Yutaka Komiyama, Noriyuki Fujikado, et al. 2009. “Differential Roles of Interleukin-17A and -17F in Host Defense against Mucoepithelial Bacterial Infection and Allergic Responses.” *Immunity* 30 (1): 108–19. https://doi.org/10.1016/j.immuni.2008.11.009.

Iwase, Tadayuki, Yoshio Uehara, Hitomi Shinji, Akiko Tajima, Hiromi Seo, Koji Takada, Toshihiko Agata, and Yoshimitsu Mizunoe. 2010. “*Staphylococcus Epidermidis* Esp Inhibits *Staphylococcus Aureus* Biofilm Formation and Nasal Colonization.” *Nature* 465 (7296): 346–49. https://doi.org/10.1038/nature09074.

Jarry, Todd M., Guido Memmi, and Ambrose L. Cheung. 2008. “The Expression of Alpha-Haemolysin Is Required for Staphylococcus Aureus Phagosomal Escape after Internalization in CFT-1 Cells.” *Cellular Microbiology* 10 (9): 1801–14. https://doi.org/10.1111/j.1462-5822.2008.01166.x.

Jiang, Xiaodong, Rachael A. Clark, Luzheng Liu, Amy J. Wagers, Robert C. Fuhlbrigge, and Thomas S. Kupper. 2012. “Skin Infection Generates Non-Migratory Memory CD8+ TRM Cells Providing Global Skin Immunity.” *Nature* 483 (7388): 227–31. https://doi.org/10.1038/nature10851.

Jorissen, Ellen, Johannes Prox, Christian Bernreuther, Silvio Weber, Ralf Schwanbeck, Lutgarde Serneels, An Snellinx, et al. 2010. “The Disintegrin/Metalloproteinase ADAM10 Is Essential for the Establishment of the Brain Cortex.” *The Journal of Neuroscience* 30 (14): 4833–44. https://doi.org/10.1523/JNEUROSCI.5221-09.2010.

Joshi, Amita, Greg Pancari, Leslie Cope, Edward P. Bowman, Daniel Cua, Richard A. Proctor, and Tessie McNeely. 2012. “Immunization with Staphylococcus Aureus Iron Regulated Surface Determinant B (IsdB) Confers Protection via Th17/IL17 Pathway in a Murine Sepsis Model.” *Human Vaccines & Immunotherapeutics* 8 (3): 336–46. https://doi.org/10.4161/hv.18946.

Kao, Kuo-Chin, Chun-Bing Chen, Han-Chung Hu, Hui-Ching Chang, Chung-Chi Huang, and Yhu-Chering Huang. 2015. “Risk Factors of Methicillin-Resistant Staphylococcus Aureus Infection and Correlation With Nasal Colonization Based on Molecular Genotyping in Medical Intensive Care Units: A Prospective Observational Study.” *Medicine* 94 (28): e1100. https://doi.org/10.1097/MD.0000000000001100.

Kaplan, Daniel H. 2010. “In Vivo Function of Langerhans Cells and Dermal Dendritic Cells.” *Trends in Immunology*, Special Focus, 31 (12): 446–51. https://doi.org/10.1016/j.it.2010.08.006.

Karauzum, Hatice, and Sandip K. Datta. 2016. “Adaptive Immunity Against <Emphasis Type="Italic">Staphylococcus Aureus</Emphasis>.” In *Staphylococcus Aureus*, 419–39. Current Topics in Microbiology and Immunology. Springer, Cham. https://doi.org/10.1007/82_2016_1.

Kautz-Neu, Kordula, Ralf G. Meyer, Björn E. Clausen, and Esther von Stebut. 2010. “Leishmaniasis, Contact Hypersensitivity and Graft-versus-Host Disease: Understanding the Role of Dendritic Cell Subsets in Balancing Skin Immunity and Tolerance.” *Experimental Dermatology* 19 (8): 760–71. https://doi.org/10.1111/j.1600-0625.2010.01116.x.

Kautz-Neu, Kordula, Madelon Noordegraaf, Stephanie Dinges, Clare L. Bennett, Dominik John, Björn E. Clausen, and Esther von Stebut. 2011. “Langerhans Cells Are Negative Regulators of the Anti-Leishmania Response.” *The Journal of Experimental Medicine* 208 (5): 885–91. https://doi.org/10.1084/jem.20102318.

Kautz-Neu, Kordula, Kirsten Schwonberg, Michael R. Fischer, Anja I. Schermann, and Esther von Stebut. 2012. “Dendritic Cells in Leishmania Major Infections: Mechanisms of Parasite Uptake, Cell Activation and Evidence for Physiological Relevance.” *Medical Microbiology and Immunology* 201 (4): 581–92. https://doi.org/10.1007/s00430-012-0261-2.

Keene, Adam, Peter Vavagiakis, Mei-Ho Lee, Kathryn Finnerty, Deborah Nicolls, Christian Cespedes, Bianca Quagliarello, Mary Ann Chiasson, David Chong, and Franklin D. Lowy. 2005. “Staphylococcus Aureus Colonization and the Risk of Infection in Critically Ill Patients.” *Infection Control and Hospital Epidemiology* 26 (7): 622–28. https://doi.org/10.1086/502591.

Kennedy, Adam D., Juliane Bubeck Wardenburg, Donald J. Gardner, Daniel Long, Adeline R. Whitney, Kevin R. Braughton, Olaf Schneewind, and Frank R. DeLeo. 2010. “Targeting of Alpha-Hemolysin by Active or Passive Immunization Decreases Severity of USA300 Skin Infection in a Mouse Model.” *The Journal of Infectious Diseases* 202 (7): 1050–58. https://doi.org/10.1086/656043.

Khader, S. A., S. L. Gaffen, and J. K. Kolls. 2009. “Th17 Cells at the Crossroads of Innate and Adaptive Immunity against Infectious Diseases at the Mucosa.” *Mucosal Immunology* 2 (5): 403–11. https://doi.org/10.1038/mi.2009.100.

Kim, Hwan Keun, Alice G. Cheng, Hye-Young Kim, Dominique M. Missiakas, and Olaf Schneewind. 2010. “Nontoxigenic Protein A Vaccine for Methicillin-Resistant Staphylococcus Aureus Infections in Mice.” *Journal of Experimental Medicine* 207 (9): 1863–70. https://doi.org/10.1084/jem.20092514.

Kim, Hwan Keun, Andrea DeDent, Alice G. Cheng, Molly McAdow, Fabio Bagnoli, Dominique M. Missiakas, and Olaf Schneewind. 2010. “IsdA and IsdB Antibodies Protect Mice against Staphylococcus Aureus Abscess Formation and Lethal Challenge.” *Vaccine* 28 (38): 6382–92. https://doi.org/10.1016/j.vaccine.2010.02.097.

Kisich, Kevin O., Charles W. Carspecken, Stephanie Fiéve, Mark Boguniewicz, and Donald Y. M. Leung. 2008. “Defective Killing of Staphylococcus Aureus in Atopic Dermatitis Is Associated with Reduced Mobilization of Human Beta-Defensin-3.” *The Journal of Allergy and Clinical Immunology* 122 (1): 62–68. https://doi.org/10.1016/j.jaci.2008.04.022.

Klevens R, Morrison MA, Nadle J, and et al. 2007. “INvasive Methicillin-Resistant Staphylococcus Aureus Infections in the United States.” *JAMA* 298 (15): 1763–71. https://doi.org/10.1001/jama.298.15.1763.

Kluytmans, J., A. van Belkum, and H. Verbrugh. 1997. “Nasal Carriage of Staphylococcus Aureus: Epidemiology, Underlying Mechanisms, and Associated Risks.” *Clinical Microbiology Reviews* 10 (3): 505–20.

Koenig, Robbin L., Jessica L. Ray, Soheila J. Maleki, Mark S. Smeltzer, and Barry K. Hurlburt. 2004. “Staphylococcus Aureus AgrA Binding to the RNAIII-Agr

Regulatory Region." *Journal of Bacteriology* 186 (22): 7549–55. https://doi.org/10.1128/JB.186.22.7549-7555.2004.

Kolata, Julia B., Iris Kühbandner, Christopher Link, Nicole Normann, Chi Hai Vu, Leif Steil, Christopher Weidenmaier, and Barbara M. Bröker. 2015. "The Fall of a Dogma? Unexpected High T-Cell Memory Response to Staphylococcus Aureus in Humans." *The Journal of Infectious Diseases* 212 (5): 830–38. https://doi.org/10.1093/infdis/jiv128.

Kolata, Julia, Lonneke G. M. Bode, Silva Holtfreter, Leif Steil, Harald Kusch, Birte Holtfreter, Dirk Albrecht, et al. 2011. "Distinctive Patterns in the Human Antibody Response to Staphylococcus Aureus Bacteremia in Carriers and Non-Carriers." *Proteomics* 11 (19): 3914–27. https://doi.org/10.1002/pmic.201000760.

Korzeniowski, O., and M. A. Sande. 1982. "Combination Antimicrobial Therapy for Staphylococcus Aureus Endocarditis in Patients Addicted to Parenteral Drugs and in Nonaddicts: A Prospective Study." *Annals of Internal Medicine* 97 (4): 496–503.

Krishna, Sheila, and Lloyd S. Miller. 2012. "Innate and Adaptive Immune Responses against <Emphasis Type="Italic">Staphylococcus Aureus</Emphasis> Skin Infections." *Seminars in Immunopathology* 34 (2): 261–80. https://doi.org/10.1007/s00281-011-0292-6.

Kuklin, Nelly A., Desmond J. Clark, Susan Secore, James Cook, Leslie D. Cope, Tessie McNeely, Liliane Noble, et al. 2006. "A Novel Staphylococcus Aureus Vaccine: Iron Surface Determinant B Induces Rapid Antibody Responses in Rhesus Macaques and Specific Increased Survival in a Murine S. Aureus Sepsis Model."

Infection and Immunity 74 (4): 2215–23. https://doi.org/10.1128/IAI.74.4.2215-2223.2006.

Lacoma, A., V. Cano, D. Moranta, V. Regueiro, D. Domínguez-Villanueva, M. Laabei, M. González-Nicolau, V. Ausina, C. Prat, and J. A. Bengoechea. 2017. "Investigating Intracellular Persistence of Staphylococcus Aureus within a Murine Alveolar Macrophage Cell Line." *Virulence* 8 (8): 1761–75. https://doi.org/10.1080/21505594.2017.1361089.

Lange, Hans, Oliver Hecht, Michael Zemlin, Ahmad Trad, Radu I. Tanasa, Harry W. Schroeder, and Hilmar Lemke. 2012. "Immunoglobulin Class Switching Appears to Be Regulated by B Cell Antigen Receptor-Specific T Cell Action." *European Journal of Immunology* 42 (4): 1016–29. https://doi.org/10.1002/eji.201141857.

Lee, Brandon, and Juliane Bubeck Wardenburg. 2018. "A Common Approach to Toxin Specificity." *Nature Microbiology* 3 (6): 644–45. https://doi.org/10.1038/s41564-018-0173-z.

Lee, Dong-Youn, Chun-Ming Huang, Teruaki Nakatsuji, Diane Thiboutot, Sun-Ah Kang, Marc Monestier, and Richard L. Gallo. 2009. "Histone H4 Is a Major Component of the Antimicrobial Action of Human Sebocytes." *The Journal of Investigative Dermatology* 129 (10): 2489–96. https://doi.org/10.1038/jid.2009.106.

Lee, Seung-Joo, James B. McLachlan, Jonathan R. Kurtz, Danhua Fan, Sebastian E. Winter, Andreas J. Baumler, Marc K. Jenkins, and Stephen J. McSorley. 2012. "Temporal Expression of Bacterial Proteins Instructs Host CD4 T Cell Expansion and Th17 Development." *PLoS Pathogens* 8 (1): e1002499. https://doi.org/10.1371/journal.ppat.1002499.

Leech, John M., Keenan A. Lacey, Michelle E. Mulcahy, Eva Medina, and Rachel M. McLoughlin. 2017. “IL-10 Plays Opposing Roles during Staphylococcus Aureus Systemic and Localized Infections.” *The Journal of Immunology Author Choice* 198 (6): 2352–65. https://doi.org/10.4049/jimmunol.1601018.

