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Phenotype and kinetics of the endogenous pulmonary CD4 T cell response to a primary influenza: A virus infection

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Abstract
Background: Viral and bacterial pneumonias are a leading killer of children and adults worldwide. Influenza A virus (IAV) is one of the most common causes of viral pneumonias, infecting up to 20% of the population each year.
Objectives: To provides insight into the specialized subsets present in the influenza A virus-specific CD4 T cell response in the lungs and demonstrates that this response is regulated by pulmonary antigen presenting cells.
Methods: Female BALB/c and C57Bl/6 mice at 8-12 weeks of age for all experiments were used. Age- and weight-matched groups of female C57Bl/6 mice were lightly anesthetized by isoflurane inhalation and infected intra nasally.
Results: A significant increase in the frequency of CD4 T cells producing IFNγ following incubation with peptide-pulsed splenic stimulator DC, compared with CD4 T cells incubated with non-peptide-pulsed splenic stimulator DC.IAV-specific CD4 and CD8 T cells specific for highly conserved epitopes within the internal proteins of IAV can provide hetero subtypic protection.
Conclusion: Authors found that IAV and GAS are individually important human pathogens that cause a significant amount of global disease and death. Understanding the immune response to these pathogens is the key to development of better treatments and vaccinations.

Keywords: CD4 cell, influenza a virus, isoflurane, mice, T cells

Introduction

Mucosal surfaces are the most common port of entry for pathogens, and the lungs in particular, boasting the largest air-exposed epithelial surface in the body, are constantly bombarded by millions of potentially infectious pathogens [1]. While millennia of natural selection have honed a sophisticated repertoire of defenses, the lung faces some unique challenges due to the primary function of the lung parenchyma: gas exchange [2].

T cells play a decisive and highly regulated role in the defense against bacterial, fungal and viral pathogens in the lungs. An inadequate T cell response during an infection can result in delayed clearance or dissemination of the pathogen to other organs, while an excessive T cell response can result in rapid clearance of the pathogen, at the expense of significant damage to host tissues [3]. The properties of the pathogen-specific T cell response vary depending on the pathogen. Antigen-presenting cells (APC) including dendrite cells (DC) play a key role in initiating the response by activating naïve T cells and then shape the response through cytokine production. The production of pro-inflammatory cytokines and chemokines, and subsequent arrival of innate immune cells represent another lung-specific [4]. Complete eradication of the pathogen while preserving the delicate architecture of the lung required for gas exchange [3]. T cells play a decisive and highly regulated role in the defense against bacterial, fungal and viral pathogens in the lungs [4].

An inadequate T cell response during an infection can result in delayed clearance or dissemination of the pathogen to other organs, while an excessive T cell response can result in rapid clearance of the pathogen, at the expense of significant damage to host tissues. The properties of the pathogen-specific T cell response vary depending on the pathogen [5].

Inhaled pathogens contain pathogen-associated molecular patterns (PAMPs) that are

recognized by respiratory epithelial cells and DC expressing pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), NOD like receptors (NLRs), and Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) (12). Immature DC triggered by recognition of a PAMP or by cytokines produced by activated epithelial cells mature and migrates to the lung-draining lymph node, where they express all the necessary signals to activate naïve T cells [1-3].

Antigen-presenting cells (APC) including dendritic cells (DC) play a key role in initiating the response by activating naïve T cells and then shape the response through cytokine production. In 1986, the first evidence of two discrete subsets of helper T cells, termed type 1 and type 2 T helper cells (Th1and Th2, respectively) was published [6].

Materials and Methods

Female BALB/c and C57Bl/6 mice were purchased from the National Cancer Institute. Mice were used at 8-12 weeks of age for all experiments.

Influenza A virus infection of mice

Age- and weight-matched groups of female C57Bl/6 mice were lightly anesthetized by isoflurane inhalation and infected intra nasally with the indicated dose of mouse-adapted A/Puerto Rico/8/1934 (H1N1) (PR8) in 50µL of Iscove's media. Virus was grown in the allantoic fluid of 10-day-old embryonated hen eggsfor 2 days at 37 ºC. Allantoic fluid was harvested and stored at -80 ºC until use.

Pulmonary APC depletion48hrs after IAV infection, mice were lightly anesthetized with isoflurane and given 75µL of clodronate liposomes intranasally.

