

Chapter 35

Pneumonia Virus of Mice (PVM): Exploring Novel Therapeutic Options In a Severe Respiratory Disease Model

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35.1 Introduction

Respiratory syncytial virus (RSV) is the most important respiratory pathogen among infants and toddlers, with infections prevalent and nearly universal in this age group. Severe infections are more common among premature infants, those with cardiac and pulmonary anomalies, and the immunosuppressed. Effective prophylactic monoclonal antibody treatment is available for high-risk infants, but there is no effective vaccine. Mouse challenge models have been used for the study of the human RSV pathogen, but the most severe forms of RSV disease are not replicated by this approach. Pneumonia virus of mice (PVM; family *Paramyxoviridae*, genus *Pneumovirus*) is a mouse pathogen of the same family as human respiratory syncytial virus. PVM replicates efficiently in mouse-lung epithelial cells *in vivo* in response to a minimal virus inoculum, and replication is accompanied by local production of proinflammatory cytokines (MIP-1 α , MIP-2, MCP-1, and IFN- γ) and granulocyte recruitment to the lung. PVM infection and the ensuing inflammatory response can lead to pulmonary edema and respiratory compromise. Our laboratories have pioneered the use of the PVM model for the study of human clinical disease, which has provided important insights into the role of the inflammatory response in the pathogenesis of severe respiratory virus infection. As part of this work, we have presented several immunomodulatory strategies that clearly reduce morbidity and mortality when administered to PVM infected, symptomatic mice, and thus hold promise as realistic therapeutic strategies for severe RSV infection in human subjects.

35.2 Human RSV Disease

Respiratory syncytial virus (RSV) infection is a near universal affliction of infancy and childhood, accounting for approximately 50% of all pneumonia and up to 90% of the reported cases of bronchiolitis in infancy. Of those infected during the first year of life, one-third develops lower respiratory

tract disease, and 2.5% are hospitalized (more than 90,000 children in the United States every year). In many previously healthy infants, RSV disease is a mild and self-limited infection involving the upper and lower respiratory tract, with varying degrees of peribronchiolar and interstitial inflammation. In others, the disease progresses to severe bronchiolitis and pneumonia, including submucosal edema and bronchiolar obstruction requiring oxygen, and in the worst cases, mechanical ventilation. Infants at particularly high risk for severe disease include those born prematurely, infants and children with cardiac or pulmonary anomalies, and the immunocompromised. Prophylactic monoclonal antibody therapy is available for high-risk infants, but no vaccine is yet approved for use. RSV has also recently been recognized as an important pathogen in the institutionalized elderly. The clinical features and pathology of RSV disease have been reviewed extensively, and the reader is referred to these excellent sources of additional information [1–4].

35.3 Identifying an Appropriate Animal Model

There is no one animal model that can replicate all features of a human disease. This is particularly true when considering infectious pathogens, many of which have particular tropisms or specificities for a limited range of hosts, and in some cases present with completely different clinical illnesses in different species. While it would perhaps be ideal to study all human pathogens in natural, relevant human or higher primate hosts, this approach is of course completely limited and impractical. Despite clear and recognized differences between human and rodent immune and inflammatory responses, various factors (availability of characterized strains, ease of handling and breeding, availability of sophisticated experimental tools) together have provided a focus on inbred strains of mice as a centerpiece for human disease research. As such, it is critical that one understands what the unique features of each infectious disease model are and that

the specific advantages and the individual limitations in each situation are clearly understood.

With these factors in mind, around 1997, we began our collaborative exploration of the pneumonia virus of mice (PVM) pathogen for the study of respiratory virus replication and the ensuing inflammatory response within a natural, evolutionarily relevant host-pathogen relationship. Further and more detailed consideration of our studies and those from other laboratories can be found in several of our recent reviews [5–8]; this article is largely excerpted from ref. 8.

