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PEARLS

Reining in the CD8+ T cell: Respiratory virus infection and PD-1-mediated T-cell impairment

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Introduction

Viral acute respiratory infections (ARIs) are associated with cluster of differentiation (CD)8$^+$ T cells that exhibit diminished production of cytokines and cytotoxic molecules. Though these cells recognize major histocompatibility complex (MHC)-I–restricted viral epitopes, ex vivo stimulation of these cells with these same viral peptides fails to elicit production of interferon (IFN)γ, interleukin (IL)-2, or tumor necrosis factor (TNF)-α; degranulation as measured by CD107a staining; or other features of functional CD8$^+$ T cells. This is a unique feature of viral ARI because T-cell dysfunction occurs in chronic rather than acute infections of many other organs. Although CD4$^+$ regulatory T cells (Tregs) contribute to CD8$^+$ T-cell dysfunction, recent work on a variety of viruses has identified inhibitory receptors as a key mediator of this phenotype. Programmed cell death 1 (PD-1) is the most well-studied inhibitory receptor in T-cell impairment, but there is growing evidence that other inhibitory receptors also play a role. The tendency of CD8$^+$ T cells to have significantly reduced functionality in the context of respiratory virus infection has been termed "T cell impairment".

What is T-cell impairment?

T-cell immunity, especially CD8$^+$ T cells, is essential to clearing acute viral lung infections. In mouse models, absence of CD8$^+$ T cells leads to delayed clearance of viruses, whereas humans that have defects in T-cell immunity associated with aging, immune suppression, or cancer tend to have more severe infections and poorer outcomes [1, 2]. However, despite this clear need for CD8$^+$ T-cell–mediated immunity, infections due to a broad range of acute viruses, including influenza virus, respiratory syncytial virus (RSV), pneumonia virus of mice, vaccinia virus (respiratory but not systemic infection), human metapneumovirus (HMPV), and others, have been associated with a state called T-cell impairment [3–11]. Broadly defined, T-cell impairment occurs when virus-specific CD8$^+$ T cells fail to produce inflammatory cytokines or perform cytotoxic functions at the site of infection, while the same virus-specific cells at other sites, such as the spleen, are completely functional (Fig 1). Epitope-specific cells in the lung-draining mediastinal lymph nodes are completely functional [7]. This suggests that the inflammatory environment of the lung parenchyma drives the process of T-cell impairment during ARI.

T-cell impairment requires cognate antigen, because CD8$^+$ T cells that are not specific to a viral antigen remain functional during primary infection [4]. It also appears to require active of which has any relation to this review. MCR declares no conflict of interest.

infection, because dendritic cell vaccination leads to functional virus-specific $CD8^+$ T cells [4]. However, adoptive transfer of cells into a naïve lung causes the transferred cells to lose some effector potential, though infection further decreases the function of these cells, indicating that the lung environment itself may program some degree of suppression even in settings without infection [12, 13]. Impairment also occurs during secondary infections [3–7, 14], which may indicate one reason why humans have poor T-cell memory to respiratory viruses and are susceptible to reinfection.

Why would the lung favor T-cell impairment?

As the site of gas exchange, the lung is essential for the long-term survival of an organism. Left unchecked, effector T cells, inflammatory cytokines, and other immune mediators could damage healthy tissue alongside virus-infected cells. Indeed, a significant portion of lung injury after serious infections is due to immunopathology rather than to the virus infecting and killing cells [15, 16]. Therefore, it is necessary for the immune system to balance virus clearance and immune-mediated damage during infection, and CD8+ T-cell impairment likely represents a host mechanism of protective immunoregulation.

