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Protective and dysregulated T cell immunity in RSV infection

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Respiratory syncytial virus (RSV) is the most important cause of infantile bronchiolitis and a major pathogen in elderly and immunosuppressed persons. Although RSV shows limited antigenic diversity, repeated infections occur throughout life. Vaccine development has been delayed by poor immunogenicity, production issues and the fear of causing enhanced disease. T cells assist in viral clearance, but immune regulation serves to limit these responses and to prevent the exaggerated inflammatory response to RSV infection seen in children with bronchiolitis. Severe RSV disease can therefore be regarded as a dysregulated response to an otherwise trivial infection. Further insights into the role of T cells (including Th17) are needed to enable the rational design of safe, effective vaccines and novel treatments.

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Introduction

Respiratory syncytial virus (RSV) is a leading cause of morbidity in infants, and is increasingly appreciated as a major cause of illness and death in susceptible adults. Globally, it is estimated that there are 33.8 million new episodes of RSV infection per year, leading to around 3.4 million hospital admissions and up to 199 000 deaths (99% of which occur in the developing world) [1]. While healthy young adults generally only suffer common cold symptoms and are at low risk of severe disease, RSV has an unusual ability to re-infect. Approximately 10% of adults suffer repeat RSV infection each year, accounting for up to 5% of admissions for community acquired pneumonia at an annual cost of $680 million in the USA for in-patient treatment alone. Although RSV causes a vast burden of disease, its name is virtually unknown by the public, or by most policymakers.

Unlike many other viruses, infection with RSV does not induce durable protective immunity. Although it is clear that most severe disease occurs during primary infantile infection, symptomatic upper respiratory tract infections occur throughout life. While infection with a given strain of influenza may protect against symptomatic disease caused by that same strain for up to seven years, even healthy adults can be repeatedly infected with an identical RSV at as little as two month intervals.

Antibody and vaccines

The reasons underlying this incomplete immunity remain unclear. Antibody can protect, since passive immunisation with the humanised anti-RSV F protein monoclonal palivizumab (Synagis^{TM}) reduces the risk of hospitalisation for bronchiolitis in high-risk infants by around 50% [2]. However, even individuals with the highest levels of natural anti-RSV neutralising antibodies are not reliably protected against nasal infection and some individuals with low antibody titres are resistant [3], suggesting that antibody-independent immunity is also important. Conversely, baseline antibody titres are well maintained in elderly adults despite the fact that disease is more frequent and severe in this population. Multiple infections are required for accumulation of these antibodies, as levels induced acutely also fall rapidly with a 4-fold or greater drop in the majority of adults by one year post-infection [4].

Thus, defects in the humoral response are probably partly responsible for the phenomenon of incomplete immunity. However, it is not clear whether this is due to an intrinsic B cell defect, due to failure of T cell help or caused by some as yet unknown influence of the virus on the immune system. In mice, the antibody response to neonatal RSV infection is weaker than that in adult life and is helper T cell independent. By contrast, the stronger adult response does depend on helper T cells. Interestingly, depletion of Natural Killer cells or CD8+ T cells during neonatal RSV infection boosts anti-RSV antibody responses. Both these cell types are major sources of IFN-γ and blocking IFN-γ causes similar effects. By contrast, providing additional IFN-γ by recombinant cytokine expression reduces antibody responses [5]. Improved understanding of the factors determining the generation of protective antibody is clearly important in the development of effective vaccines for the neonate.

In young children, particularly preterm babies and neonates, RSV may cause bronchiolitis, characterised by an

exuberant inflammatory response. This results in airway oedema, cellular infiltration and tissue necrosis. It remains uncertain why some individuals are at higher risk of this exaggerated immune response. Formalin-inactivated RSV vaccine (FI-RSV), tested in children in the 1960s, not only failed to protect, but also induced unexpectedly severe disease in young children during subsequent natural infection. In those trials, FI-RSV vaccinees suffered an 80% hospitalisation rate following natural infection with RSV and two of those children died. Infantile bronchiolitis and lung disease augmented by FI-RSV both seem to represent an uncontrolled immunologically mediated tissue damage, though differing significantly in the type of pathology that is seen.