35.4 The Pneumonia Virus of Mice Pathogen

PVM was originally discovered in 1939 by Frank Horsfall and Richard Hahn at the Rockefeller University as part of an attempt to identify pathogens from human clinical samples that would replicate in lung tissues of inbred mice [9]. Interestingly, PVM was isolated from lung tissue from the control (thought to be uninfected) mice, which yielded an infectious isolate after undergoing serial mouse-to-mouse passage. Choppin and colleagues [10] presented the first electron micrographs of these newly discovered virions (Fig. 35.1),

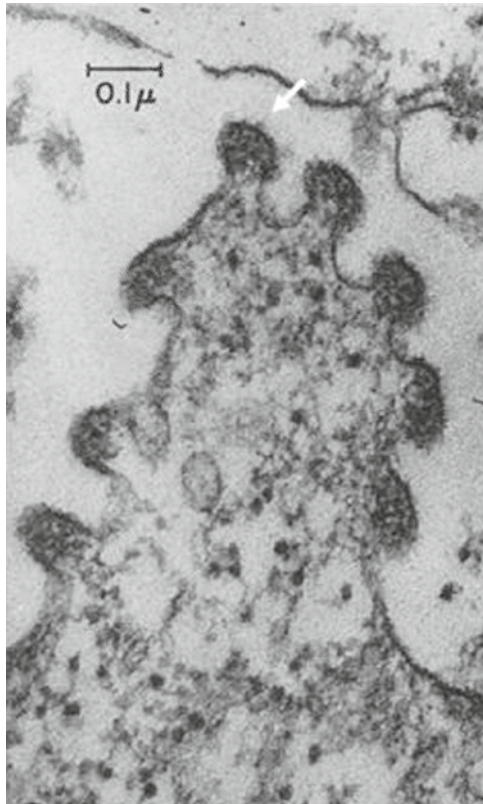


Fig. 35.1 Electron microscopic image of nascent PVM virions. Virions as shown budding (at arrow) from BHK-21 cells in culture. Reprinted with permission from Compans, R. W., et al. [10]

which they described as defining a new, third subgroup of myxoviruses. The polymorphic virions formed spheres of 80–120 millimicrons in diameter to filaments up to three microns in length, and replicated over a period of 24–30 hours in mouse lung tissue *in vivo*, with virus amplification proceeding at ~16-fold per cycle. Perhaps most interestingly, Ginsberg and Horsfall [11] recognized the potential of PVM for the exploration of acute respiratory virus infection in an evolutionarily relevant host. Among several studies, these authors were the first to relate the development of lung lesions to ongoing virus replication and to evaluate altered morbidity and mortality in response to rudimentary immunomodulatory therapy, specifically in response to administration of bacterial capsular polysaccharide [11–13].

PVM has since been classified as a pneumovirus (Family Paramyxoviridae, genus Pneumovirus), together with the human and bovine respiratory syncytial virus pathogens. Viruses of this family are enveloped, and have non-segmented, negative-sense RNA genomes [14]. The molecular organization of the PVM genome has been elucidated primarily by Easton and colleagues [15–18], and has been the subject of several recent reviews [5,6]. The genomic organizations of PVM and RSV are shown in Fig. 35.2, which highlights the similarities in gene structure and gene order.

There are two major characterized strains of PVM in general use, although there is not complete clarity on all details related to their origin and maintenance. The original studies by Horsfall and colleagues [9,11–13] were performed on an isolate known as strain 15, which was at that time highly pathogenic in mice. Since that time, PVM strain 15 has reportedly undergone tissue-culture passage and lost some of its pathogenicity *in vivo*, although the extent to which this is so, and in which specific isolates, remains uncertain. A second strain, PVM strain J3666, also developed at the Rockefeller University, has been reportedly maintained in mice with minimal tissue-culture passage, and has recently been shown to be highly pathogenic in nearly all inbred strains of mice [19]. In our hands, PVM strain 15 from Dr. Andrew Easton's laboratory (PVM strain 15 Warwick) is highly attenuated and elicits

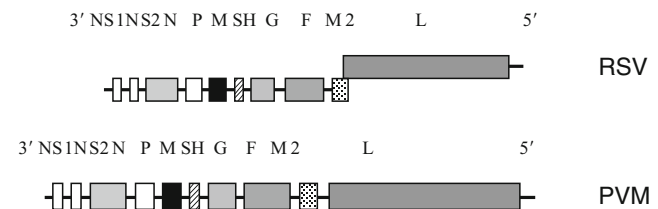


Fig. 35.2 Genomic organization of RSV and PVM. Shown are the 3' to 5' linear order of PVM and RSV protein encoding genes, including non-structural proteins (NS1 and NS2), nucleoprotein (N), phosphoprotein (P), matrix protein (M), surface hydrophobic protein (SH), attachment protein (G), fusion protein (F), the M2 genes, and polymerase protein (L). Reprinted with permission from Rosenberg, et al. [5]