Similarly, once a respiratory virus is cleared, normal lung homeostasis must be restored. T-cell impairment likely represents a regulatory mechanism to restore the normal state by reducing function or survival of cytotoxic virus-specific $CD8^+$ T cells. Some strategies to reverse T-cell impairment, discussed below, can be associated with increased immunopathology or

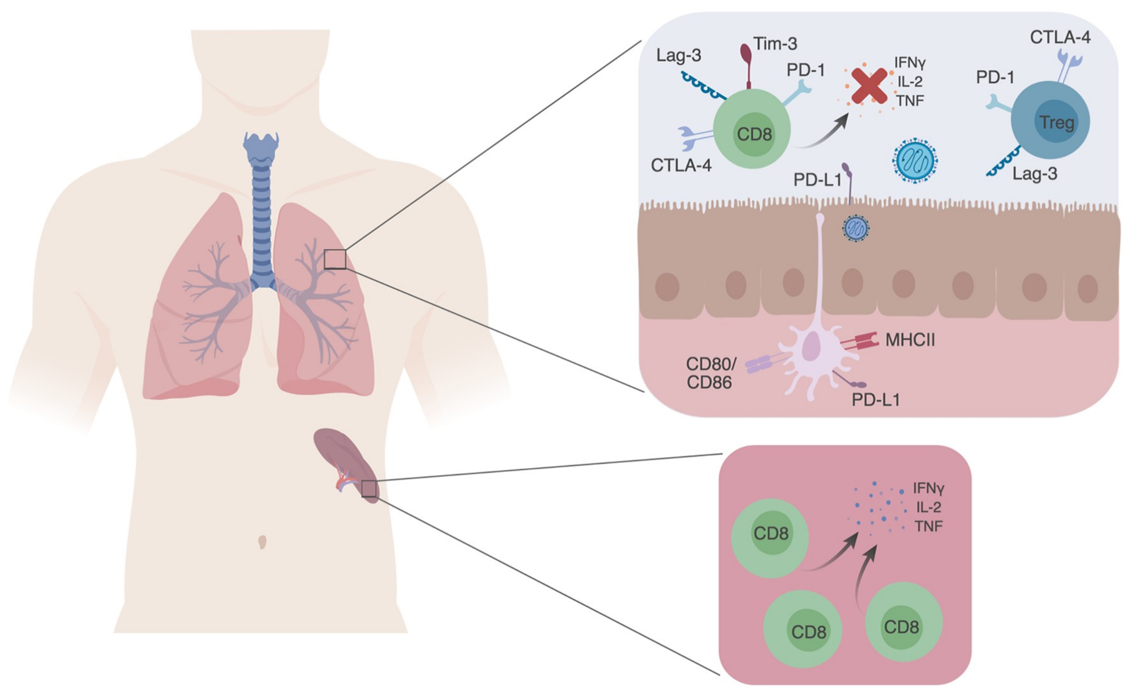

Fig 1. Respiratory virus infection is associated with T-cell impairment. During respiratory virus infection, impaired virus-specific T cells in the lung fail to produce pro-inflammatory cytokines or perform cytotoxic functions. T-cell impairment is at least partially mediated by expression of inhibitory receptors, which decrease effector T-cell function and promote Treg activity. In contrast, virus-specific T cells in the spleen rarely express inhibitory receptors and are functional. PD-1 and other inhibitory receptors are expressed in the lung during both primary infection and reinfection. CD8, cluster of differentiation 8; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IFNγ, interferon gamma; IL-2, interleukin 2; Lag-3, Lymphocyte-activation gene 3; MHCII, major histocompatibility complex class II; PD-1, programmed cell death 1; PD-L1,; Tim-3, T-cell immunoglobulin and mucin-domain containing-3; TNF, tumor necrosis factor; Treg, regulatory T cells.

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disease severity and delayed recovery from weight loss [4, 17, 18] at time points after viral inoculation when the virus has almost entirely been cleared.

Another tissue that appears to favor T-cell impairment is the brain. Some neurotropic infections, such as the JHM strain of murine coronavirus, cause dysfunctional virus-specific T cells [19]. Considering the critical and delicate nature of neural tissue and the limited healing capacity of the brain, it seems likely that T-cell impairment in both organs represents an adaptation to preserve vital organs during infection and recovery. Reversal of T-cell dysfunction in neurotropic infection also causes increased immunopathology and disease severity [19].

What mediates T-cell impairment?