Li, Zhigang, Adam G. Peres, Andreea C. Damian, and Joaquín Madrenas. 2015. “Immunomodulation and Disease Tolerance to Staphylococcus Aureus.” *Pathogens* 4 (4): 793–815. https://doi.org/10.3390/pathogens4040793.

Liang, Jie, Hsin-I Huang, Fernanda P. Benzatti, Amelia B. Karlsson, Junyi J. Zhang, Nourhan Youssef, Averil Ma, Laura P. Hale, and Gianna E. Hammer. 2016. “Inflammatory Th1 and Th17 Cells in Small Intestine Are Each Driven by Functionally-Specialized Dendritic Cells with Distinct Requirements for MyD88.” *Cell Reports* 17 (5): 1330–43. https://doi.org/10.1016/j.celrep.2016.09.091.

Lin, Hsi-Hsien, Douglas E. Faunce, Martin Stacey, Ania Terajewicz, Takahiko Nakamura, Jie Zhang-Hoover, Marilyn Kerley, Michael L. Mucenski, Siamon Gordon, and Joan Stein-Streilein. 2005. “The Macrophage F4/80 Receptor Is Required for the Induction of Antigen-Specific Efferent Regulatory T Cells in Peripheral Tolerance.” *The Journal of Experimental Medicine* 201 (10): 1615–25. https://doi.org/10.1084/jem.20042307.

Lin, Lin, Ashraf S. Ibrahim, Xin Xu, Joshua M. Farber, Valentina Avanesian, Beverlie Baquir, Yue Fu, Samuel W. French, John E. Edwards, and Brad Spellberg. 2009. “Th1-Th17 Cells Mediate Protective Adaptive Immunity against Staphylococcus Aureus and Candida Albicans Infection in Mice.” *PLoS Pathogens* 5 (12): e1000703. https://doi.org/10.1371/journal.ppat.1000703.

Liu, Qiang, Yuan-hong Zhou, and Zhan-qiu Yang. 2016. “The Cytokine Storm of Severe Influenza and Development of Immunomodulatory Therapy.” *Cellular and Molecular Immunology* 13 (1): 3–10. https://doi.org/10.1038/cmi.2015.74.

Lizak, Miroslaw, and Timur O. Yarovinsky. 2012. “Phospholipid Scramblase 1 Mediates Type i Interferon-Induced Protection against Staphylococcal α-Toxin.” *Cell Host & Microbe* 11 (1): 70–80. https://doi.org/10.1016/j.chom.2011.12.004.

Löffler, Bettina, Lorena Tuchscherr, Silke Niemann, and Georg Peters. 2014. “Staphylococcus Aureus Persistence in Non-Professional Phagocytes.” *International Journal of Medical Microbiology*, Pathophysiology of Staphylococci in the Post-Genomic Era, 304 (2): 170–76. https://doi.org/10.1016/j.ijmm.2013.11.011.

Lu, P., Y. L. Wang, and P. S. Linsley. 1997. “Regulation of Self-Tolerance by CD80/CD86 Interactions.” *Current Opinion in Immunology* 9 (6): 858–62.

Lyon, Gholson J., Jesse S. Wright, Tom W. Muir, and Richard P. Novick. 2002. “Key Determinants of Receptor Activation in the Agr Autoinducing Peptides of Staphylococcus Aureus.” *Biochemistry* 41 (31): 10095–104.

Ma, Cindy S., Elissa K. Deenick, Marcel Batten, and Stuart G. Tangye. 2012. “The Origins, Function, and Regulation of T Follicular Helper Cells.” *Journal of Experimental Medicine* 209 (7): 1241–53. https://doi.org/10.1084/jem.20120994.

MacLeod, Megan KL, John W Kappler, and Philippa Marrack. 2010. “Memory CD4 T Cells: Generation, Reactivation and Re-Assignment.” *Immunology* 130 (1): 10–15. https://doi.org/10.1111/j.1365-2567.2010.03260.x.

Manicassamy, Santhakumar, and Bali Pulendran. 2011. “Dendritic Cell Control of Tolerogenic Responses.” *Immunological Reviews* 241 (1): 206–27. https://doi.org/10.1111/j.1600-065X.2011.01015.x.

Matsui, K., and A. Nishikawa. 2002. “Lipoteichoic Acid from Staphylococcus Aureus Induces Th2-Prone Dermatitis in Mice Sensitized Percutaneously with an Allergen.” *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 32 (5): 783–88.

McCaig, Linda F., L. Clifford McDonald, Sanjay Mandal, and Daniel B. Jernigan. 2006. “Staphylococcus Aureus–Associated Skin and Soft Tissue Infections in Ambulatory Care.” *Emerging Infectious Diseases* 12 (11): 1715–23. https://doi.org/10.3201/eid1211.060190.

McLachlan, James B., Drew M. Catron, James J. Moon, and Marc K. Jenkins. 2009. “Dendritic Cell Antigen Presentation Drives Simultaneous Cytokine Production by Effector and Regulatory T Cells in Inflamed Skin.” *Immunity* 30 (2): 277–88. https://doi.org/10.1016/j.immuni.2008.11.013.

McNeely, Tessie B., Najaf A. Shah, Arthur Fridman, Amita Joshi, Jonathan S. Hartzel, Ravi S. Keshari, Florea Lupu, and Mark J. DiNubile. 2014. “Mortality among Recipients of the Merck V710 Staphylococcus Aureus Vaccine after Postoperative S. Aureus Infections: An Analysis of Possible Contributing Host Factors.” *Human Vaccines & Immunotherapeutics* 10 (12): 3513–16. https://doi.org/10.4161/hv.34407.

Menzies, Barbara E, and Iordanka Kourteva. 2000. “Staphylococcus Aureus α-Toxin Induces Apoptosis in Endothelial Cells.” *FEMS Immunology and Medical Microbiology* 29 (1): 39–45. https://doi.org/10.1016/S0928-8244(00)00185-1.

Merica, R., A. Khoruts, K. A. Pape, R. L. Reinhardt, and M. K. Jenkins. 2000. “Antigen-Experienced CD4 T Cells Display a Reduced Capacity for Clonal Expansion in Vivo That Is Imposed by Factors Present in the Immune Host.” *Journal of Immunology (Baltimore, Md.: 1950)* 164 (9): 4551–57.

Miller, Lloyd S. 2008. “Toll-like Receptors in Skin.” *Advances in Dermatology* 24: 71–87.

Miller, Lloyd S., Ryan M. O’Connell, Miguel A. Gutierrez, Eric M. Pietras, Arash Shahangian, Catherine E. Gross, Ajaykumar Thirumala, Ambrose L. Cheung, Genhong Cheng, and Robert L. Modlin. 2006. “MyD88 Mediates Neutrophil Recruitment Initiated by IL-1R but Not TLR2 Activation in Immunity against Staphylococcus Aureus.” *Immunity* 24 (1): 79–91. https://doi.org/10.1016/j.immuni.2005.11.011.

Miller, Loren G., Samantha J. Eells, Michael Z. David, Nancy Ortiz, Alexis R. Taylor, Neha Kumar, Denise Cruz, Susan Boyle-Vavra, and Robert S. Daum. 2015. “Staphylococcus Aureus Skin Infection Recurrences Among Household Members: An Examination of Host, Behavioral, and Pathogen-Level Predictors.” *Clinical Infectious Diseases* 60 (5): 753–63. https://doi.org/10.1093/cid/ciu943.

Miller, Loren G., Clifford Quan, Anthony Shay, Katayoun Mostafaie, Kiran Bharadwa, Nelly Tan, Kelli Matayoshi, et al. 2007. “A Prospective Investigation of Outcomes after Hospital Discharge for Endemic, Community-Acquired Methicillin-Resistant and -Susceptible Staphylococcus Aureus Skin Infection.” *Clinical Infectious*

Diseases: An Official Publication of the Infectious Diseases Society of America 44 (4): 483–92. https://doi.org/10.1086/511041.

Miller, W. G., and S. E. Lindow. 1997. “An Improved GFP Cloning Cassette Designed for Prokaryotic Transcriptional Fusions.” *Gene* 191 (2): 149–53.

Mongini, P. K., W. E. Paul, and E. S. Metcalf. 1982. “T Cell Regulation of Immunoglobulin Class Expression in the Antibody Response to Trinitrophenyl-Ficoll. Evidence for T Cell Enhancement of the Immunoglobulin Class Switch.” *The Journal of Experimental Medicine* 155 (3): 884–902.

Montgomery, Christopher P., Melvin Daniels, Fan Zhao, Maria-Luisa Alegre, Anita S. Chong, and Robert S. Daum. 2014. “Protective Immunity against Recurrent Staphylococcus Aureus Skin Infection Requires Antibody and Interleukin-17A.” *Infection and Immunity* 82 (5): 2125–34. https://doi.org/10.1128/IAI.01491-14.

Murakami, Masamoto, Takaaki Ohtake, Robert A. Dorschner, Birgit Schittek, Claus Garbe, and Richard L. Gallo. 2002. “Cathelicidin Anti-Microbial Peptide Expression in Sweat, an Innate Defense System for the Skin.” *The Journal of Investigative Dermatology* 119 (5): 1090–95. https://doi.org/10.1046/j.1523-1747.2002.19507.x.

Nagy, István, Andor Pivarcsi, Kornélia Kis, Andrea Koreck, László Bodai, Andrew McDowell, Holger Seltmann, Sheila Patrick, Christos C. Zouboulis, and Lajos Kemény. 2006. “Propionibacterium Acnes and Lipopolysaccharide Induce the Expression of Antimicrobial Peptides and Proinflammatory Cytokines/Chemokines in Human Sebocytes.” *Microbes and Infection* 8 (8): 2195–2205. https://doi.org/10.1016/j.micinf.2006.04.001.

Naik, Shruti, Nicolas Bouladoux, Jonathan L. Linehan, Seong-Ji Han, Oliver J. Harrison, Christoph Wilhelm, Sean Conlan, et al. 2015. “Commensal-Dendritic-Cell Interaction Specifies a Unique Protective Skin Immune Signature.” *Nature* 520 (7545): 104–8. https://doi.org/10.1038/nature14052.

Naik, Shruti, Nicolas Bouladoux, Christoph Wilhelm, Michael J. Molloy, Rosalba Salcedo, Wolfgang Kastenmuller, Clayton Deming, et al. 2012. “Compartmentalized Control of Skin Immunity by Resident Commensals.” *Science (New York, N.Y.)* 337 (6098): 1115–19. https://doi.org/10.1126/science.1225152.

Nakatsuji, Teruaki, Mandy C. Kao, Liangfang Zhang, Christos C. Zouboulis, Richard L. Gallo, and Chun-Ming Huang. 2010. “Sebum Free Fatty Acids Enhance the Innate Immune Defense of Human Sebocytes by Upregulating Beta-Defensin-2 Expression.” *The Journal of Investigative Dermatology* 130 (4): 985–94. https://doi.org/10.1038/jid.2009.384.

Narita, Kouji, Dong-Liang Hu, Fumiaki Mori, Koichi Wakabayashi, Yoichiro Iwakura, and Akio Nakane. 2010. “Role of Interleukin-17A in Cell-Mediated Protection against Staphylococcus Aureus Infection in Mice Immunized with the Fibrinogen-Binding Domain of Clumping Factor A.” *Infection and Immunity* 78 (10): 4234–42. https://doi.org/10.1128/IAI.00447-10.

Nguyen, Dao M., Laurene Mascola, and Elizabeth Brancoft. 2005. “Recurring Methicillin-Resistant Staphylococcus Aureus Infections in a Football Team.” *Emerging Infectious Diseases* 11 (4): 526–32. https://doi.org/10.3201/eid1104.041094.

Nielsen, Morten M., Deborah A. Witherden, and Wendy L. Havran. 2017. “Γδ T Cells in Homeostasis and Host Defence of Epithelial Barrier Tissues.” *Nature Reviews Immunology* 17 (12): 733–45. https://doi.org/10.1038/nri.2017.101.