a minimal inflammatory response in the highly susceptible BALB/c strain of mice [20]. In contrast, PVM strain 15 from the American Type Culture collection (PVM strain 15 ATCC) is pathogenic in BALB/c mice (unpublished findings), but results in little to no disease in the less susceptible C57BL/6 strain at similar inoculating doses [<100 pfu/mouse [21]]. Kreml and colleagues [22] likewise found PVM strain 15 (ATCC) to be highly pathogenic in the BALB/c strain. Complete sequence data are available for both PVM strain J3666 and PVM strain 15 [23,24]. The most remarkable differences are in selected regions of the G (attachment protein) and throughout the sequence of the SH small hydrophobic glycoprotein.

35.5 RSV and PVM: Inflammatory Responses and Disease Severity

We became interested in PVM in order to pursue studies of inflammatory responses to respiratory virus infections in a natural, evolutionarily relevant host. We initially recapitulated the aforementioned findings of Horsfall and colleagues and reported robust virus replication *in situ* (to titers $>10^8$ pfu/gm lung tissue), progressing to marked morbidity (hunching, fur ruffling), weight loss, and mortality, in our case in response to a minimal virus inoculum of the highly pathogenic strain PVM J3666 [25,26]. We have localized immunoreactive PVM to the bronchiolar epithelium [27], in a distribution similar to what has been observed for RSV in human post-mortem specimens [28] (Fig. 35.3). Microscopic examination of bronchoalveolar lavage fluid and lung tissue from morbid mice revealed profound inflammation, most notable for

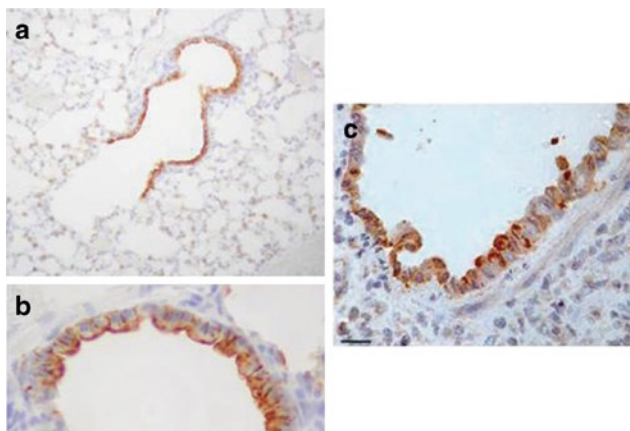


Fig. 35.3 Detection of immunoreactive PVM and RSV in bronchiolar epithelial cells. (a) Immunoreactive PVM is detected in infected mouse lung tissue by probing with convalescent mouse sera, original magnification 20X, (b) as in (a), original magnification 40X. (c) Detection of immunoreactive RSV in post-mortem human lung tissue, original magnification 40X. PVM images reprinted with permission from Bonville, et al. [27]; RSV images reprinted with permission from Welliver, et al. [28]

recruitment of granulocytes and for severe pulmonary edema consistent with the clinical findings characteristic of acute respiratory distress syndrome (ARDS; Fig. 35.4). Interestingly, severe inflammation, edema and recruitment of granulocytes have also been characterized in a recent series of RSV-diagnosed post-mortem samples evaluated by Welliver and colleagues [28]. PVM replication *in situ* results in local production of proinflammatory mediators, including MIP-1 α , MIP-2, MCP-1 and IFN- γ [27]. A similar subset of proinflammatory mediators is produced in association with the more severe forms of RSV in human infants (reviewed in ref. 29).