Preparation of cells

Lungs were harvested into 10mL Iscove' s DMEM, mashed through a wiremesh and filtered through a nylon mesh to obtain a single cell suspension inIscove' s media. When indicated, lungs were minced and digested in Iscove' smedia containing 1mg/mL collagenase (Sigma) and 0.02mg/mL DNAse (Sigma for 15 minutes at 37 ºC prior to mashing. Lymphocytes were quantified using trypan blue exclusion and a hemo cytometer.

Flow Cytometry

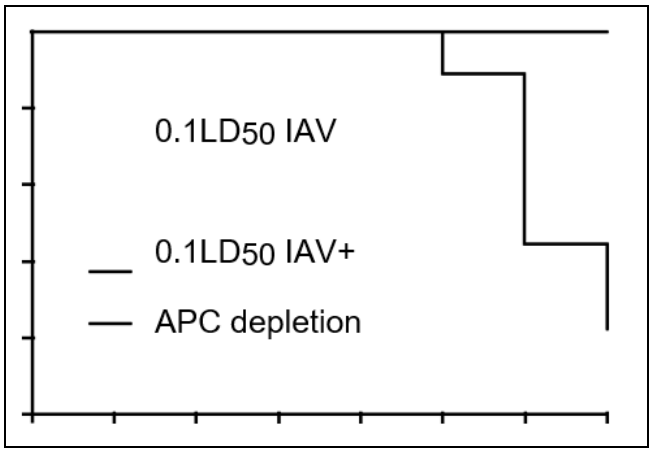
The following monoclonal antibodies were used for these studies: anti-CD4 (GK1.5 and RM4-5), anti-CD8α (53-6.7). For surface staining, cells were suspended at $1x10^6$ cells/100µL FACS buffer (sterile PBS, 2% heat-inactivated fetal calf serum, 0.02% Sodium Azide) with the indicated antibodies and incubated on ice for 30 minutes. Cells werethen fixed with BD FACS Lysing Solution (BD Biosciences, San Diego, CA). For intracellular staining for transcription factors, surface staining was performed as described, followed by fixation, permeabilization and staining according to manufacturer instructions in the e-Bioscience Foxp3 intracellular staining kit (eBioscience, San Diego CA).

Results

Table I: MHC class II molecule binding for published CD4 T cell epitopes

Mouse Strain	Specificity	Sequence	MHC	tested	outcome
	HA48 - 67	Tgkicnnphridgidctli	I-A^d	no	n/a
	HA71-91	Lgnphcdvfqnetwdlfvers	I-A^d	no	n/a
	HA72 - 92	Hrildgidctlidallgdphc	I-A^d	no	n/a
	HA81 - 97	Netwdlfverskafsnc	I-A^d	no	n/a
	HA110-120	Sferfeifpke	I-E^d	yes	-
	HA141-153	Hntngvtaacshe	I-A^d	yes	+++
BALB/c	HA159-170	Klknsyvnkkgk	I-E^d	yes	+
	HA 178 - 195	Yiwgihhpstnqeqtsly	I-E^d	no	n/a
	HA 186 - 200	Stnqeqtslyvqasg	I-E^d	no	n/a
	HA195-209	Nayvsvvtsnynrrf	I-A^d	yes	-
	HA306-318	Pkyvrsaklrmvt	I-E^d	yes	+
	HA317-329	Vtglrnipsiqsr	I-E^d	yes	-
	NA69 - 89	Iltgnsslcpirgwaiyskdn	I-E^d	yes	+++
	NA79 - 86	Irgwaiys	I-E^d	yes	-
	NP218 - 229	Ayermcnilkgk	I-A^d	yes	+++
	NP136-150	Mmiwhsnlndatyqr	I-A^b	yes	++
C57Bl/6	NP201-215	Indrnfwrgengrkt	I-A^b	yes	-
	NP276-290	Lpacvygpavasgyd	I-A^b	yes	-
	NP311-325	Qvyslirpnenpahk	I-A^b	yes	++++
	NP316-330	Irpnenpahksqlvw	I-A^b	yes	-

Table I shows sequence and MHC class II molecule binding for published CD4 T cell epitopes in A/Puerto Rico/8/1934 in the indicated mouse strain. A significant increase in the frequency of CD4 T cells producing IFNγ following incubation with peptide-pulsed splenic stimulator DC, compared with CD4 T cells incubated with non-peptide-pulsed splenic stimulator DC.