Much work remains to fully elucidate the mechanisms of impairment. One essential component is the action of inhibitory receptors, the most well-described being PD-1. PD-1 and other inhibitory receptors interfere with signaling through the T-cell receptor and thus have far reaching consequences on T-cell function, from cytokine release to metabolism [20–22]. PD-1 signaling alters metabolism in multiple ways: it suppresses PI3k (phosphatidylinositide 3-kinase/Akt and mTOR (mammalian target of rapamycin) activation, diverts metabolic pathways away from glycolysis and towards fatty acid oxidation, and increases reactive oxygen species that promote apoptosis [21, 23, 24]. PD-1 is expressed on T and B cells, as well as occasionally on antigen-presenting cells (APCs) [25]. PD-1 has two ligands, PD-L1 (expressed on almost all cell types) and PD-L2 (expressed on APCs and B cells). PD-1 is up-regulated by antigen stimulation, while inhibitory ligands are induced by interferons [25]. Because antigen stimulation and interferon release tend to occur simultaneously only at the site of infection, this facilitates impairment specifically in the infected tissue and explains why T cells in the spleen or lymph node are not impaired in ARI.

Blockade of PD-L1 or genetic depletion of PD-1 increases the functionality of virus-specific $CD8^+$ T cells. In infections such as HMPV or influenza, blockade also leads to faster virus clearance [4, 26]. Other inhibitory receptors, including Lag-3, Tim-3, 2B4, and CTLA-4 are up-regulated in response to respiratory virus infection and appear to contribute to some degree of T-cell impairment, but thus far PD-1 appears to be the dominant inhibitory receptor for ARI [14]. Lag-3 mediates later T-cell impairment, but blockade of Lag-3 increases immunopathology without reducing viral titer [17]. Although other inhibitory receptors have been discovered and characterized, to date, they have not been evaluated for a role in T-cell impairment in viral ARI.

Is T-cell impairment the same as T-cell exhaustion?

Although many of the same inhibitory receptors have been implicated in both impairment and exhaustion, these two states differ in a few significant ways. T-cell exhaustion is defined by antigen-unresponsive T cells after a prolonged antigenic stimulus, such as during chronic infections or cancer. Inhibitory receptor blockade and other strategies to restore T-cell function are licensed strategies for cancer treatment and are in clinical trials for chronic infection. T-cell exhaustion has been well characterized using the lymphocytic choriomeningitis virus (LCMV) model of chronic infection and is associated with progressive hierarchical loss of T-cell function [27] as well as virus persistence. Pulmonary T-cell impairment is also associated with dysfunction of multiple T-cell capacities (IL-2, TNF, granzyme B, and IFNγ production, and degranulation as measured by CD107a,) and defects in virus clearance [4].

However, one major difference between T-cell impairment and exhaustion is timing. T-cell exhaustion requires long-term antigen exposure before inhibitory receptors become up-regulated. Acute LCMV infection is associated with transient PD-1 expression, whereas chronic

LCMV infection leads to sustained PD-1 expression on epitope-specific T cells [28]. In contrast, T-cell impairment is seen relatively early (day 7–8) in the adaptive immune response to ARI, and PD-1 expression remains high on lung lymphocytes weeks after virus is cleared [4]. Additionally, during T-cell exhaustion, there are subsets of exhausted T cells that can be rescued by anti–PD-1 blockade or that remain terminally differentiated and unresponsive to PD-1 blockade [29, 30]. It is not yet known whether similar subsets of impaired T cells exist in the lung.

It seems likely that the particular inflammatory environment of the pulmonary tissue triggers the cascade of PD-1 and PD-L1/2 up-regulation more rapidly, accounting for a level of impairment at day 7 of acute infection that by microarray bears a striking similarity to exhaustion at day 30 of chronic LCMV [14, 28]. For instance, alveolar macrophages express high levels of PD-L1 even at baseline [31], which may allow the lung to rapidly trigger impairment, whereas induction of PD-L1 on other APCs requires infection and inflammation.

How might our knowledge of T-cell impairment impact drug or vaccine design?

It is important to consider lung T-cell impairment when developing vaccines or therapeutics for respiratory viruses as well as anticipating potential side effects of PD-L1 antibodies and other checkpoint blockade therapies for cancer.