35.5.1 MIP-1 α (CCL3) Is a Crucial Component of the Virus-Induced Inflammatory Response

Similar to findings from the mouse model of influenza virus [30] we found that the chemokine, MIP-1 α (CCL3), is crucial for granulocyte recruitment in response to PVM infection [26]. Specifically, MIP-1 α gene-deleted mice are readily infected with PVM, although 10^5 -fold fewer granulocytes are recruited to the lung tissue in response to the identical initial inoculum. Similar results were obtained upon infecting mice devoid of CCR1, the major receptor for MIP-1 α on neutrophils and eosinophils. We have used this observation to design specific immunomodulatory strategies for the virus-induced inflammatory response and its associated pathology [31,32]

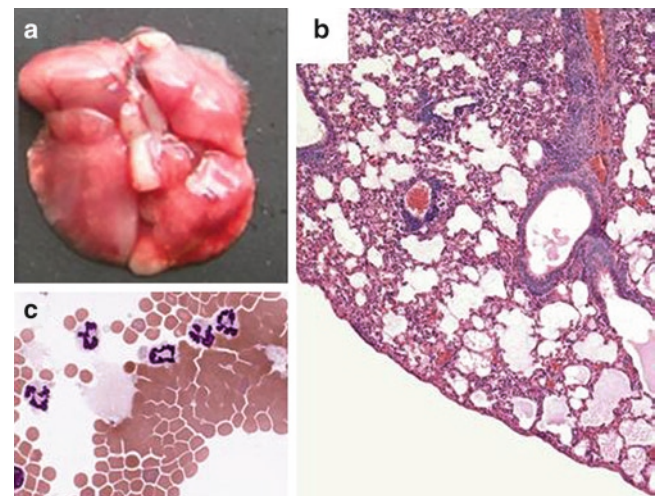


Fig. 35.4 Lung pathology. (a) Lung tissue from infected mice, with region of typical grey discoloration and multiple hemorrhagic foci; reprinted with permission from Ellis, et al. [21]; (b) Microscopic pathology, with evidence of profound cellular inflammatory response and developing pulmonary edema; original magnification, 20X, reprinted with permission from Garvey, et al. [36]; (c) Bronchoalveolar lavage fluid, with recruited neutrophils, original magnification, 63X

(discussed further below). Interestingly, although MIP-1 α is clearly crucial for granulocyte recruitment in response to PVM infection, this chemokine, acting alone, even at high concentrations, does not induce ARDS, nor does it recruit granulocytes effectively in the absence of IFN- γ [33].

35.5.2 Inflammatory Responses In Aged Mice

As noted above, respiratory virus infection is also a growing problem in the aging population [34]. In a study performed using young adult (eight–12 weeks) through aged (up to 78 weeks), but otherwise immunologically naïve mice, we observed no change in the kinetics of PVM replication, but diminished local production of several proinflammatory mediators, including MIP-1 α , MCP-1, and IFN- γ , along with diminished recruitment of granulocytes to the lung tissue [35]. The differences observed when comparing these results to those reported among elderly human subjects, who tend to have more severe, rather than less severe forms of RSV infection, may be related to virus re-exposure and its impact on the ensuing biochemical and cellular inflammatory responses. Interestingly, there is no published data on PVM replication and disease pathogenesis in neonatal mice, a critical target population given the prevalence of pneumovirus infection among human infants and toddlers.

35.5.3 Differential Responses In Type I IFN Receptor Gene-Deleted Mice

Pneumoviruses have unusual anti-interferon strategies, as they do not limit IFN production, nor do they interfere with receptor binding or signal transduction. Of particular recent interest are the ways in which IFNs and IFN-mediated signaling mechanisms interact with proinflammatory pathways and modulate the production of chemoattractant cytokines. As part of a larger exploration of the inflammatory responses to PVM, we examined the differential expression of cytokine genes in wild-type mice and mice devoid of the receptor for type I interferons (IFN $\alpha\beta$ R gene-deleted mice) [36]. As anticipated, PVM infection induces transcription of IFN antiviral response genes preferentially in lung tissue of wild-type over IFN $\alpha\beta$ R gene-deleted mice. However, we demonstrate that PVM infection also results in enhanced expression of eotaxin-2, TARC, and the proinflammatory RNase mouse eosinophil-associated RNase (mEar) 11, and we observe paradoxically prolonged survival among the IFN $\alpha\beta$ R gene-deleted mice.