Nomura, Ichiro, Bifeng Gao, Mark Boguniewicz, Marc A. Darst, Jeffrey B. Travers, and Donald Y. m Leung. 2003. “Distinct Patterns of Gene Expression in the Skin Lesions of Atopic Dermatitis and Psoriasis: A Gene Microarray Analysis.” *The Journal of Allergy and Clinical Immunology* 112 (6): 1195–1202. https://doi.org/10.1016/j.jaci.2003.08.049.

Novick, R. P., H. F. Ross, S. J. Projan, J. Kornblum, B. Kreiswirth, and S. Moghazeh. 1993. “Synthesis of Staphylococcal Virulence Factors Is Controlled by a Regulatory RNA Molecule.” *The EMBO Journal* 12 (10): 3967–75.

Ohlsen, Knut, and Udo Lorenz. 2010. “Immunotherapeutic Strategies to Combat Staphylococcal Infections.” *International Journal of Medical Microbiology: IJMM* 300 (6): 402–10. https://doi.org/10.1016/j.ijmm.2010.04.015.

O’Keeffe, Kate M., Mieszko M. Wilk, John M. Leech, Alison G. Murphy, Maisem Laabei, Ian R. Monk, Ruth C. Massey, et al. 2015. “Manipulation of Autophagy in Phagocytes Facilitates Staphylococcus Aureus Bloodstream Infection.” *Infection and Immunity* 83 (9): 3445–57. https://doi.org/10.1128/IAI.00358-15.

Palmqvist, Niklas, Timothy Foster, Andrzej Tarkowski, and Elisabet Josefsson. 2002. “Protein A Is a Virulence Factor in Staphylococcus Aureus Arthritis and Septic Death.” *Microbial Pathogenesis* 33 (5): 239–49.

Pasparakis, Manolis, Ingo Haase, and Frank O. Nestle. 2014. “Mechanisms Regulating Skin Immunity and Inflammation.” *Nature Reviews. Immunology* 14 (5): 289–301. https://doi.org/10.1038/nri3646.

Patel, Dhavalkumar D., and Vijay K. Kuchroo. 2015. “Th17 Cell Pathway in Human Immunity: Lessons from Genetics and Therapeutic Interventions.” *Immunity* 43 (6): 1040–51. https://doi.org/10.1016/j.immuni.2015.12.003.

Pauli, Noel T., Hwan Keun Kim, Fabiana Falugi, Min Huang, John Dulac, Carole Henry Dunand, Nai-Ying Zheng, et al. 2014. “Staphylococcus Aureus Infection Induces Protein A–Mediated Immune Evasion in Humans.” *The Journal of Experimental Medicine* 211 (12): 2331–39. https://doi.org/10.1084/jem.20141404.

Peng, H. L., R. P. Novick, B. Kreiswirth, J. Kornblum, and P. Schlievert. 1988. “Cloning, Characterization, and Sequencing of an Accessory Gene Regulator (Agr) in Staphylococcus Aureus.” *Journal of Bacteriology* 170 (9): 4365–72.

Pfundt, R., I. van Vlijmen-Willems, M. Bergers, M. Wingens, W. Cloin, and J. Schalkwijk. 2001. “In Situ Demonstration of Phosphorylated C-Jun and P38 MAP Kinase in Epidermal Keratinocytes Following Ultraviolet B Irradiation of Human Skin.” *The Journal of Pathology* 193 (2): 248–55. https://doi.org/10.1002/1096-9896(2000)9999:9999<::AID-PATH780>3.0.CO;2-Y.

Pickard, S., G. Shankar, and K. Burnham. 1994. “Langerhans’ Cell Depletion by Staphylococcal Superantigens.” *Immunology* 83 (4): 568–72.

Pivarcsi, A., L. Kemény, and A. Dobozy. 2004. “Innate Immune Functions of the Keratinocytes. A Review.” *Acta Microbiologica Et Immunologica Hungarica* 51 (3): 303–10. https://doi.org/10.1556/AMicr.51.2004.3.8.

Platt, Andrew M., Joseph M. Rutkowski, Catherine Martel, Emma L. Kuan, Stoyan Ivanov, Melody A. Swartz, and Gwendalyn J. Randolph. 2013. “Normal Dendritic Cell Mobilization to Lymph Nodes under Conditions of Severe Lymphatic Hypoplasia.” *Journal of Immunology (Baltimore, Md. : 1950)* 190 (9): 4608–20. https://doi.org/10.4049/jimmunol.1202600.

Powers, Michael E., Russell E. N. Becker, Anne Sailer, Jerrold R. Turner, and Juliane Bubeck Wardenburg. 2015. “Synergistic Action of Staphylococcus Aureus α-Toxin on Platelets and Myeloid Lineage Cells Contributes to Lethal Sepsis.” *Cell Host & Microbe* 17 (6): 775–87. https://doi.org/10.1016/j.chom.2015.05.011.

Pozzi, Clarissa, Katarzyna Wilk, Jean C. Lee, Marina Gening, Nikolay Nifantiev, and Gerald B. Pier. 2012. “Opsonic and Protective Properties of Antibodies Raised to Conjugate Vaccines Targeting Six Staphylococcus Aureus Antigens.” *PloS One* 7 (10): e46648. https://doi.org/10.1371/journal.pone.0046648.

Prendergast, Andrew, Julia G. Prado, Yu-hoi Kang, Fabian Chen, Lynn A. Riddell, Graz Luzzi, Philip Goulder, and Paul Klenerman. 2010. “Hiv-1 Infection Is Characterized by Profound Depletion of Cd161+ Th17 Cells and Gradual Decline in Regulatory T Cells.” *Aids* 24 (4): 491–502. https://doi.org/10.1097/QAD.0b013e3283344895.

Price, Jeremy G., Juliana Idoyaga, Hélène Salmon, Brandon Hogstad, Carolina L. Bigarella, Saghi Ghaffari, Marylene Leboeuf, and Miriam Merad. 2015a. “CDKN1A Regulates Langerhans Cell Survival and Promotes Treg Cell Generation upon Exposure to Ionizing Irradiation.” *Nature Immunology* 16 (10): 1060–68. https://doi.org/10.1038/ni.3270.

Proksch, Ehrhardt, Johanna M. Brandner, and Jens-Michael Jensen. 2008. “The Skin: An Indispensable Barrier.” *Experimental Dermatology* 17 (12): 1063–72. https://doi.org/10.1111/j.1600-0625.2008.00786.x.

Rabe, Hardis, Inger Nordström, Kerstin Andersson, Anna-Carin Lundell, and Anna Rudin. 2014. “Staphylococcus Aureus Convert Neonatal Conventional CD4+ T Cells into FOXP3+ CD25+ CD127low T Cells via the PD-1/PD-L1 Axis.” *Immunology* 141 (3): 467–81. https://doi.org/10.1111/imm.12209.

Ramaswamy, Kalyanasundaram, Pawan Kumar, and Yi-Xun He. 2000. “A Role for Parasite-Induced PGE2 in IL-10-Mediated Host Immunoregulation by Skin Stage Schistosomula of Schistosoma Mansoni.” *The Journal of Immunology* 165 (8): 4567–74. https://doi.org/10.4049/jimmunol.165.8.4567.

Rauch, Philipp J., Aleksey Chudnovskiy, Clinton S. Robbins, Georg F. Weber, Martin Etzrodt, Ingo Hilgendorf, Elizabeth Tiglao, et al. 2012. “Innate Response Activator B Cells Protect against Microbial Sepsis.” *Science (New York, N.Y.)* 335 (6068): 597–601. https://doi.org/10.1126/science.1215173.

Rebholz, Bernd, Ingo Haase, Birgit Eckelt, Stephan Paxian, Michael J. Flaig, Kamran Ghoreschi, Sergei A. Nedospasov, et al. 2007. “Crosstalk between Keratinocytes and Adaptive Immune Cells in an IκBα Protein-Mediated Inflammatory Disease of the Skin.” *Immunity* 27 (2): 296–307. https://doi.org/10.1016/j.immuni.2007.05.024.

Reyes, Dindo, Diego O. Andrey, Antoinette Monod, William L. Kelley, Gongyi Zhang, and Ambrose L. Cheung. 2011. “Coordinated Regulation by AgrA, SarA, and

SarR to Control Agr Expression in Staphylococcus Aureus.” *Journal of Bacteriology* 193 (21): 6020–31. https://doi.org/10.1128/JB.05436-11.

Rieg, S., C. Garbe, B. Sauer, H. Kalbacher, and B. Schittek. 2004. “Dermcidin Is Constitutively Produced by Eccrine Sweat Glands and Is Not Induced in Epidermal Cells under Inflammatory Skin Conditions.” *The British Journal of Dermatology* 151 (3): 534–39. https://doi.org/10.1111/j.1365-2133.2004.06081.x.

Rieg, Siegbert, Silke Seeber, Heiko Steffen, Andreas Humeny, Hubert Kalbacher, Stefan Stevanovic, Akihiko Kimura, Claus Garbe, and Birgit Schittek. 2006. “Generation of Multiple Stable Dermcidin-Derived Antimicrobial Peptides in Sweat of Different Body Sites.” *The Journal of Investigative Dermatology* 126 (2): 354–65. https://doi.org/10.1038/sj.jid.5700041.

Ritter, Uwe, Anja Meissner, Christina Scheidig, and Heinrich Körner. 2004. “CD8 Alpha- and Langerin-Negative Dendritic Cells, but Not Langerhans Cells, Act as Principal Antigen-Presenting Cells in Leishmaniasis.” *European Journal of Immunology* 34 (6): 1542–50. https://doi.org/10.1002/eji.200324586.

Romani, Nikolaus, Patrick M. Brunner, and Georg Stingl. 2012a. “Changing Views of the Role of Langerhans Cells.” *The Journal of Investigative Dermatology* 132 (3 Pt 2): 872–81. https://doi.org/10.1038/jid.2011.437.

Rose, Frank, Gabriele Dahlem, Bernd Guthmann, Friedrich Grimminger, Ulrich Maus, Jörg Hänze, Nils Duemmer, Ulrich Grandel, Werner Seeger, and Hossein Ardeschir Ghofrani. 2002. “Mediator Generation and Signaling Events in Alveolar Epithelial Cells Attacked by S. Aureus Alpha-Toxin.” *American Journal of*

Physiology. Lung Cellular and Molecular Physiology 282 (2): L207-214. https://doi.org/10.1152/ajplung.00156.2001.

Safdar, Nasia, and Elisa A. Bradley. 2008. “The Risk of Infection after Nasal Colonization with Staphylococcus Aureus.” *The American Journal of Medicine* 121 (4): 310–15. https://doi.org/10.1016/j.amjmed.2007.07.034.

Sampedro, Georgia R., Andrea C. DeDent, Russell E. N. Becker, Bryan J. Berube, Michael J. Gebhardt, Hongyuan Cao, and Juliane Bubeck Wardenburg. 2014. “Targeting Staphylococcus Aureus α-Toxin as a Novel Approach to Reduce Severity of Recurrent Skin and Soft-Tissue Infections.” *Journal of Infectious Diseases*, April, jiu223. https://doi.org/10.1093/infdis/jiu223.

Sanford, James A., and Richard L. Gallo. 2013a. “Functions of the Skin Microbiota in Health and Disease.” *Seminars in Immunology*, Microbiota and the immune system, an amazing mutualism forged by co-evolution, 25 (5): 370–77. https://doi.org/10.1016/j.smim.2013.09.005.

Schaffer, Adam C., and Jean C. Lee. 2008. “Vaccination and Passive Immunisation against Staphylococcus Aureus.” *International Journal of Antimicrobial Agents* 32 Suppl 1 (November): S71-78. https://doi.org/10.1016/j.ijantimicag.2008.06.009.

Scharschmidt, Tiffany C., Kimberly S. Vasquez, Hong-An Truong, Sofia V. Gearty, Mariela L. Pauli, Audrey Nosbaum, Iris K. Gratz, et al. 2015. “A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes.” *Immunity* 43 (5): 1011–21. https://doi.org/10.1016/j.immuni.2015.10.016.

Schenkel, Jason M., and David Masopust. 2014. “Tissue-Resident Memory T Cells.” *Immunity* 41 (6): 886–97. https://doi.org/10.1016/j.immuni.2014.12.007.