There are no licensed vaccines against many common respiratory viruses (including RSV, HMPV, and parainfluenza viruses) despite extensive research. Vaccines that elicit T-cell immunity are thought to enhance protection against respiratory viruses, but whether T-cell impairment contributes to the failure of successful vaccine development in humans requires further study. The vaccine adjuvant alum induced high PD-1 expression on $CD8^+$ T cells in a mouse model of influenza [32], and virus-like particle vaccination of mice against HMPV did not protect from $CD8^+$ T-cell impairment and inhibitory receptor expression in secondary challenge [33]. T-cell impairment may represent a barrier to vaccination for ARI, but pharmacologic restoration of T-cell function can have detrimental effects in the lungs as well.

A rare but serious and often fatal side effect of checkpoint inhibitors is pneumonitis, or inflammation of the lung [34]. Although an exact mechanism has not been established, aberrant T-cell activation to self or foreign antigen in the lung may contribute. Humans receiving checkpoint inhibition therapy who then acquire common respiratory viral infections may be at increased risk for pneumonitis. A future improvement to checkpoint therapy would be the ability to specifically target tumor-specific T cells. Moreover, studies of respiratory virus infection in patients treated with checkpoint inhibitors would help us understand and anticipate potential risks associated with these treatments.

Conclusion

T-cell impairment, mediated by PD-1 and other inhibitory receptors, represents a regulatory adaptation by the immune system to preserve healthy lung tissue during acute viral infection. Though antibody blockade of PD-1 and other strategies to reverse T-cell impairment offer exciting treatments for cancer, the use of these drugs can pose risks to lung function and therefore warrant further study. Pulmonary T-cell impairment contributes to memory and vaccine responses, though the ramifications of these interactions are not yet clear. Finding the perfect balance between immunoprotection and immunopathology remains elusive.

