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Review

Respiratory syncytial virus suppression of the antiviral immune response: Implications for evaluation of candidate vaccines

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ABSTRACT

Respiratory syncytial virus infections recur throughout life despite induction of immunity by the first natural infection. Results of an extensive series of studies indicate that the virus adversely affects the human antiviral recall response to challenge, although subsequent infections are less severe than the initial illness. The observations suggest that candidate vaccines for respiratory syncytial virus should not be expected to prevent clinical illness upon subsequent exposure. Candidate vaccines may be considered effective if they render a subsequent natural infection less severe. This is what would be expected from an initial and commonly more severe natural infection and sensitization.

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1. Introduction

Respiratory syncytial virus (RSV) is the major cause of respiratory illness in infants and young children and, in the USA alone, 100,000 children may require hospitalization for RSV infection in a single year [1]. In children under 5 years of age, it is estimated that as many as 118, 200 deaths were attributable to RSV worldwide in 2015 [2]. RSV infects virtually all children by the age of three years and then repeatedly infects throughout life [3].

RSV has been associated with over 11,000 deaths yearly in the USA, and older individuals are most likely to die as a result of the infection [4,5]. Symptomatic re-infection with RSV is common, although repeat infections are associated with less morbidity and mortality than the initial infection [6]. RSV infection is common and more serious in infants and the elderly and in individuals with underlying medical conditions [5]. The first infection of an infant can even be associated with detection of viral antigens in circulating leukocytes [7] whereas that is not the case with subsequent infections.

2. The problem

There is currently no effective and licensed vaccine available. The desired roadmap for the development as well as the

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characteristics of an RSV vaccine have been described recently by WHO [8]. Mazur and colleagues recently reviewed the current state of RSV vaccine development with particular attention to recent and current human clinical trials of candidate vaccines [9].

It is important to review and update studies [1] of adverse effects of RSV on the human antiviral immune response that are relevant to recurring clinically evident RSV infections despite immunity. Such a review should extend the concepts important to vaccine evaluation reviewed recently by Mazur and colleagues [9]. The studies that are cited herein examined the effects of RSV on human peripheral blood mononuclear cells (PBMC, containing both monocytes-macrophages and lymphocytes) and compared responses to those elicited by influenza A virus (IAV). The PBMC, in all studies but one, were donated by healthy young adults who would have been exposed to both RSV and IAV multiple times in the past. The one study that examined RSV infection of PBMC from infants and children demonstrated RSV infection of the PBMC both in vitro and in vivo, including infection of circulating cells during a first infection [7]. The reported existence of circulating RSV-infected T cells in young children has recently been replicated [10]. The adult volunteer donors of the PBMC were shown to have similar percentages of RSV-specific and IAV-specific lymphocytes [11], identified also by deletion and reconstitution experiments [12]. Immune responses to inactivated RSV and IAV were both vigorous, as were responses to infectious IAV but not to infectious RSV [13,14].

2.1. In vitro studies of RSV and the human PBMC anamnestic immune response, compared to responses to IAV

Both RSV and IAV may infect human PBMC during the immune response to viral challenge as the cells are recruited to the respiratory tract [15,16]. Exposure of the PBMC to RSV or to IAV results in substantial differences in both innate immune responses, such as interferon production [17,18], and adaptive immune responses, such as T lymphocyte expression of activation markers and cell proliferation after exposure [11,14,19]. It is important to note that the observed adaptive immune responses to either RSV or IAV are produced by a small subset of lymphocytes that are specific for the virus, with later recruitment of additional PBMC [11,12,14].

The recall response of the sensitized adaptive immune system is responsible for limiting the extent of infection and its consequences upon re-challenge by a previously encountered pathogen. Data from a series of studies (Table 1) support the concept that recurrent RSV infection may be related at least in part to early RSV effects on lymphocyte function [1,11] rather than due only to inadequate generation or persistence of immunity after previous infections as suggested by some animal models [20]. The observed results are due to the responses of the small percentages of PBMC (specifically, T lymphocytes) that are virus-specific. As noted above, the studies mostly used cells from adult subjects who would have been exposed to RSV multiple times in the past, and the results provided evidence of immune sensitization to RSV as well as to IAV [11,12,14]. Virus-induced early responses of human lymphocytes measured after exposure to RSV were clearly demonstrated but consistently reduced or delayed compared with determinations of cell function in response to exposure to IAV. Early effects on an anamnestic immune response such as observed in the studies, especially the reduced clonal expansion of RSV-specific lymphocytes, might allow RSV re-infection to progress to clinically evident illness.