The development of an effective and safe vaccine that protects against RSV has long been constrained by these two factors: poor immune protection and heightened immunopathology. As our understanding of the mechanisms behind these opposing factors in RSV has increased, it has become clear that more subtle, less empirical approaches to vaccine design will be required to overcome these problems. It is also increasingly obvious that the effector and regulatory capabilities of T cells must be balanced in order to induce a coordinated adaptive response capable of providing long-term protective immunity (Figure 1).

T cells in viral clearance and protection

T cells are believed to play an essential role in clearance of RSV from the lungs. In the BALB/c mouse, up to 40% of CD8+ T cells infiltrating the lung may be specific for an epitope from the M2 protein at the peak of infection [6]. Early studies in athymic nude or irradiated mice showed that persistent infection with prolonged RSV shedding could be cleared by adoptive transfer of memory T cells from previously primed animals [7]. In BALB/c mice depleted of CD4+ or CD8+ T cells, both T cell subsets play a role in shortening the duration of RSV shedding [8]. Conversely, stimulation of epitope-specific CD8+ T cells using peptide immunisation of HLA-A*0201 transgenic mice ameliorates disease and improves viral control [9]. Immunisation using peptide-loaded dendritic cells (DCs) from the closely related pneumonia virus of mice confers partial protection against subsequent RSV challenge [10••]. Similar findings have been observed in other animal models such as RSV infection (e.g. calves) in which depletion of CD8+ T cells delays viral clearance.

In human observational studies, T cells are also implicated in viral clearance. In children with severe infection, CD8+ antigen-specific T cells accumulate in the peripheral blood and airways, peaking at around nine days after symptom onset [11]. These display an activated phenotype, but their abundance does not correlate with disease severity. In studies of adults, numbers of activated CD4+ and CD8+ T cells do correlate with disease severity [12••]. A role for T cells in viral clearance is also implied by a prospective study examining children under the age of five, in which individuals immunocompromised due to chemotherapy or with primary immunodeficiencies affecting T cell function were shown to suffer more severe disease and shed virus at higher levels for several months, compared with 7–21 days in normal children [13]. This is also true of adult bone marrow transplant recipients. The increased frequency and risk of severe RSV disease seen in elderly adults may similarly be related to the lower numbers and decreased proliferative and functional capacity of IFN-γ producing RSV F-protein specific T cells seen in that population [14].

However, despite these data suggesting the apparent importance of T cells, it remains to be seen whether measuring T cell responses provides an additional correlate of protection in man that might be of use in vaccine development.