Schlievert, Patrick M., Kristi L. Strandberg, Ying-Chi Lin, Marnie L. Peterson, and Donald Y. M. Leung. 2010. “Secreted Virulence Factor Comparison between Methicillin-Resistant and Methicillin-Sensitive Staphylococcus Aureus, and Its Relevance to Atopic Dermatitis.” *The Journal of Allergy and Clinical Immunology* 125 (1): 39–49. https://doi.org/10.1016/j.jaci.2009.10.039.

Schmaler, Mathias, Naja J. Jann, Fabrizia Ferracin, and Regine Landmann. 2011a. “T and B Cells Are Not Required for Clearing Staphylococcus Aureus in Systemic Infection despite a Strong TLR2-MyD88-Dependent T Cell Activation.” *Journal of Immunology (Baltimore, Md.: 1950)* 186 (1): 443–52. https://doi.org/10.4049/jimmunol.1001407.

Schnaith, Annabelle, Hamid Kashkar, Sonja A. Leggio, Klaus Addicks, Martin Krönke, and Oleg Krut. 2007. “Staphylococcus Aureus Subvert Autophagy for Induction of Caspase-Independent Host Cell Death.” *The Journal of Biological Chemistry* 282 (4): 2695–2706. https://doi.org/10.1074/jbc.M609784200.

Schreiner, Jens, Dorothee Kretschmer, Juliane Klenk, Michael Otto, Hans-Jörg Bühring, Stefan Stevanovic, Ji Ming Wang, Sandra Beer-Hammer, Andreas Peschel, and Stella E. Autenrieth. 2013. “Staphylococcus Aureus Phenol-Soluble Modulin Peptides Modulate Dendritic Cell Functions and Increase in Vitro Priming of Regulatory T Cells.” *Journal of Immunology (Baltimore, Md.: 1950)* 190 (7): 3417–26. https://doi.org/10.4049/jimmunol.1202563.

Seneschal, Julien, Rachael A. Clark, Ahmed Gehad, Clare M. Baecher-Allan, and Thomas S. Kupper. 2012. “Human Epidermal Langerhans Cells Maintain Immune Homeostasis in Skin by Activating Skin Resident Regulatory T Cells.” *Immunity* 36 (5): 873–84. https://doi.org/10.1016/j.immuni.2012.03.018.

Sharma-Kuinkel, Batu K., Yuling Wu, David E. Tabor, Hoyin Mok, Bret R. Sellman, Amy Jenkins, Li Yu, et al. 2015. “Characterization of Alpha-Toxin Hla Gene Variants, Alpha-Toxin Expression Levels, and Levels of Antibody to Alpha-Toxin in Hemodialysis and Postsurgical Patients with Staphylococcus Aureus Bacteremia.” *Journal of Clinical Microbiology* 53 (1): 227–36. https://doi.org/10.1128/JCM.02023-14.

Shaulov, Angela, and Kaja Murali-Krishna. 2008. “CD8 T Cell Expansion and Memory Differentiation Are Facilitated by Simultaneous and Sustained Exposure to Antigenic and Inflammatory Milieu.” *The Journal of Immunology* 180 (2): 1131–38. https://doi.org/10.4049/jimmunol.180.2.1131.

Shinefield, Henry, Steven Black, Ali Fattom, Gary Horwith, Scott Rasgon, Juan Ordonez, Hock Yeoh, et al. 2002. “Use of a Staphylococcus Aureus Conjugate Vaccine in Patients Receiving Hemodialysis.” *The New England Journal of Medicine* 346 (7): 491–96. https://doi.org/10.1056/NEJMoa011297.

Small, P. M., and H. F. Chambers. 1990. “Vancomycin for Staphylococcus Aureus Endocarditis in Intravenous Drug Users.” *Antimicrobial Agents and Chemotherapy* 34 (6): 1227–31.

Smith, CI Edvard, and Anna Berglöf. 1993. “X-Linked Agammaglobulinemia.” In *GeneReviews*®, edited by Margaret P. Adam, Holly H. Ardinger, Roberta A.

Pagon, Stephanie E. Wallace, Lora JH Bean, Karen Stephens, and Anne Amemiya. Seattle (WA): University of Washington, Seattle. http://www.ncbi.nlm.nih.gov/books/NBK1453/.

Song, L., M. R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, and J. E. Gouaux. 1996. "Structure of Staphylococcal Alpha-Hemolysin, a Heptameric Transmembrane Pore." *Science (New York, N.Y.)* 274 (5294): 1859–66.

Steinhoff, Martin, Thomas Brzoska, and Thomas A. Luger. 2001. "Keratinocytes in Epidermal Immune Responses." *Current Opinion in Allergy and Clinical Immunology* 1 (5): 469–76.

Stentzel, Sebastian, Nandakumar Sundaramoorthy, Stephan Michalik, Maria Nordengrün, Sarah Schulz, Julia Kolata, Peggy Kloppot, et al. 2015. "Specific Serum IgG at Diagnosis of Staphylococcus Aureus Bloodstream Invasion Is Correlated with Disease Progression." *Journal of Proteomics* 128 (October): 1–7. https://doi.org/10.1016/j.jprot.2015.06.018.

Stephan, J. L., V. Vlekova, F. Le Deist, S. Blanche, J. Donadieu, G. De Saint-Basile, A. Durandy, C. Griscelli, and A. Fischer. 1993. "Severe Combined Immunodeficiency: A Retrospective Single-Center Study of Clinical Presentation and Outcome in 117 Patients." *The Journal of Pediatrics* 123 (4): 564–72.

Stranger-Jones, Yukiko K., Taeok Bae, and Olaf Schneewind. 2006. "Vaccine Assembly from Surface Proteins of Staphylococcus Aureus." *Proceedings of the National Academy of Sciences of the United States of America* 103 (45): 16942–47. https://doi.org/10.1073/pnas.0606863103.

Stryjewski, Martin E., and Henry F. Chambers. 2008. “Skin and Soft-Tissue Infections Caused by Community-Acquired Methicillin-Resistant Staphylococcus Aureus.” *Clinical Infectious Diseases* 46 (Supplement_5): S368–77. https://doi.org/10.1086/533593.

Suttorp, N., M. Fuhrmann, S. Tannert-Otto, F. Grimminger, and S. Bhadki. 1993. “Pore-Forming Bacterial Toxins Potently Induce Release of Nitric Oxide in Porcine Endothelial Cells.” *The Journal of Experimental Medicine* 178 (1): 337–41.

Tobkes, N., B. A. Wallace, and H. Bayley. 1985. “Secondary Structure and Assembly Mechanism of an Oligomeric Channel Protein.” *Biochemistry* 24 (8): 1915–20.

Tong, Steven Y. C., Joshua S. Davis, Emily Eichenberger, Thomas L. Holland, and Vance G. Fowler. 2015. “Staphylococcus Aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management.” *Clinical Microbiology Reviews* 28 (3): 603–61. https://doi.org/10.1128/CMR.00134-14.

Toshkova, K., C. Annemüller, null Akineden O, and C. Lämmler. 2001. “The Significance of Nasal Carriage of Staphylococcus Aureus as Risk Factor for Human Skin Infections.” *FEMS Microbiology Letters* 202 (1): 17–24.

Tromp, Angelino T., Michiel Gent, Pauline Abrial, Amandine Martin, Joris P. Jansen, Carla J. C. Haas, Kok P. M. Kessel, et al. 2018. “Human CD45 Is an F-Component-Specific Receptor for the Staphylococcal Toxin Panton–Valentine Leukocidin.” *Nature Microbiology* 3 (6): 708–17. https://doi.org/10.1038/s41564-018-0159-x.

Tweten, R. K., K. K. Christianson, and J. J. Iandolo. 1983. “Transport and Processing of Staphylococcal Alpha-Toxin.” *Journal of Bacteriology* 156 (2): 524–28.

Valeva, Angela, Nadja Hellmann, Iwan Walev, Dennis Strand, Markus Plate, Fatima Boukhallouk, Antje Brack, Kentaro Hanada, Heinz Decker, and Sucharit Bhakdi. 2006a. “Evidence That Clustered Phosphocholine Head Groups Serve as Sites for Binding and Assembly of an Oligomeric Protein Pore.” *The Journal of Biological Chemistry* 281 (36): 26014–21. https://doi.org/10.1074/jbc.M601960200.

Vermaelen, Karim Y., Ines Carro-Muino, Bart N. Lambrecht, and Romain A. Pauwels. 2001. “Specific Migratory Dendritic Cells Rapidly Transport Antigen from the Airways to the Thoracic Lymph Nodes.” *The Journal of Experimental Medicine* 193 (1): 51–60.

Vignali, Dario A. A., Lauren W. Collison, and Creg J. Workman. 2008. “How Regulatory T Cells Work.” *Nature Reviews Immunology* 8 (7): 523–32. https://doi.org/10.1038/nri2343.

Wan, Y. S., Z. Q. Wang, Y. Shao, J. J. Voorhees, and G. J. Fisher. 2001. “Ultraviolet Irradiation Activates PI 3-Kinase/AKT Survival Pathway via EGF Receptors in Human Skin in Vivo.” *International Journal of Oncology* 18 (3): 461–66.

Wanke, Ines, Heiko Steffen, Christina Christ, Bernhard Krismer, Friedrich Götz, Andreas Peschel, Martin Schaller, and Birgit Schittek. 2011. “Skin Commensals Amplify the Innate Immune Response to Pathogens by Activation of Distinct Signaling Pathways.” *Journal of Investigative Dermatology* 131 (2): 382–90. https://doi.org/10.1038/jid.2010.328.

Wardenburg, Juliane Bubeck, and Olaf Schneewind. 2008. “Vaccine Protection against Staphylococcus Aureus Pneumonia.” *The Journal of Experimental Medicine* 205 (2): 287–94. https://doi.org/10.1084/jem.20072208.

Watanabe, M., T. Tomita, and T. Yasuda. 1987. “Membrane-Damaging Action of Staphylococcal Alpha-Toxin on Phospholipid-Cholesterol Liposomes.” *Biochimica Et Biophysica Acta* 898 (3): 257–65.

Weber, Silvio, Michaela T. Niessen, Johannes Prox, Renate Lüllmann-Rauch, Annika Schmitz, Ralf Schwanbeck, Carl P. Blobel, et al. 2011. “The Disintegrin/Metalloproteinase Adam10 Is Essential for Epidermal Integrity and Notch-Mediated Signaling.” *Development (Cambridge, England)* 138 (3): 495–505. https://doi.org/10.1242/dev.055210.

Weisman, Leonard E., Helen M. Thackray, Robin H. Steinhorn, William F. Walsh, Herbert A. Lassiter, Ramasubbareddy Dhanireddy, Beverly S. Brozanski, et al. 2011. “A Randomized Study of a Monoclonal Antibody (Pagibaximab) to Prevent Staphylococcal Sepsis.” *Pediatrics* 128 (2): 271–79. https://doi.org/10.1542/peds.2010-3081.

Weissmann, G., G. Sessa, and A. W. Bernheimer. 1966. “Staphylococcal Alpha-Toxin: Effects on Artificial Lipid Spherules.” *Science (New York, N.Y.)* 154 (3750): 772–74.

Wertheim, Heiman F. L., Damian C. Melles, Margreet C. Vos, Willem van Leeuwen, Alex van Belkum, Henri A. Verbrugh, and Jan L. Nouwen. 2005. “The Role of Nasal Carriage in Staphylococcus Aureus Infections.” *The Lancet. Infectious Diseases* 5 (12): 751–62. https://doi.org/10.1016/S1473-3099(05)70295-4.

Wertheim, Heiman FL, Margreet C. Vos, Alewijn Ott, Alex van Belkum, Andreas Voss, Jan AJW Kluytmans, Peter HJ van Keulen, Christina MJE Vandenbroucke-Grauls, Marlene HM Meester, and Henri A. Verbrugh. 2004. “Risk and Outcome of Nosocomial Staphylococcus Aureus Bacteraemia in Nasal Carriers versus Non-Carriers.” *The Lancet* 364 (9435): 703–5. https://doi.org/10.1016/S0140-6736(04)16897-9.