Earlier studies [12–14,19,21] combined with more recent data [11] suggest that the earliest activation/proliferation response to IAV is exhibited by the small percent of virus-specific T cells, followed by the recruitment of bystander cells in response to the cytokines produced by monocytes-macrophages, evolving into a virus-specific proliferative and effector response. Such a complex response overall is reduced or delayed after exposure to RSV. Fewer proliferating T lymphocytes were observed in the RSV-exposed PBMC than in the IAV-exposed population in spite of similar percentages of RSV- and IAV-specific lymphocytes [11]. Reduced RSV-induced proliferation was not due to lymphocyte apoptosis, which was itself reduced relative to that of IAV-exposed T lymphocytes [11].

Table 1
Summary of published observations regarding differential responses of human PBMC to RSV and influenza virus challenge.

Parameter	RSV	Influenza Virus	References
Cell infection			[7,16,21,35,36]
Monocytes-macrogphages	+++	+++	
Lymphocytes	+	+	
Cytokine production			
IL 1	++	+++	[37]
IL-1 inhibitor (IL-1inh)	+++	++	[37]
Net effect	IL-1inh	IL-1	[14,19,21,37]
IFN-α/β	+/-	+++	[17,18,38–40]
IFN-γ	+	+++	[19,40]
IL-2	+	+++	[11]
Cell collaboration			[16,19]
ICAM-1 expression			
Monocytes-macrophages	–	↑	
Lymphocytes	–	↑	
LFA-1 expression			
Monocytes-macrophages	–	↑	
Lymphocytes	–	↑	
Cell clustering	+/-	+++	
Lymphocyte activation			
Fos expression	+/-	+++	[11]
CD69 expression	+/-	+++	[11]
HLA-DR expression, day 3	–	+	[13,14]
Day 7	++	+++	
Cell size changes, day 2–3	++	++	[12,21]
Proliferative responses			
Virus-specific			[11–14,39,41]
To Inactivated virus	+++	+++	
To Infectious virus	+	+++	
Alternate (Mg, Ag)	↓	↓	
IL-2R (CD25) expression, day3	–	+	[14]
Day 7	–	+++	
TFR (CD71) expression, day 3	–	+	[14]
Day 7	–	+++	
Cell cycle progression, d3	–	++	[14]
Apoptosis	+	+++	[11,42]

Results are organized according to related aspects of immune response. Responses relative to sham-exposed (control) cells are indicated. Results are due to the responses of the small percentages of PBMC (lymphocytes) that are virus-specific, with later recruitment of additional cells.
Abbreviations used: HLA = human leukocyte antigen; ICAM-1 = intercellular adhesion molecule-1; IFN = interferon; IL-2R = IL-2 receptor; LFA-1 = lymphocyte function-associated antigen-1; TFR = transferrin receptor.

Earlier studies also documented the existence of RSV-specific lymphocytes in PBMC from adults, with robust proliferation in response to inactivated RSV but markedly reduced proliferation after exposure to infectious RSV [14]. In fact, exposure to infectious RSV effectively inhibits the proliferative T cell response to inactivated RSV [22]. In contrast, PBMC mount a strong proliferative response to both inactivated and infectious IAV. RSV infection of adult PBMC appears to be recognized as vigorously as IAV as measured by early biophysical changes in the small populations of lymphocytes that were in turn shown to be virus-specific by deletion and reconstitution experiments [12], although the IAV-exposed cells proliferated and the RSV-exposed cells showed cell cycle arrest [13,14].

2.2. Representative in vivo observations illustrating reduced human recall immune responses

Longitudinal studies of RSV infections in children showed that children can be naturally re-infected with the same strain of virus

[23] although subsequent disease severity is usually reduced, especially as the child ages [24]. Thus, there is some generation of partially protective immunity in infants following natural infection [25]. Furthermore, a single RSV isolate has been shown to cause repeated experimental symptomatic infections in adult volunteers [6], effectively eliminating strain variation as the singular determinant of re-infection. Habibi and colleagues more recently studied antibody-mediated protection using intranasal challenge of healthy adults with RSV and found that more than half of the volunteers became infected, and most of those developed symptomatic colds, despite moderately high levels of preexisting serum antibody [26]. In contrast to RSV, IAV infection generally induces adequate immune protection against subsequent clinical infection with homotypic strains [27]. There is, however, one very recent report from a human challenge study using the most recent pandemic strain of IAV (H1N1pdm09) that described clinical re-infection with a second challenge with that same strain [28]. Nonetheless, most IAV re-infections result from exposure to drifted or shifted strains of the virus.