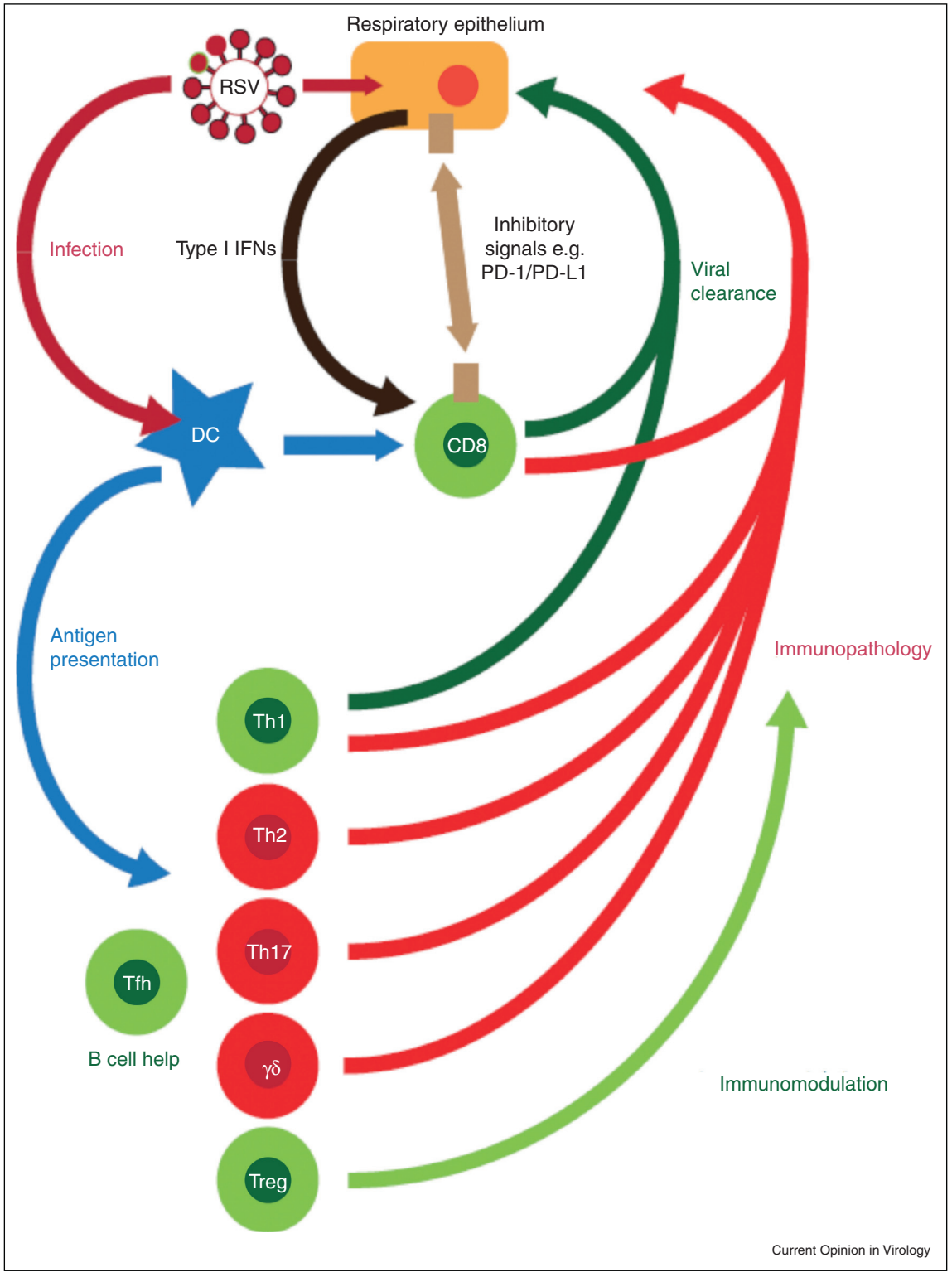
Modulation of T cell activity by RSV

The relationship between T cell numbers or function and protection from RSV re-infection may not be simple, and is complicated by checks and balances in the immune system. Although some individuals are protected from RSV re-infection in the absence of high levels of neutralising antibody, most are not [3]. In a prospective study of infants with RSV bronchiolitis, virus-specific T cell responses could be demonstrated during the convalescent phase, but these did not correlate with protection from infection the following year and were not boosted on secondary infection [15]. It is now increasingly clear that RSV takes advantage of several mechanisms that lead to the modulation of T cell function in order to permit these productive re-infections.

Mechanisms for immune evasion are common in many pathogens that have undergone prolonged co-evolution with their hosts. A number of RSV proteins have therefore been implicated in immune modulation. For example, both bovine and human RSV nonstructural proteins NS1 and NS2 have been shown to suppress the production of type I interferons by epithelial cells [16]. In addition, modulation of effector functions may occur as a consequence of changes in the pulmonary microenvironment, which is generally maintained in a relatively anti-inflammatory state [6,17]. CD8+ T cells from infected murine lungs (but not spleen) are impaired in their cytolytic ability and capacity to produce IFN-γ [18]. In the RSV-infected airway, feedback mechanisms such as the upregulation of PD-1/PD-L1 are activated and inhibit CD8+ T cells in order to prevent immune-related tissue damage [19]. It is probably that these mechanisms all contribute to impaired RSV-specific T cell immunity.

Recently, the effect of RSV on interactions between T cells and DCs has come under scrutiny. DCs are major

Figure 1



The role of T cells in RSV clearance, protective immunity and immunopathology. RSV infects respiratory epithelial cells but impairs innate immune responses by inhibiting type I interferons and upregulating inhibitory molecules in the lung. Direct infection of dendritic cells also causes dysregulation of antigen presentation, leading to impaired T cell function, reduced viral clearance and memory formation. The T cell response is also skewed towards T cell subsets that enhance immunopathology but which may be limited by regulatory T cells. Proposed mechanisms by which RSV deranges the T cell response are shown in red text.

professional antigen-presenting cells and act at the interface between the innate and adaptive arms of the immune system. *In vitro*, RSV can infect DCs and undergo active replication [20,21]. In the mouse, conventional and plamacytoid DCs do migrate to the lung during RSV infection and transport antigen to the draining lymph nodes [22]. However, despite undergoing phenotypic maturation, RSV-infected human DCs are poor inducers of T cell effector function *in vitro*. Compared with human metapneumovirus, they significantly impair CD4+ T cell proliferation [20] and in several different models, they have been shown to inhibit the production of cytokines, including IFN-γ [23].