References

1. Widmer K, Zhu Y, Williams JV, Griffin MR, Edwards KM, Talbot HK. Rates of Hospitalizations for Respiratory Syncytial Virus, Human Metapneumovirus, and Influenza Virus in Older Adults. Journal of Infectious Diseases. 2012; 206(1):56–62. https://doi.org/10.1093/infdis/jis309 PMID: 22529314
2. Matias G, Taylor R, Haguinet F, Schuck-Paim C, Lustig R, Shinde V. Estimates of hospitalization attributable to influenza and RSV in the US during 1997–2009, by age and risk status. BMC public health. 2017; 17(1):271. Epub 2017/03/23. https://doi.org/10.1186/s12889-017-4177-z PMID: 28320361
3. Chang J, Braciale TJ. Respiratory syncytial virus infection suppresses lung CD8+ T-cell effector activity and peripheral CD8+ T-cell memory in the respiratory tract. Nat Med. 2002; 8(1):54–60. Epub 2002/01/12. https://doi.org/10.1038/nm0102-54 PMID: 11786907.
4. Erickson JJ, Gilchuk P, Hastings AK, Tollefson SJ, Johnson M, Downing MB, et al. Viral acute lower respiratory infections impair CD8+ T cells through PD-1. J Clin Invest. 2012; 122(8):2967–82. Epub 2012/07/17. https://doi.org/10.1172/JCI62860 PMID: 22797302 mc3408742.
5. Claassen EA, van der Kant PA, Rychnavska ZS, van Bleek GM, Easton AJ, van der Most RG. Activation and inactivation of antiviral CD8 T cell responses during murine pneumovirus infection. Journal of immunology (Baltimore, Md: 1950). 2005; 175(10):6597–604. Epub 2005/11/08. PMID: 16272314.
6. DiNapoli JM, Murphy BR, Collins PL, Bukreyev A. Impairment of the CD8+ T cell response in lungs following infection with human respiratory syncytial virus is specific to the anatomical site rather than the virus, antigen, or route of infection. Virology journal. 2008; 5:105. Epub 2008/09/26. https://doi.org/10.1186/1743-422X-5-105 PMID: 18816384 mc2561024.
7. Gray PM, Arimilli S, Palmer EM, Parks GD, Alexander-Miller MA. Altered function in CD8+ T cells following paramyxovirus infection of the respiratory tract. J Virol. 2005; 79(6):3339–49. Epub 2005/02/26. https://doi.org/10.1128/JVI.79.6.3339-3349.2005 PMID: 15731228 mc1075682.
8. Lukens MV, Claassen EA, de Graaff PM, van Dijk ME, Hoogerhout P, Toebes M, et al. Characterization of the CD8+ T cell responses directed against respiratory syncytial virus during primary and secondary infection in C57BL/6 mice. Virology. 2006; 352(1):157–68. Epub 2006/05/30. https://doi.org/10.1016/j.virol.2006.04.023 PMID: 16730775.
9. Vallbracht S, Unsold H, Ehl S. Functional impairment of cytotoxic T cells in the lung airways following respiratory virus infections. European journal of immunology. 2006; 36(6):1434–42. Epub 2006/05/19. https://doi.org/10.1002/eji.200535642 PMID: 16708402.
10. Rutigliano JA, Sharma S, Morris MY, Oguin TH 3rd, McClaren JL, Doherty PC, et al. Highly pathological influenza A virus infection is associated with augmented expression of PD-1 by functionally compromised virus-specific CD8+ T cells. J Virol. 2014; 88(3):1636–51. Epub 2013/11/22. https://doi.org/10.1128/JVI.02851-13 PMID: 24257598 mc3911576.
11. Telcian AG, Laza-Stanca V, Edwards MR, Harker JA, Wang H, Bartlett NW, et al. RSV-induced bronchial epithelial cell PD-L1 expression inhibits CD8+ T cell nonspecific antiviral activity. The Journal of infectious diseases. 2011; 203(1):85–94. Epub 2010/12/15. https://doi.org/10.1093/infdis/jiq020 PMID: 21148500 mc3086441.
12. Arimilli S, Palmer EM, Alexander-Miller MA. Loss of function in virus-specific lung effector T cells is independent of infection. Journal of leukocyte biology. 2008; 83(3):564–74. Epub 2007/12/15. https://doi.org/10.1189/jlb.0407215 PMID: 18079210.
13. Fulton RB, Olson MR, Varga SM. Regulation of Cytokine Production by Virus-Specific CD8 T Cells in the Lungs. Journal of Virology. 2008; 82(16):7799–811. https://doi.org/10.1128/JVI.00840-08 PMID: 18524828
14. Erickson JJ, Lu P, Wen S, Hastings AK, Gilchuk P, Joyce S, et al. Acute Viral Respiratory Infection Rapidly Induces a CD8+ T Cell Exhaustion-like Phenotype. Journal of immunology (Baltimore, Md: 1950). 2015; 195(9):4319–30. Epub 2015/09/25. https://doi.org/10.4049/jimmunol.1403004 PMID: 26401005
15. Cannon MJ, Openshaw PJ, Askonas BA. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. The Journal of experimental medicine. 1988; 168(3):1163–8. Epub 1988/09/01. PMID: 3262705
16. Alwan WH, Kozlowska WJ, Openshaw PJ. Distinct types of lung disease caused by functional subsets of antiviral T cells. The Journal of experimental medicine. 1994; 179(1):81–9. Epub 1994/01/01. PMID: 8270885
17. Erickson JJ, Rogers MC, Tollefson SJ, Boyd KL, Williams JV. Multiple Inhibitory Pathways Contribute to Lung CD8+ T Cell Impairment and Protect against Immunopathology during Acute Viral Respiratory Infection. Journal of immunology (Baltimore, Md: 1950). 2016; 197(1):233–43. Epub 2016/06/05. https://doi.org/10.4049/jimmunol.1502115 PMID: 27259857
18. Yao S, Jiang L, Moser EK, Jewett LB, Wright J, Du J, et al. Control of pathogenic effector T-cell activities in situ by PD-L1 expression on respiratory inflammatory dendritic cells during respiratory syncytial virus

infection. Mucosal immunology. 2014. Epub 2014/12/04. https://doi.org/10.1038/mi.2014.106 PMID: 25465101.