Both antibody and CD8+T cell responses are established during a child's primary response to RSV but ex vivo analyses of PBMC from infected children suggest that responses to the virus may not be boosted by episodes of re-infection [29]. In studies by Bont and colleagues, RSV-specific T-cell responses did not provide protection against re-infection. Moreover, re-infection did not boost RSV-specific T-cell proliferation. To explain both findings, the investigators hypothesized that RSV-specific T cells fail to expand in vivo upon re-infection [29]. In like manner, Habibi and colleagues found that RSV-specific IgA+ memory B cells were not induced by the infection in their intranasal challenge study of healthy adults [26]. Such evidence suggests that RSV during natural infections reduces human innate immune responses and, especially, recall adaptive immune responses acutely but incompletely, allowing clinically evident re-infection, albeit less severe than the first natural infection [30].

Studies by Sananez and colleagues [31] suggest that both the production and function of IL-2 is compromised in the course of severe RSV infection in infants. They reported that conventional CD4 + T cells from RSV-infected infants produce low amounts of IL-2. They also found that exogenous IL-2 was unable to fully restore IL-2–dependent functions such as the proliferative response of CD4 + T cells.

2.3. Implications for vaccine development

The existence and participation of a protective recall response to RSV is supported by the studies cited above, showing clear although diminished proliferative responses, and induced but low IL-2 production, as well as IFN-γ production, compared to IAV [11,19]. It is clear that the host does have anamnestic immune responses that render the second and subsequent infections with RSV less severe than the initial infection. Although the cited studies provide evidence supporting the potential for natural RSV infection to acutely and adversely affect human innate immune responses and, especially, recall adaptive immune responses upon infection or re-infection, such a concept does not in any way diminish the need for a vaccine for RSV.

Progress is being made in the efforts to develop RSV vaccines. For example, a trial of a candidate vaccine that was developed through rational design based on increased knowledge of RSV gene function showed not only that a candidate was immunogenic but that immunity was boosted over a subsequent seasonal epidemic period without clinically evident illness [32], an encouraging result. The investigators had previously determined that deletion of the coding sequence for the viral M2-2 protein (ΔM2-2) down-regulated viral RNA replication and up-regulated gene transcription and antigen synthesis, raising the possibility of development of an attenuated vaccine with enhanced immunogenicity. RSV MEDI ΔM2-2 was then evaluated as a live intranasal vaccine in adults, RSV-seropositive children, and RSV-seronegative children. Notably, surveillance during the subsequent RSV season showed that several of the seronegative RSV MEDI ΔM2-2 recipients had substantial antibody rises without reported illness, suggesting that the vaccine was protective against even clinically evident infection yet primed the recipients for recall responses to RSV [32]. As noted in an accompanying editorial, however, live attenuated vaccines will not be suitable for all populations at risk for RSV [33].

Vaccines generally cannot be expected to prevent infection but can be expected to protect from progression to clinical illness, or at least protect from severe disease. Furthermore, vaccines may never effectively prevent clinically evident infection or re-infection if natural infection by the virus does not lead to such protection because of suppression of a recall response rather than due to insufficient induction of immunity, such as is the case with RSV. The cellular and humoral defenses that are induced by an RSV vaccine are likely to be offset at least partially by the ability of the naturally encountered infectious virus to counteract innate and especially adaptive immune responses. However, there is benefit to be gained from rendering a first natural infection less severe, as would be expected with a re-infection, or from rendering a re-infection less likely to require medical attention, result in hospitalization, or result in mortality.

It is difficult to conceive of a candidate vaccine, live or inactivated, that could induce a recall antiviral immunity that would be less susceptible to the adverse effects of a subsequent natural RSV infection. Thus, the WHO appropriately seeks greater than 70% vaccine efficacy against confirmed severe RSV disease, rather than protection from all clinically evident RSV infection [8]. Furthermore, limited immune function at the extremes of life (infancy and old age), and with immunosuppression due to disease or its therapy, combined with RSV effects on the recall immune response make a continued search for effective anti-RSV antiviral agents an important partner to vaccine development. It is possible that different vaccines will be optimal for infants; older children; young to middle-age adults (including pregnant women); and elderly persons [3]. The elderly may even require annual vaccination with a chosen RSV vaccine before the RSV season due to increased risk from the potential waning of immune memory [5,34], rather than because of drifts of the virus as is the case with current IAV vaccines. The studies cited in this review provide strong support for the limited but important goal for RSV candidate vaccines, to prevent severe RSV disease, that has been proposed by the WHO [8]. Prevention of any clinically evident illness after subsequent exposure to RSV is an unwarranted and excessive, although desired, criteria for further development of a candidate vaccine in view of the observations described in this report. Furthermore, effective therapeutic agents for RSV are likely to be needed, especially for high-risk populations, even after effective vaccine development.

The author attests that he meets the ICMJE criteria for authorship, and declares that he has no conflicts of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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