The mechanisms underlying this phenomenon remain unclear. The ability of DCs to carry antigen for presentation to T cells may allow F protein to directly inhibit T cell proliferation. Some strains of RSV may also interfere with the production of type I interferons by plasmacytoid DCs [24], although this conflicts with other studies that suggest that IFN-α and IFN-λ produced by monocyte-derived DCs infected with live RSV suppress CD4+ T cell proliferation [25]. *In vitro* culture of human T cells with DCs infected with RSV deletion mutants lacking NS1 protein has demonstrated increased proliferation of CD103+ mucosa-homing CD8+ T cell with concurrent increase in Th17 and Th2-like CD4+ T cells unrelated to the effect of NS1 on type I interferons [26••]. Finally, it has been proposed that RSV infection of DCs renders them unable to activate T cells (even causing those T cells to be refractory to further stimulation) due to failure of assembly of the immunological synapse [27]. This may also be affected by the differential expression on DCs of co-stimulatory molecules such as CD40 and OX40L in response to individual RSV proteins and their downstream effects on cytokine production by T cells [28].

The role of T cells in RSV immunopathology

Despite the importance of T cells in viral clearance, cellular immunity is also paradoxically associated with increased severity of disease. In the mouse model, depletion of CD8+ (and to a lesser extent CD4+) T cells has been shown to reduce weight loss following RSV infection despite delaying clearance of virus [7,8].

Following the events surrounding the failure of FI-RSV, major efforts were made to understand the resultant vaccine-enhanced disease. More severely affected children were found to have increased pulmonary inflammation and eosinophilia, thus implicating an overexuberant immune response as the cause. It was noted that these features mirrored those of a CD4+ Th2 response and therefore a potential role for T cells in regulating this phenomenon. Although Th2 cytokines are not consistently found in children with bronchiolitis [29,30], FI-RSV can induce skewing towards a Th2 cytokine response in a variety of animal models [31,32]. IL-27, which enhances Th1 differentiation and suppresses Th2 and Th17-type responses in mice, can prevent vaccine-enhanced inflammation if overexpressed [33•]. Using recombinant vaccinia viruses expressing individual RSV proteins, it was found that while both F and N protein induce inflammatory infiltrates in the lung, only G protein causes pulmonary eosinophilia [34]. Furthermore, T cell responses made by mice against the G protein are directed against a dominant MHC class II-restricted epitope; by comparison, F induces both CD4+ and CD8+ T cell responses [35].

The absence of a normal IFN-γ producing CD8+ T cell response may therefore be involved in skewing towards a Th2 phenotype. This is particularly pertinent as many vaccine candidates involve the administration of adjuvanted RSV proteins that are expected to present primarily via MHC class II and to a much lesser extent by cross-presentation. In mice, when purified F or G adsorbed to alum (which was also in the FI-RSV) is used to immunise, both proteins induce a Th2 response, suggesting that the mode of presentation or the nature of the adjuvant may play a significant role [36]. A construct in which G protein was expressed along with a known immunodominant class I-restricted epitope induced no pulmonary eosinophilia, implying that the co-expansion of antigen-specific CD8+ T cells abrogated the Th2 response [37]. This has been further tested in the BALB/c mouse in which stimulation of epitope-specific CD8+ T cells using a class I-restricted peptide vaccine mixture protected against the immunopathologic features observed during infection with RSV line 19 [38]. RSV-specific CD8+ T cells therefore seem to play an essential role both in viral clearance and the generation of a balanced T cell response in spite of their demonstrated potential for unwanted tissue damage.

Other T cell subsets have been implicated in the immunopathology seen during RSV infection. These include nonclassical T cells, such as γδ T cells, seen in BALB/c mice after priming with recombinant vaccinia expressing F protein followed by RSV challenge [39]. Increased γδ T cell numbers had a minor effect on viral clearance but are associated with increased disease severity. Interestingly, these cells make Th1-like cytokines early post infection, but more IL-4, IL-5 and IL-10 at later time-points.