Graph 1: Pulmonary APC depletion

Graph 1 shows pulmonary APC depletion 48hpi with two normally sub lethal doses of IAV results in increased mortality. Female C57Bl/6 mice were infected i.n. with a $0.1LD_{50}$ (A) or $0.05LD^{50}$ (B) inoculums of PR8 and half were depleted of pulmonary APC by i.n. administration of clodronate liposomes 48hpi. Both IAV -infected mice (solid line) and IAV-infected, pulmonary APC-depleted mice (dashed line) were monitored daily for survival. Data are representative of at least two independent experiments. n=5-10 mice/group for both doses.

Discussion

In humans, analysis of pulmonary immune response to IAV infection is limited by the inability to take samples of the lungs themselves [7]. A thorough understanding of the immune response in the lungs of mice can help to focus human studies on cell types found in the bronchio-alveolar lavage fluid or blood that perform crucial functions in the lung tissue [8]. First, the extent of plasticity of IAV-specific CD4 T cells in the lungs during infection. Studies in other infection models have clearly shown that specific signals are required during the priming stage of CD4 T cell activation, but additional signals later on have been shown to change or reinforce the lineage commitment of this cells [9].

Whether within individual CD4 T cells there are changes in lineage determining transcription factor expression during the course of IAV infection is an area ripe for future study. My expectation is that rather than a complete shift in expression of a lineage-determining transcription factor (e.g. from T-bethiFoxp3- to T-bet-Foxp3hi), up regulation of a second lineage-determining transcription factor (e.g. T-bet+/intFoxp3+/int) could occur during IAV infection. Foxp3+T-bet+ cells arise during a murine model of M. tuberculosis and are very effective at trafficking to the site of Th1-mediated inflammation and suppressing proliferation and cytokine production by Th1 cells [10].

During IAV infection, a pro-inflammatory, anti-viral environment is important when virus is still present within the lungs, but later, resolution of inflammation and repair functions become more important [11]. During the transition from the pro-inflammatory phase to the resolution/restoration phase, Th1-specific suppression (i.e. by IAV-specific Foxp3+T-bet+ cells) would permit other subsets to perform their functions as necessary. The benefits of such a "finely-tuned" IAV-specific CD4 T cell response are evident considering that within the lung, excessive cellular infiltrate and inflammation can interfere with gas-exchange [12].

In support of IAV-specific CD4 T cells co-expressing lineage determining transcription factors, my own preliminary data (Figure 30) shows that at day 10 post-IAV infection, about 80% of NP311+ cells are T-bet+, and about 60% of NP311+ CD4 T cells are Bcl-6+, which is a mathematical impossibility unless some of the NP311+ cells are expressing both transcription factors [13].

These results are consistent with other reports that limiting IL-2 favors Tfh development during IAV infection, and the timelines in these studies (i.e. 7-20dpi) include the time point at which my data suggest Bcl-6+ Tbet+ cells appear (i.e. 10dpi). Furthermore, Bcl-6+T-bet+ cells would not be unexpected in the context of an influenza virus infection, given the Th polarization of the response and the iso type bias of the antibody response [14].

Further clarification could come from tracking the kinetics of IAV-specific T-bet+Bcl-6+ cells in the lungs and secondary lymphoid organs relative to the kinetics of germinal center formation using both flow cytometry and microscopy. A quantitative analysis of the proportion of IAV-specific CD4 T cells in the lungs, LN and spleen expressing T-bet and Bcl-6 over time, combined with data about localization of IAV-specific T-bet+Bcl-6+ cells relative to germinal centers could indicate the specific function of these cells following IAV infection [15].

Conclusion

Authors found that IAV and GAS are individually important human pathogens that cause a significant amount of global disease and death. Understanding the immune response to these pathogens is the key to development of better treatments and vaccinations. During IAV infection, IAV-specific CD4 T cells accumulate in the lung beginning at 6dpi, and they peak in numbers at 10dpi. During this time, the majority of IAV-specific CD4 T cells are T-bet+, and more produce IFNγ than IL-2, IL-13 or IL-17. In both C57Bl/6 mice and BALB/c mice there are also substantial numbers of IAV-specific Foxp3+ or Bcl-6+ cells, but only very small numbers of RORγt+ cells and virtually no GATA-3+ cells.

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