19. Phares TW, Ramakrishna C, Parra GI, Epstein A, Chen L, Atkinson R, et al. Target-dependent B7-H1 regulation contributes to clearance of central nervous system infection and dampens morbidity. Journal of immunology (Baltimore, Md: 1950). 2009; 182(9):5430–8. Epub 2009/04/22. https://doi.org/10.4049/jimmunol.0803557 PMID: 19380790

20. Nurieva R, Thomas S, Nguyen T, Martin-Orozco N, Wang Y, Kaja MK, et al. T-cell tolerance or function is determined by combinatorial costimulatory signals. The EMBO journal. 2006; 25(11):2623–33. Epub 2006/05/26. https://doi.org/10.1038/sj.emboj.7601146 PMID: 16724117

21. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun. 2015; 6:6692. Epub 2015/03/27. https://doi.org/10.1038/ncomms7692 PMID: 25809635

22. Patsoukis N, Brown J, Petkova V, Liu F, Li L, Boussiotis VA. Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. Science signaling. 2012; 5(230):ra46. Epub 2012/06/29. https://doi.org/10.1126/scisignal.2002796 PMID: 22740686

23. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Molecular and cellular biology. 2005; 25(21):9543–53. Epub 2005/10/18. https://doi.org/10.1128/MCB.25.21.9543-9553.2005 PMID: 16227604

24. Tkachev V, Goodell S, Opipari AW, Hao L-Y, Franchi L, Glick GD, et al. Programmed death-1 controls T cell survival by regulating oxidative metabolism. Journal of immunology (Baltimore, Md: 1950). 2015; 194(12):5789–800. https://doi.org/10.4049/jimmunol.1402180 PMID: 25972478

25. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. The Journal of experimental medicine. 2006; 203(4):883–95. https://doi.org/10.1084/jem.20051776 PMID: 16606670

26. McNally B, Ye F, Willette M, Flano E. Local blockade of epithelial PDL-1 in the airways enhances T cell function and viral clearance during influenza virus infection. J Virol. 2013; 87(23):12916–24. Epub 2013/09/27. https://doi.org/10.1128/JVI.02423-13 PMID: 24067957 mc3838157.

27. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol. 2003; 77(8):4911–27. Epub 2003/03/29. https://doi.org/10.1128/JVI.77.8.4911-4927.2003 PMID: 12663797

28. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006; 439(7077):682–7. https://doi.org/10.1038/nature04444 PMID: 16382236

29. Blackburn SD, Shin H, Freeman GJ, Wherry EJ. Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(39):15016–21. Epub 2008/09/24. https://doi.org/10.1073/pnas.0801497105 PMID: 18809920

30. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature. 2016; 537(7620):417–21. Epub 2016/08/09. https://doi.org/10.1038/nature19330 PMID: 27501248

31. Hastings AK, Erickson JJ, Schuster JE, Boyd KL, Tollefson SJ, Johnson M, et al. Role of type I interferon signaling in human metapneumovirus pathogenesis and control of viral replication. J Virol. 2015; 89(8):4405–20. Epub 2015/02/06. https://doi.org/10.1128/JVI.03275-14 PMID: 25653440.

32. MacLeod MK, McKee AS, David A, Wang J, Mason R, Kappler JW, et al. Vaccine adjuvants aluminum and monophosphoryl lipid A provide distinct signals to generate protective cytotoxic memory CD8 T cells. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108 (19):7914–9. Epub 2011/04/27. https://doi.org/10.1073/pnas.1104588108 PMID: 21518876

33. Wen SC, Schuster JE, Gilchuk P, Boyd KL, Joyce S, Williams JV. Lung CD8+ T Cell Impairment Occurs during Human Metapneumovirus Infection despite Virus-Like Particle Induction of Functional CD8+ T Cells. J Virol. 2015; 89(17):8713–26. Epub 2015/06/13. https://doi.org/10.1128/JVI.00670-15 PMID: 26063431

34. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine. 2012; 366(26):2443–54. Epub 2012/06/05. https://doi.org/10.1056/NEJMoa1200690 PMID: 22658127