35.6 Immunity to PVM Generated via Mucosal Inoculation

Using PVM strain 15 (ATCC), which, as noted above, is attenuated (replication-competent but does not induce a profound inflammatory response) in the C57BL/6 mouse strain, we explored the development of acquired immunity in response to a mucosal vaccination strategy. Neutralizing antibodies were detected at 14 days after inoculation in mice receiving live attenuated virus (but not heat-inactivated virus), which correlated with protection against subsequent challenge with the highly pathogenic strain J3666 [21]. Among the interesting questions, we are not certain of the duration of protective immunity. Interestingly, the responses of IFN γ R gene-deleted mice were indistinguishable from the wild-type mice, indicating that any role for IFN- γ in generating acquired immunity in this setting is at least somewhat dispensable (Fig. 35.5). Among our other findings, PVM antigens, when prepared and administered in a manner analogous to the earlier hRSV lot 100 vaccine [37], will induce a Th2-mediated hypersensitivity response [38].

35.7 Therapeutic Strategies

Among the primary reasons to explore respiratory virus infection using the PVM model is to improve our understanding of the molecular basis of severe disease so as to design novel therapeutic strategies.

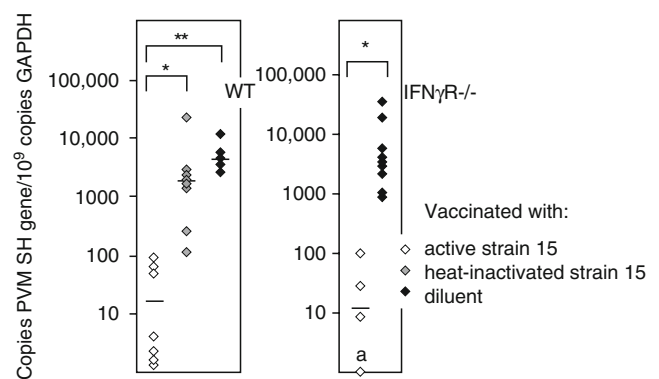


Fig. 35.5 Virus replication in lung tissue of vaccinated mice. Wild type and IFN γ R gene-deleted mice were vaccinated with live-attenuated PVM strain 15 (ATCC), heat-inactivated strain 15, or diluent alone and challenged with virulent strain J3666. Replication was measured by a quantitative RT-PCR assay designed to detect the PVM SH gene. We observed no differences in the kinetics of virus replication, and no differences in responses to mucosal vaccination between the wild type and IFN γ R gene-deleted mouse strains. Reprinted with permission from Ellis, et al. [21]

35.7.1 Antiviral Agents

The antiviral agent, ribavirin, is very effective at blocking replication of RSV in both tissue culture and in human subjects, yet the impact of ribavirin therapy alone on the course of actual clinical disease is insignificant [39]. The PVM model replicates this scenario, as ribavirin at concentrations of $>10 \mu\text{g/ml}$ is effective at blocking virus replication in tissue culture, and at concentrations of $75 \mu\text{g/kg/day}$, at blocking virus replication in mouse lung tissue *in vivo*. Yet, analogous to observations made in clinical studies, administration of effective doses of ribavirin alone to PVM-infected, symptomatic mice, has little impact on the ultimate outcome of disease, when measured in terms of morbidity and mortality [31,32]. In conjunction with this observation, we found that, although ribavirin was quite effective at blocking virus replication, it had no impact on the ongoing production of proinflammatory chemokines and associated recruitment of granulocytes to the lung tissue. Clearly, there is some disconnect between virus replication and the ensuing inflammatory response, in that inflammation is not necessarily controlled effectively at all given points in time by reducing or eliminating the primary stimulus.

35.7.2 Glucocorticoids

Glucocorticoids are in general use as broad-spectrum, anti-inflammatory agents, yet overall analysis suggests that they have only limited benefit for the treatment of severe hRSV-

associated inflammation [40]. Although we have not evaluated specific combinations of ribavirin and glucocorticoids in PVM-infected mice, we have documented the effects of hydrocortisone alone on the inflammatory response. We have determined that hydrocortisone therapy has no effect on the production of MIP-1 α or on the influx of neutrophils; PVM-infected mice responded to hydrocortisone with enhanced viral replication and slightly accelerated mortality [41]. These results suggest several mechanisms to explain why glucocorticoid therapy may be of limited benefit in the overall picture of pneumovirus infection. Interestingly, Thomas and colleagues [42] also determined that glucocorticoids had no impact on the virus-induced chemokine response in hRSV infection in human subjects.