It has more recently been shown that Th17 cells may also play a role in regulating inflammation in the RSV-infected lung. This CD4+ T cell subset is enriched at mucosal sites and characterised by production of IL-17, a cytokine that induces chemokine production by respiratory epithelial cells and resultant leukocyte infiltration [40]. *In vitro*, bronchial epithelial cells infected with RSV cause the differentiation of peripheral blood lymphocytes to Th2 and Th17 phenotypes [41]. In mouse models, members of the IL-17 family are induced following RSV infection and cause neutrophil recruitment to the airway,

mucus production, and impairment of viral clearance, possibly via reduction in CD8+ T cell numbers and function in lung and draining lymph nodes [42•]. IL-17 may also be involved in airway hyper-responsiveness seen following RSV infection via induction of tachykinin and complement activation [43•]. In humans, the role of Th17 cells remains unclear. While IL-17 has been detected in tracheal aspirates from mechanically ventilated children with bronchiolitis, in non-ventilated patients IL-17 peaks later during convalescence [42•,44]. It therefore remains to be determined whether Th17 cells are involved in the immunopathology related to RSV or during recovery.

Regulatory T cells in the modulation of RSV immunopathology

It has recently become evident that there are sophisticated and elegant systems that curtail antiviral effector mechanisms and thus limit inflammation and tissue damage. For T cells, these include programmed cell death following the peak of antigen-induced proliferation and upregulation of inhibitory molecules such as PD-1 [45••]. Additionally, the control of T cell-associated autoimmunity and immunopathology is mediated by the regulatory T cell (Treg) subsets, the importance of which has recently been demonstrated in RSV disease.

Tregs are essential modulators of the adaptive immune response, making up 5–10% of CD4+ T cells in the mouse and often (but not invariably) characterised by the expression of the transcription factor FoxP3. Absence of CD4+ FoxP3+ Treg cells in both mice and humans leads to autoimmunity, and defective or suboptimal Treg function during RSV infection may cause immunopathology. In RSV-infected mice, Tregs proliferate and accumulate in the lungs, upregulating activation markers and CTLA-4 [46]. Depletion of Tregs leads to enhanced viral clearance but also to disease exacerbation and increased numbers of antigen-specific IFN-γ and TNF-α producing CD8+ T cells [46,47]. Recent evidence has also implicated Tregs in maintaining tolerance, which RSV infection in infant mice then breaks, thus predisposing towards allergic airways disease [48]. Conversely, increasing Tregs by administration of preformed IL-2/anti-IL-2 immune complexes reduces pulmonary inflammation without inhibition of viral clearance [49].

Our recent studies demonstrate a remarkable decline of Tregs in RSV-infected mice with vaccine-enhanced lung disease. Passive transfer of conventional virus-specific CD4+ T cells replicates the augmented disease, showing that these cells are pathogenic. Moreover, selective recruitment of Tregs into the RSV-infected airway by inhalational administration of CCL17/22 attenuates vaccine-enhanced disease [50•].

Further details of how Tregs inhibit lung inflammation continue to emerge, but studies in our group have shown an unexpected functional requirement for granzyme B expression by pulmonary Tregs [49]. Anti-inflammatory cytokines such as IL-10 are also produced in abundance by this subset and limit pulmonary inflammation [51•]. It remains to be determined whether Tregs are the primary source [52,53].

As yet, there is no data in humans as to the role of Tregs in modulating inflammatory responses in primary or secondary RSV infection. However, it does seem clear that coordinated induction of Tregs is essential for the control of mucosal inflammation during RSV infection. Thus, Tregs appear to limit immunopathology in RSV disease and may provide new opportunities for interventions to limit inflammatory disease.

Conclusion

T cells are essential for the clearance of viral infection and generation of protective immunity. However, infection with RSV causes a dysregulated antiviral immune response with impaired T cell function as well as exaggerated inflammation via multiple mechanisms (Figure 1). RSV vaccine development has been hindered by these two opposing constraints. An effective vaccine should stimulate a balanced immune response in which T cells assist in the generation of cytotoxic and humoral memory responses without causing enhanced immune-related inflammation. Our understanding of the cell types and processes that participate in this process remains incomplete, especially in man. Further studies that address the role of T cells both as mediators of protection and modulators of immunopathology are therefore urgently required.

Conflict of interest

The authors declare no conflicts of interest.

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- • of special interest
- •• of outstanding interest

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