West, Heather C., and Clare L. Bennett. 2018. “Redefining the Role of Langerhans Cells As Immune Regulators within the Skin.” *Frontiers in Immunology* 8 (January). https://doi.org/10.3389/fimmu.2017.01941.

Whitmire, Jason K., Nicola Benning, and J. Lindsay Whitton. 2006. “Precursor Frequency, Nonlinear Proliferation, and Functional Maturation of Virus-Specific CD4+ T Cells.” *Journal of Immunology (Baltimore, Md.: 1950)* 176 (5): 3028–36.

Wilke, Georgia A., and Juliane Bubeck Wardenburg. 2010a. “Role of a Disintegrin and Metalloprotease 10 in Staphylococcus Aureus Alpha-Hemolysin-Mediated Cellular Injury.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (30): 13473–78. https://doi.org/10.1073/pnas.1001815107.

Xiong, Yan Q., Julie Willard, Michael R. Yeaman, Ambrose L. Cheung, and Arnold S. Bayer. 2006. “Regulation of Staphylococcus Aureus Alpha-Toxin Gene (Hla) Expression by Agr, SarA, and Sae in Vitro and in Experimental Infective Endocarditis.” *The Journal of Infectious Diseases* 194 (9): 1267–75. https://doi.org/10.1086/508210.

Zhao, Xinyan, Eszter Deak, Kelly Soderberg, Melissa Linehan, David Spezzano, Jia Zhu, David M. Knipe, and Akiko Iwasaki. 2003a. “Vaginal Submucosal Dendritic

Cells, but Not Langerhans Cells, Induce Protective Th1 Responses to Herpes Simplex Virus-2." *The Journal of Experimental Medicine* 197 (2): 153–62. https://doi.org/10.1084/jem.20021109.

Zielinski, Christina E., Federico Mele, Dominik Aschenbrenner, David Jarrossay, Francesca Ronchi, Marco Gattorno, Silvia Monticelli, Antonio Lanzavecchia, and Federica Sallusto. 2012. "Pathogen-Induced Human TH17 Cells Produce IFN-γ or IL-10 and Are Regulated by IL-1β." *Nature* 484 (7395): 514–18. https://doi.org/10.1038/nature10957.

APPENDIX

FIGURES

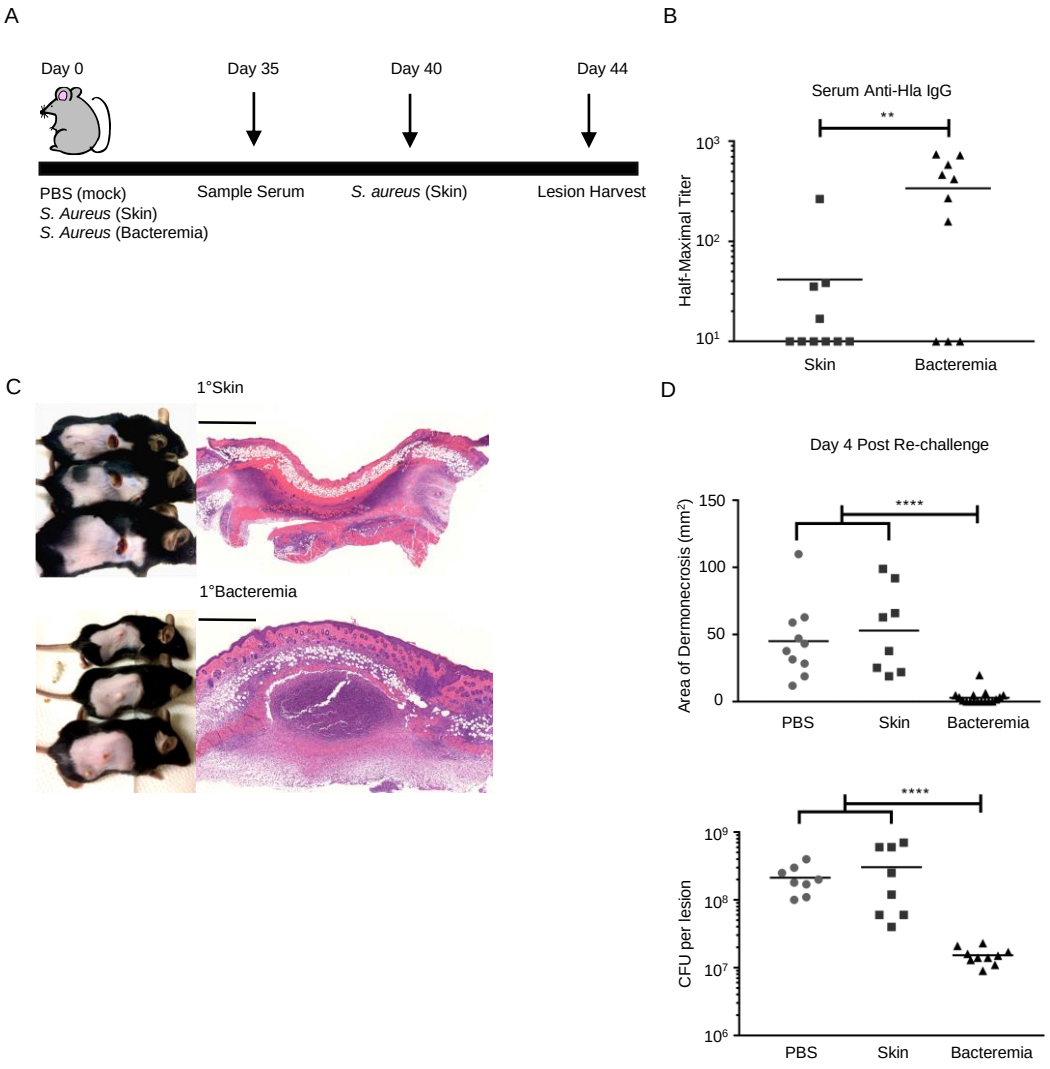

Figure 1. Primary *S. aureus* intravenous challenge promotes the development of protective immunity to secondary skin challenge. A) Experimental approach, outlining timing of primary subcutaneous (S.C), retro-orbital (R.O) infections and skin re-challenge. Lesions were measured and harvested. Colony forming units (CFU) were enumerated by plating. The limit of detection was 1000 CFU. B) Representative gross (left) and H&E (right) images of lesions day 4 post re-challenge. Scale bars, 1000 μm. C) Area of dermonecrosis (top), CFU per lesion (center), and kidney CFU (bottom) enumerated. **** = $p<0.0001$ determined by one-way ANOVA with Sidak's multiple comparison test. Data are representative of three independent experiments.

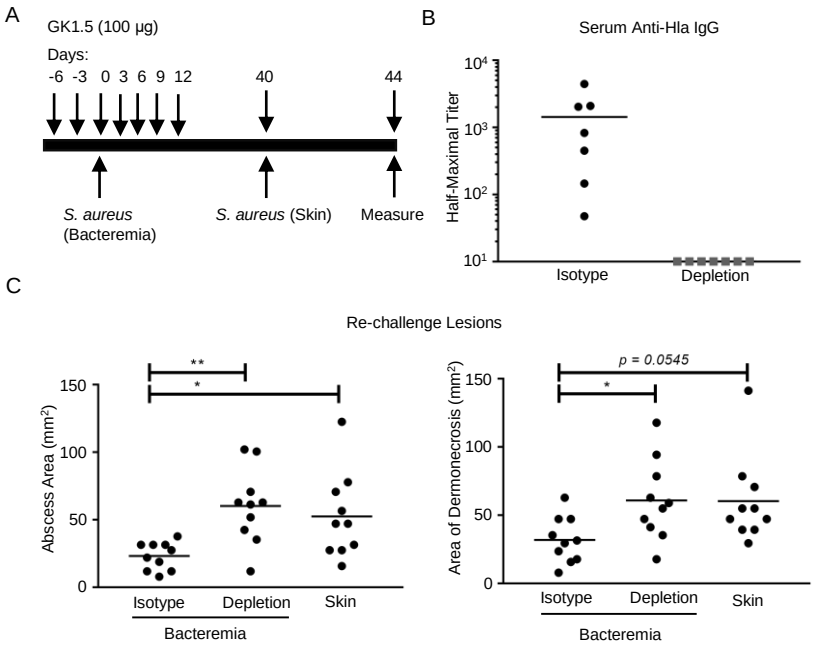

Figure 2. T cells are required for protection. A) Experimental timeline for CD4 T cell depletion during intravenous infection. Mice were given 100 µg of GK1.5 or isotype control for the times indicated. B) Anti-hla IgG half maximal serum titers day 35 post primary bacteremia. C) Abscess area and area of dermonecrosis (AOD) were assessed 4 days post re-challenge. Lesions of primary bacteremic mice were compared to re-challenge lesions of primary skin infected hosts. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ****= $p < 0.0001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of two independent experiments.

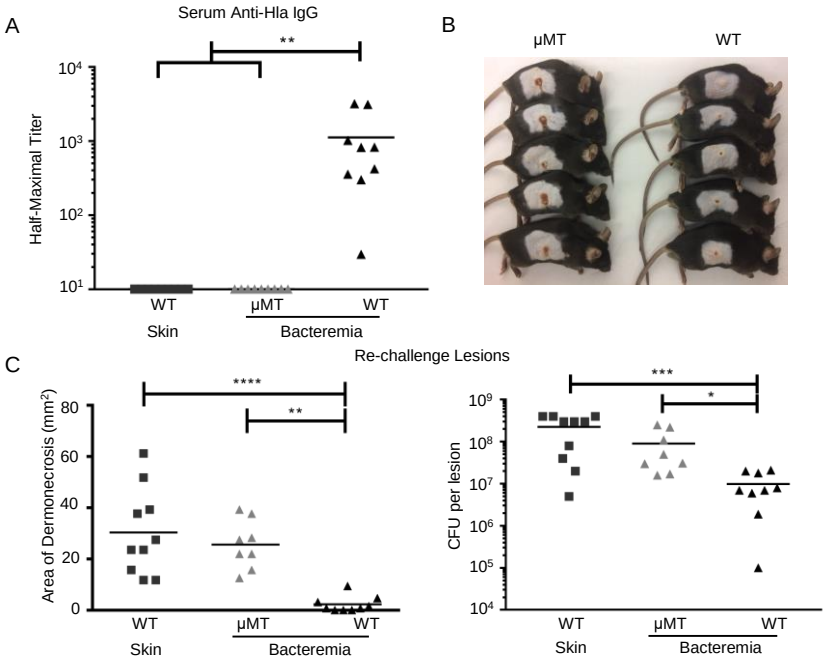

Figure 3. B cells are required for protective immunity
A) Serum Anti-Hla IgG titers, day 35 post primary infection. B) WT and μMT mice were retro-orbitally infected with S. aureus and subcutaneously re-challenged on Day 40 post primary infection. C) Area of dermonecrosis and lesion colony forming units (CFU) quantification demonstrates loss of protection in the absence of B cells. Absence of protection and anti-hla IgG titers are correlated. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ****= $p < 0.0001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of three independent experiments.