35.7.3 Combination Therapy with Ribavirin and Specific Immunomodulatory Agents

Given our earlier observation on the crucial nature of the chemokine, MIP-1 α , in promoting granulocyte recruitment in response to virus infection, we considered the possibility that blockade of this chemokine itself, or its signaling via its major receptor, CCR1, might provide appropriate immunomodulatory control in this setting. In a series of studies, we found significant improvements in long-term survival when ribavirin was administered to symptomatic mice in conjunction with anti-MIP-1 α antibodies, or with small molecule blockade (met-RANTES) of the MIP-1 α receptor, CCR1 [31,32] (Fig. 35.6).

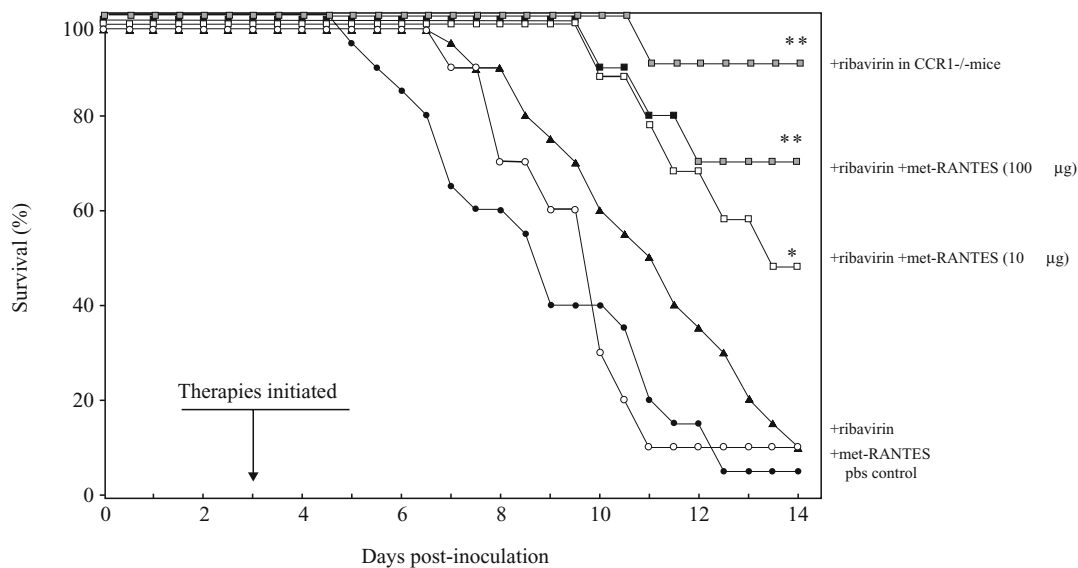


Fig. 35.6 Survival of PVM-infected mice treated with combined antiviral and immunomodulatory therapy. Improved survival resulted from treatment with ribavirin and met-RANTES (* $p < 0.05$) compared individually to the groups treated with Met-RANTES alone, ribavirin alone, or PBS. Further improvement was observed in response to combination

of ribavirin with the higher met-RANTES dose, $100 \mu\text{g/day}$, over that observed in response to ribavirin-met-RANTES at $10 \mu\text{g/day}$ (** $p < 0.05$), approaching that observed for CCR1 gene-deleted (CCR1 $-/-$) mice treated with ribavirin, representing theoretical complete receptor blockade. Reprinted with permission from Bonville, et al. [32]

A similar study documented the effectiveness of ribavirin in conjunction with the cysteinyl-leukotriene inhibitor montelukast [43]. Interestingly, although neither agent was effective at reducing morbidity or mortality as single-agent therapy, together, administered to infected, symptomatic mice, significant improvements in long-term survival were observed (50% vs. 10% for PBS control). Interestingly, montelukast had little impact overall on neutrophil recruitment, suggesting that the presence of neutrophils alone does not indicate inevitable progression to intractable disease.

35.8 Conclusions

The PVM model holds great promise for the elucidation of inflammatory mechanisms associated with virus infection and acute inflammatory responses in the lung. Studies carried out to date have provided an explanation for the lack of clinical efficacy of antiviral therapy, and have indicated that chemokine and/or chemokine-receptor blockade in conjunction with appropriate antiviral therapy might be more effective than antiviral therapy alone. Likewise, PVM is an excellent system in which to explore the molecular mechanisms through which natural immunity to pneumovirus infection develops, information which may assist in the development of novel vaccines and other prevention strategies.

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