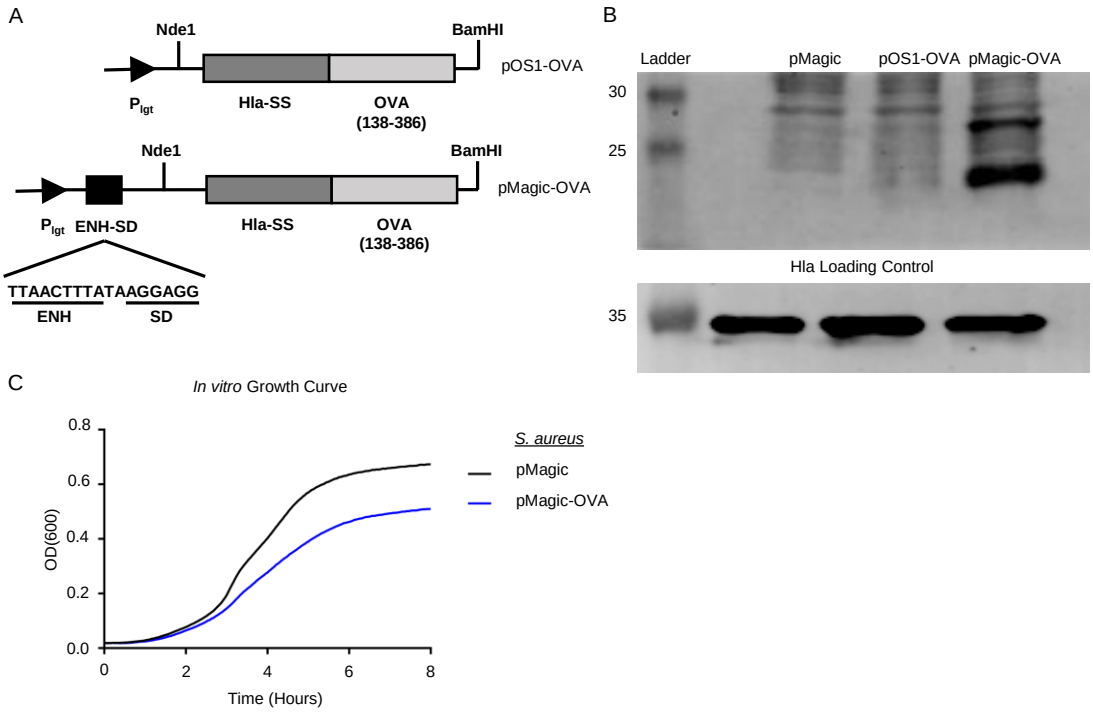

Figure 4. Generating OVA expressing *S. aureus* strain.
A) Plasmid map of candidate constructs pOS1-OVA and pMagic-OVA. pMagic-OVA includes an improved translation initiation region, containing an optimized Shine-Dalgarno (SD) sequence, spacer region and translational enhancer (ENH) from gene 10 of phage T7 (based on the TIR sequence of Cheng and Patterson, 1992). Chicken egg ovalbumin sequence was codon optimized (Thermo Fisher) for *S. aureus*. B) OVA(138-386) expression by Western blot. Overnight culture supernatants of pMagic, pOS1-OVA, and pMagic-OVA were TCA precipitated and blotted for OVA. Hla was blotted as a loading control. The expected molecular weight of OVA(138-386) was 27.5 kDA. C) Growth kinetics of pMagic and pMagic-OVA *S. aureus* strains were assessed in TSB alone or supplemented with 20 µg/ml of chloramphenicol.

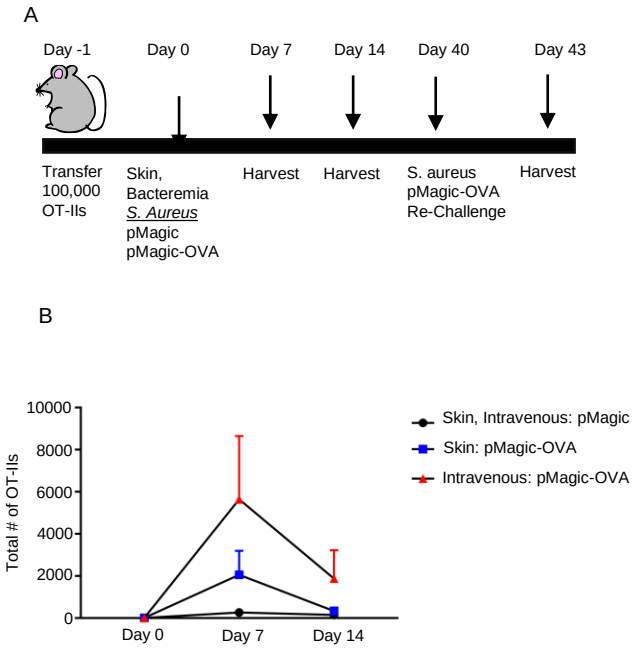

Figure 5. T cell kinetics are tissue dependent.
A) Experimental timeline. Mice received 100,000 CD45.1+ OT-IIs via retro-orbital injection one day prior to skin or intravenous infection with S-EV or S-OVA. B) OT-II priming kinetics were tracked 7 and 14 days after infection. The spleen and skin draining lymph nodes (brachial, axillary and superficial inguinal), on the same side, were harvested from both intravenously and skin infected mice, and total OT-IIs were enumerated by flow cytometry. Numbers from both sites were added to calculated the total number recovered per mouse.

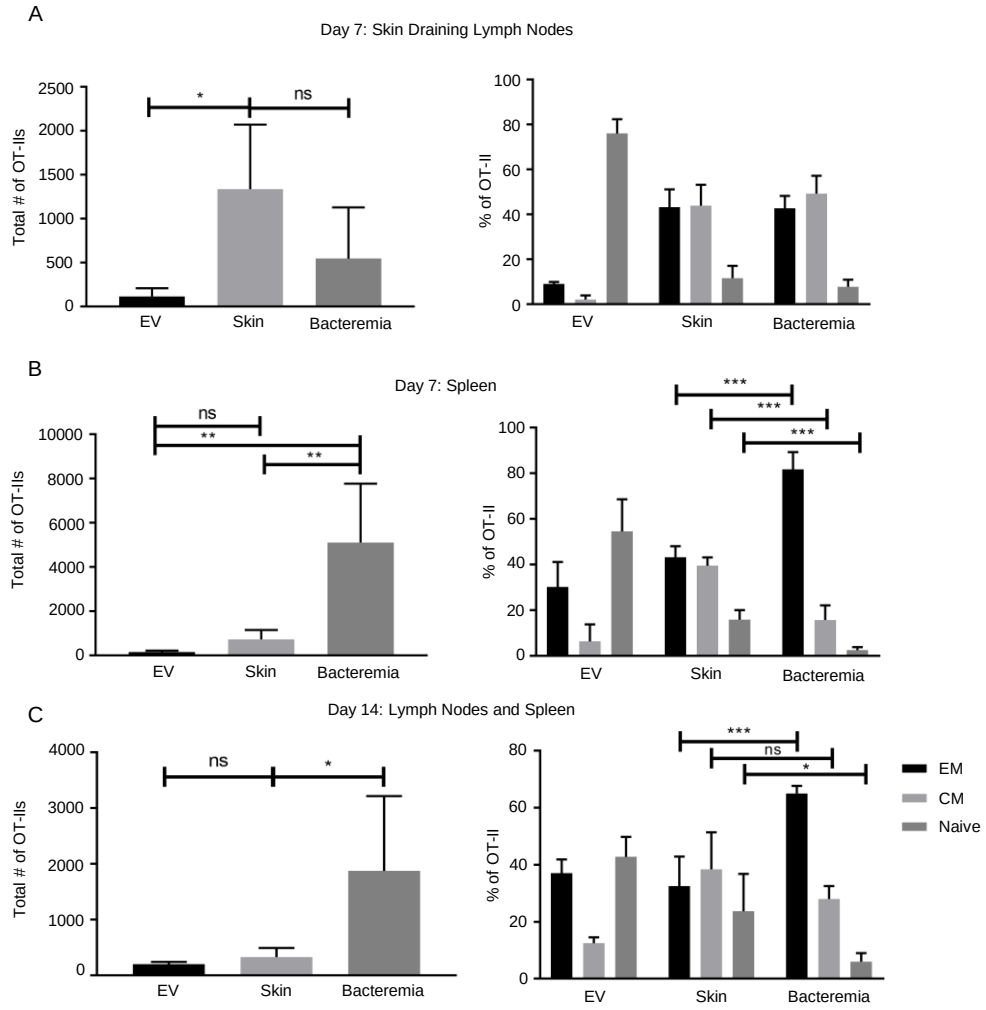

Figure 6. Tissue dependence of antigen-specific CD4 T cell priming. A) OT-II numbers were analyzed at the skin draining lymph nodes for differences in site-specific accumulation on day 7. B) OT-II numbers were analyzed at the spleen for differences in site-specific accumulation on day 7. C) OT-IIs were quantified at day 14 post infection. Lymph nodes and spleen counts were pooled for analysis. OT-IIs were classified into T helper effector memory (EM), central memory (CM) and naïve cell phenotypes, as determined by CD44 and CD62L surface expression (bottom). EM = $CD44^{high}$ $CD62L^{low}$; CM = $CD44^{high}$ $CD62L^{high}$; Naive = $CD44^{low}$ $CD62L^{high}$. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of three independent experiments.

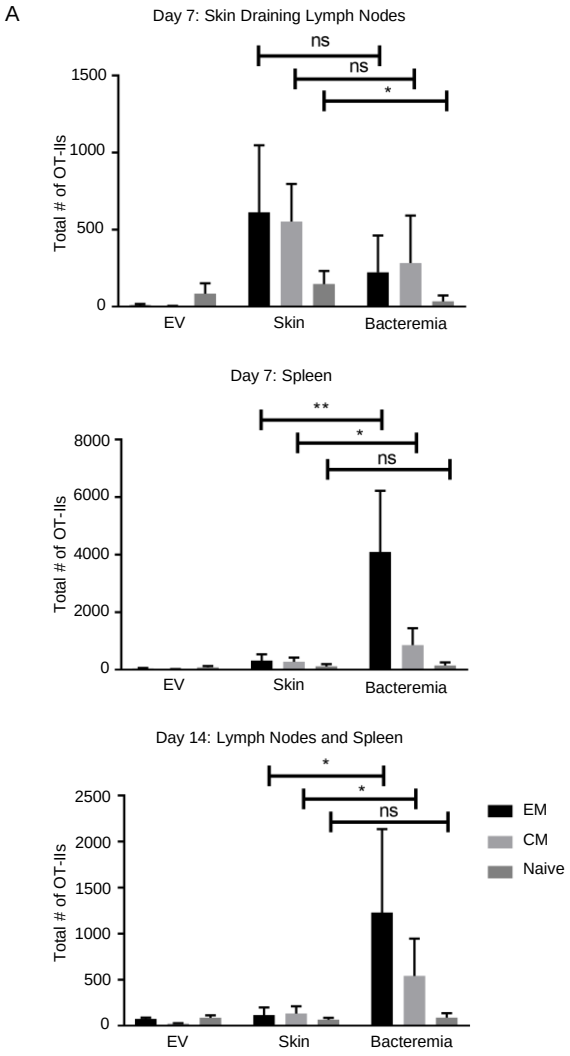

Figure 7. Bacteremia induces T memory cells A) OT-II numbers were analyzed at the skin draining lymph nodes and spleen for differences in site-specific accumulation on day 7 and 14. On day 14, LN and spleen cells were pooled for quantificationOT-IIs were classified into T helper effector memory (EM), central memory (CM) and naïve cell phenotypes, as determined by CD44 and CD62L surface expression (bottom). EM = $CD44^{high}$ $CD62L^{low}$; CM = $CD44^{high}$ $CD62L^{high}$; Naive = $CD44^{low}$ $CD62L^{high}$. * = $p < 0.05$; ** = $p < 0.01$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of three independent experiments.

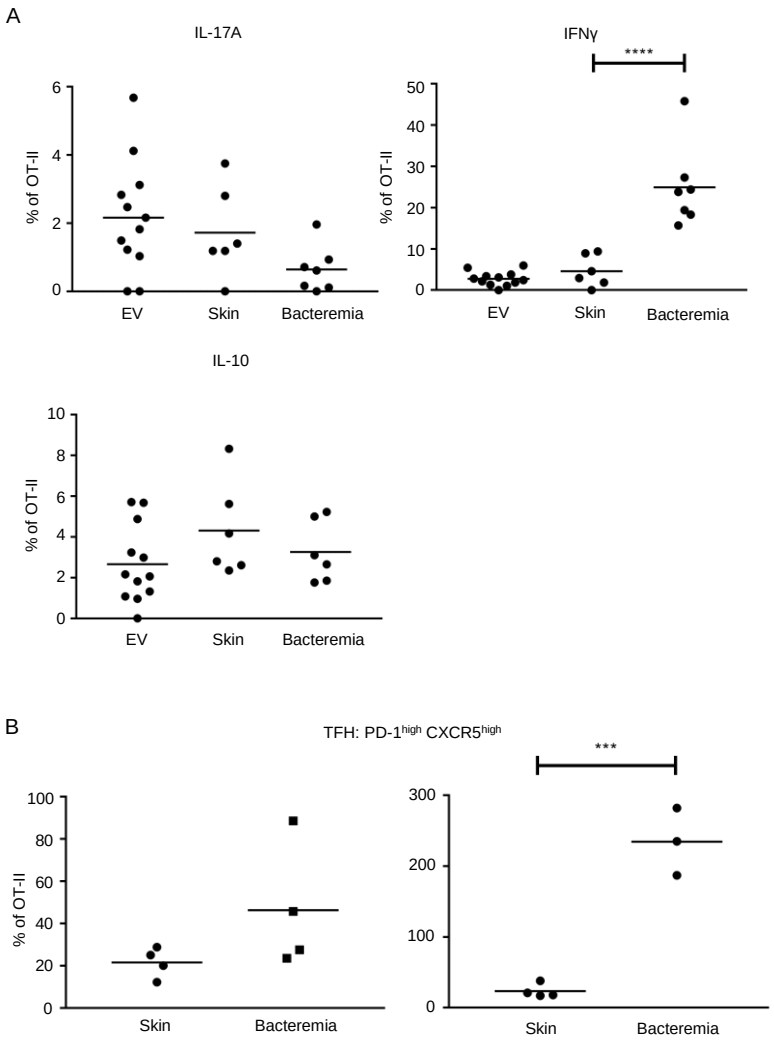

Figure 8. Skin infection fails to induce T helper subset differentiation A) Day 14 OT-II cytokine responses. Mice received 100,000 CD45.1+ OT-IIs via retro-orbital injection one day prior to skin or septic infection with pMagic or pMagic-OVA S. aureus strains. OT-IIs were phenotyped by IFNγ, IL-17A, and IL-10 responses. B) T follicular helper cell subsets were identified by PD-1 and CXCR5 expression. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$, **** = $p < 0.0001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of two independent experiments.

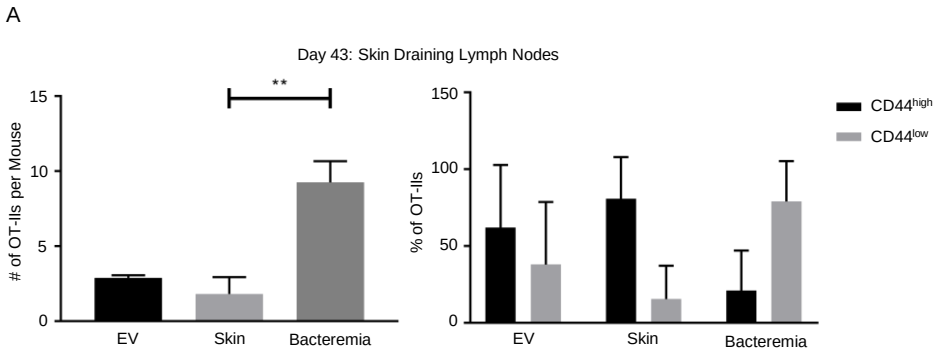

Figure 9. Skin infection fails to produce responsive memory T cells
A) Recall effector and central memory T cell responses were evaluated by *S. aureus* pMagic-OVA skin re-challenge 40 days post primary infection. Skin draining lymph nodes, and skin biopsies, were excised 3 days post re-challenge and analyzed by flow cytometry. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of two independent experiments.

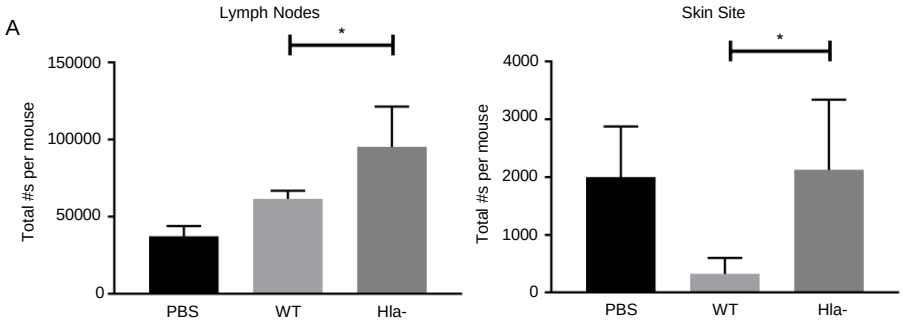

Figure 10. Hla targets skin dendritic cells
A) Total migratory dendritic cell (mDC) accumulation in skin draining lymph nodes and infection site. Axillary, brachial and superficial inguinal lymph nodes were harvested for mDC analysis and enumeration by flow cytometry. Infection site was extracted using an 8 mm punch biopsy.. * = $p < 0.05$; ** = $p < 0.01$; determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of three independent experiments.

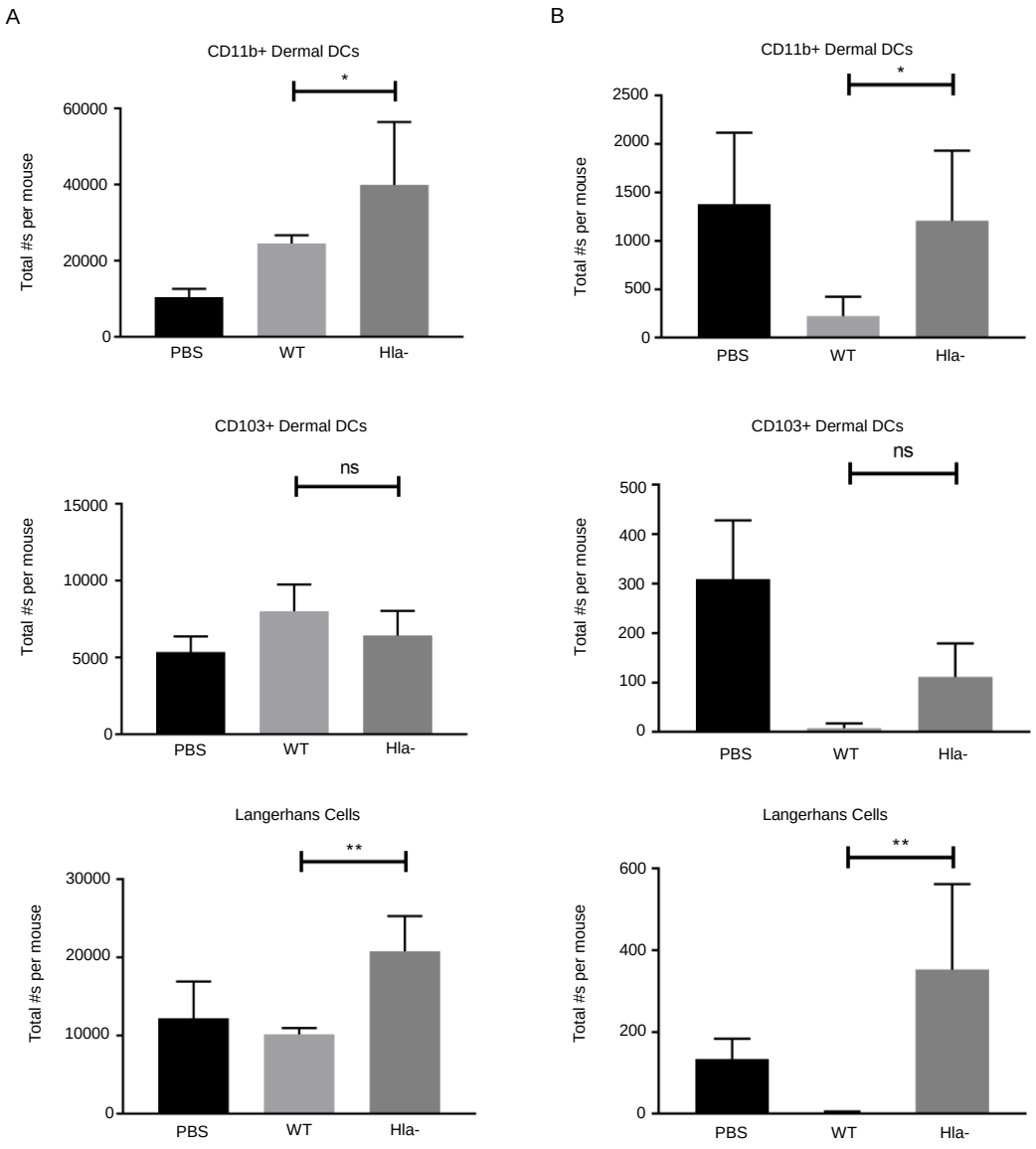

Figure 11. Hla reduces Langerhans cell and CD11b dermal dendritic cell accumulation

A) Lymph node CD11b+ and CD103+ dermal dendritic cells (DCs) and Langerhans Cells were analyzed from the draining lymph nodes and site of infection. B) Skin CD11b+ and CD103+ dermal dendritic cells (DCs) and Langerhans Cells were analyzed from the site of infection. Total numbers were enumerated by flow cytometry. * = $p < 0.05$; ** = $p < 0.01$; determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of three independent experiments.

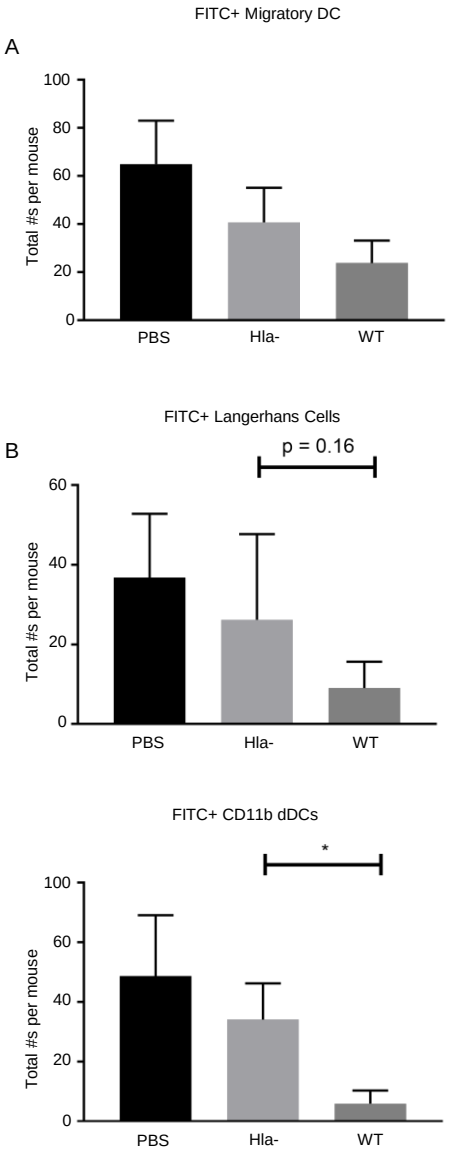

Figure 12. Skin DC migration is improved during LAC *hla::erm* infection A) FITC^{+} DCs in skin draining lymph nodes 4 days post paint and infection. Total numbers were enumerated by flow cytometry. B) FITC+ Migratory dendritic cell subsets in the skin draining lymph node after infection. Lymph nodes were harvested, digested and analyzed 4 days post infection. * = $p < 0.05$; determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of on pilot experiment

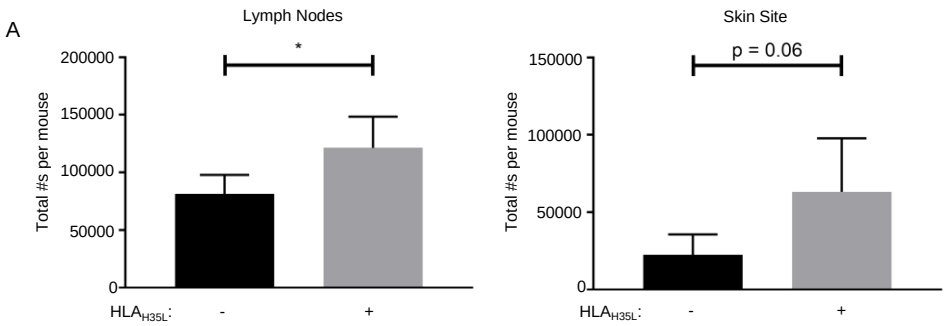

Figure 13. Active immunization against Hla protects skin mDCs. A) Total migratory DC counts in the skin and skin draining lymph nodes 4 days post infection B) Migratory dendritic cell subsets in the skin draining lymph node after infection. Lymph nodes were harvested, digested and analyzed 4 days post infection. C) Migratory dendritic cell subsets in the skin after infection. Skin was harvested, digested and analyzed 4 days post infection. Total numbers were enumerated by flow cytometry. * = $p < 0.05$; ** = $p < 0.01$; determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of two independent experiments.

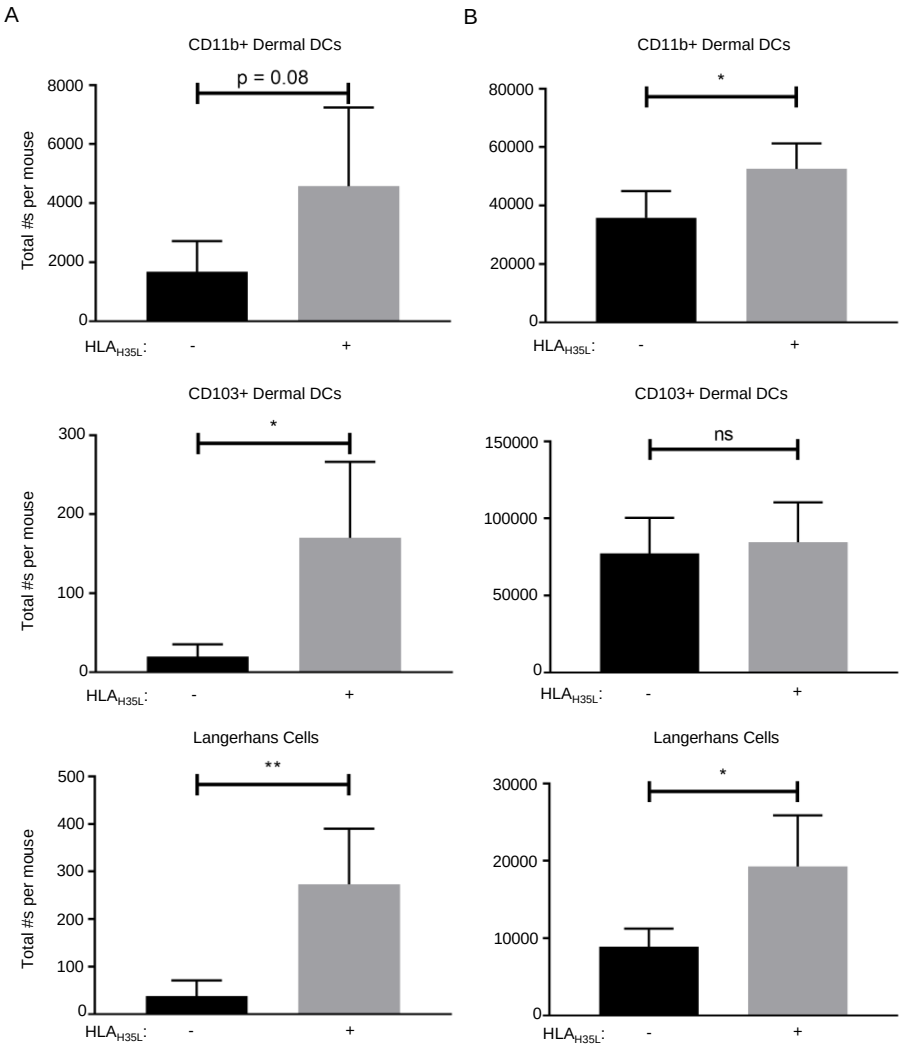

Figure 14. Active immunization against Hla protects all DC subsets. A) CD11b and CD103 dermal dendritic cells and Langerhans cells in the skin were analyzed 4 days post infection. B). CD11b and CD103 dermal dendritic cells and Langerhans cells from the skin draining lymph nodes were analyzed 4 days post infection. Total numbers were enumerated by flow cytometry. * = $p < 0.05$; ** = $p < 0.01$; determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of two independent experiments.

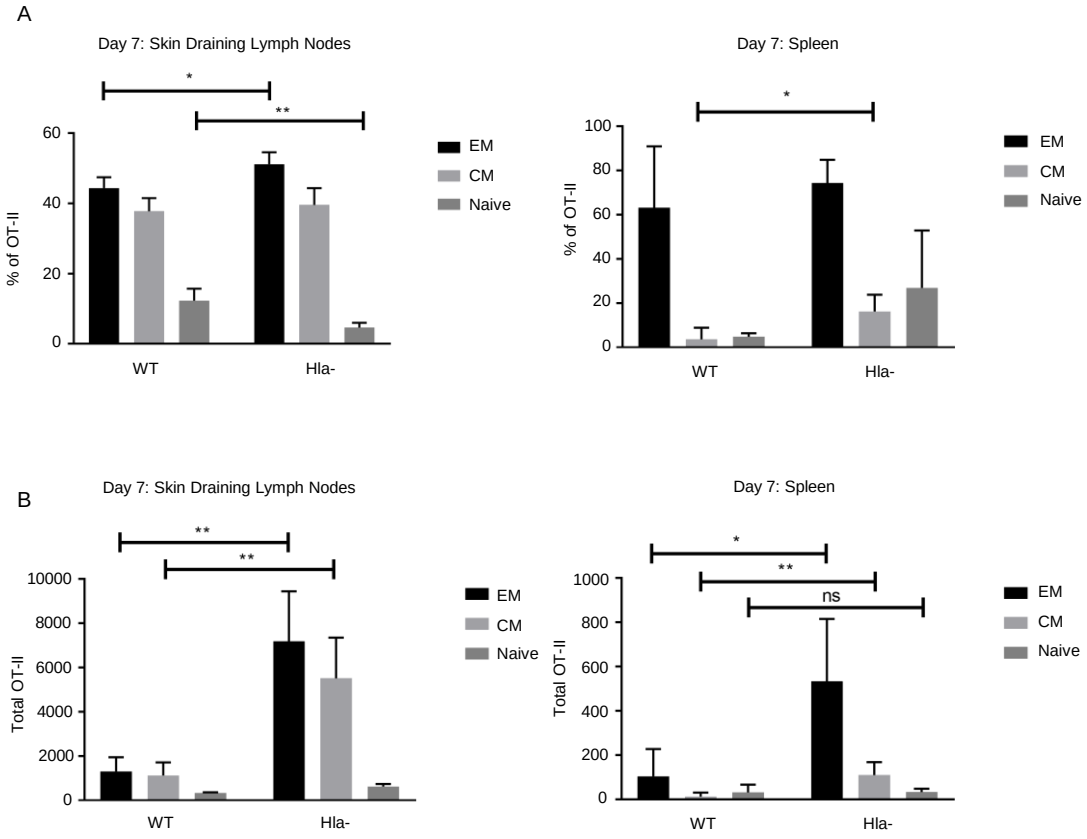

Figure 15. Hla suppresses T cell priming. A) OT-II priming kinetics were tracked 7 after infection with WT or LAC *hla::erm* pMagic-OVA on the skin. The spleen and skin draining lymph nodes (brachial, axillary and superficial inguinal), on the same side, were harvested total OT-IIs were enumerated by flow cytometry. B) Total numbers of memory and naïve OT-IIs in the skin draining lymph nodes and spleens * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of one experiment.

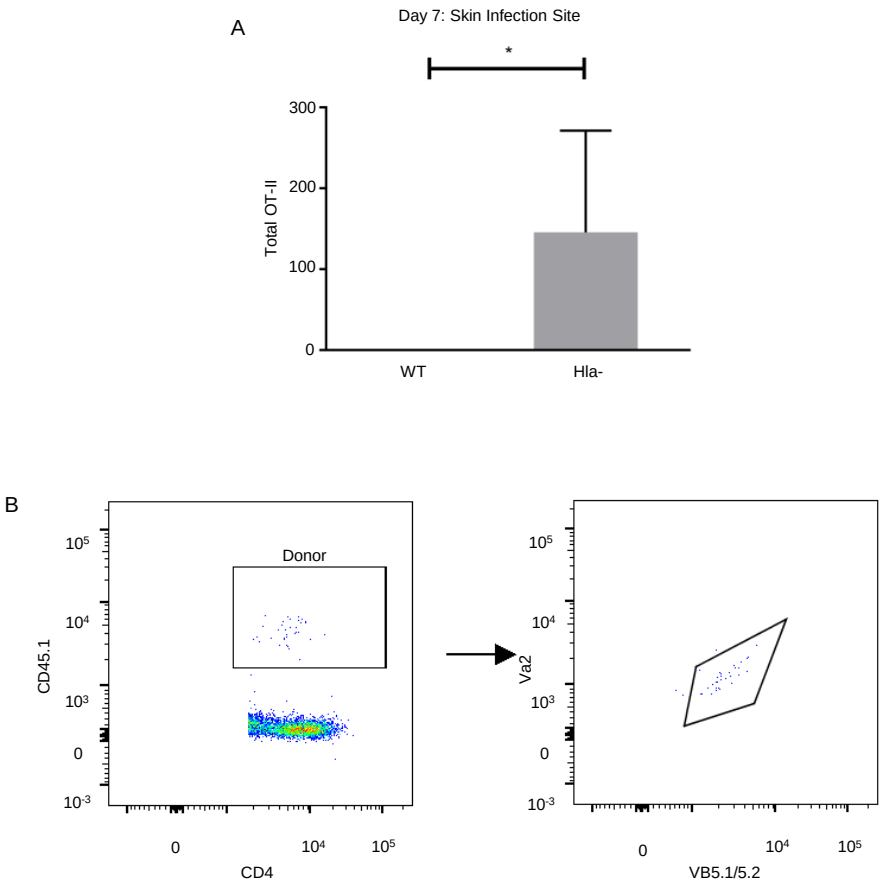

Figure 16. Hla suppresses T cell skin site accumulation. A) OT-II priming kinetics were tracked 7 after infection with WT or LAC *hla::erm* pMagic-OVA on the skin. An 8 mm punch biopsy of the infection site was harvested and total OT-IIs were enumerated by flow cytometry. B) Representative flow plot of gating strategy in the skin. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of one experiment.

A

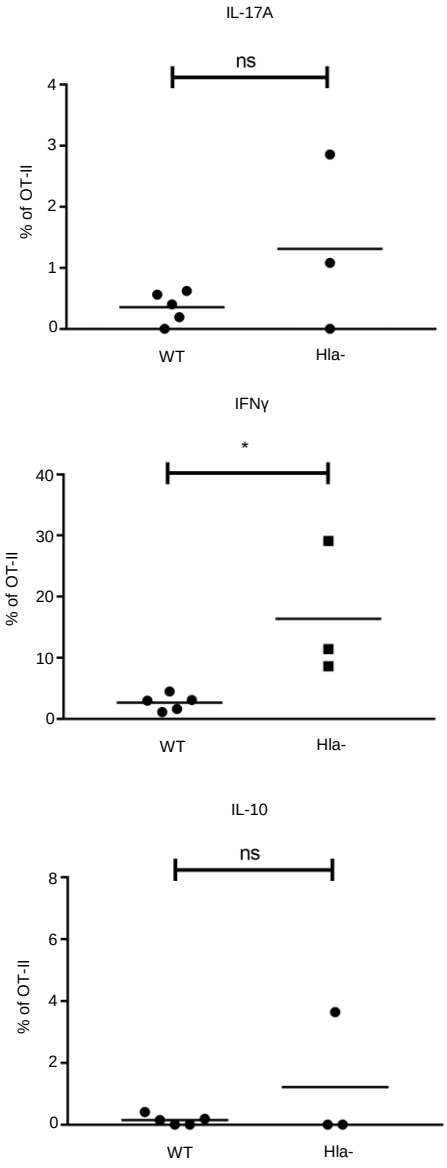

Figure 17. Hla suppresses T cell cytokine production A) OT-IIs were positively enriched from skin draining lymph nodes and spleen 14 days post infection, and stimulated with PMA and Ionomycin for 4 hours. IFNγ, IL-4, IL-10 and IL-17A expression was analyzed by flow cytometry. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ determined by one-way ANOVA with Sidak's multiple comparison test, or t test where appropriate. Data are representative of one experiment.