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Short communication

The contribution of type I interferon signaling to immunity induced by alphavirus replicon vaccines

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ABSTRACT

The type I interferon (IFN) system is critical for protecting the mammalian host from numerous virus infections and plays a key role in shaping the antiviral adaptive immune response. In this report, the importance of type I IFN signaling was assessed in a mouse model of alphavirus-induced humoral immune induction. Venezuelan equine encephalitis virus replicon particles (VRP) expressing the hemagglutinin (HA) gene from influenza virus (HA-VRP) were used to vaccinate both wildtype (wt) and IFN α/β receptor knockout (RKO) mice. HA-VRP vaccination induced equivalent levels of flu-specific systemic IgG, mucosal IgG, and systemic IgA antibodies in both wt and IFN RKO mice. In contrast, HA-VRP vaccination of IFN RKO mice failed to induce significant levels of flu-specific mucosal IgA antibodies at multiple mucosal surfaces. In the VRP adjuvant system, co-delivery of null VRP with ovalbumin (OVA) protein significantly increased the levels of OVA-specific serum IgG, fecal IgG, and fecal IgA antibodies in both wt and RKO mice, suggesting that type I IFN signaling plays a less significant role in the VRP adjuvant effect. Taken together, these results suggest that (1) at least in regard to IFN signaling, the mechanisms which regulate alphavirus-induced immunity differ when VRP are utilized as expression vectors as opposed to adjuvants, and (2) type I IFN signaling is required for the induction of mucosal IgA antibodies directed against VRP-expressed antigen. These results shed new light on the regulatory networks which promote immune induction, and specifically mucosal immune induction, with alphavirus vaccine vectors.

1. Introduction

The type I interferons (IFNs) are a family of pleiotropic cytokines which were originally identified for their ability to interfere with virus replication [1], and are now known to provide the first line of defense against numerous viral pathogens [2]. Type I IFNs, which include IFNα and IFNβ, signal through a common receptor, the type I IFN receptor, which is expressed in almost all cell types [3]. The importance of IFN signaling in antiviral defense is evidenced by the observation that animals with an engineered genetic deficiency in the IFNα/β receptor (IFNα/β receptor knockouts, or RKOs) are acutely susceptible to numerous viral infections [4–7].

In addition to its role in innate immunity, it has recently been appreciated that type I IFN signaling is required for the activation of adaptive immune responses [8–12]. Type I IFN provides a powerful activation signal to differentiated dendritic cells (DCs), promoting co-stimulatory molecule expression and their antigen-presenting-cell (APC) function [12]. Moreover, interferon-treated DCs, upon interacting with B cells, activate immunoglobulin (Ig) class switch recombination and Ig secretion [13] to multiple isotypes, including IgA [14]. In addition to effects on DCs, IFN signaling is directly required for complete activation of B cells [15], $CD4^+$ T cells [16], $CD8^+$ T cells [17], and natural killer cells [18].

Vaccine vectors based on the alphavirus, Venezuelan equine encephalitis virus (VEE), contain a message-sense, single-stranded RNA genome [19] and have proven to be efficacious inducers of antigen-specific immunity in several pre-clinical vaccination models. VEE replicon particles (VRP) function as antigen expression vectors, encoding a modified genome in which the structural genes are replaced with a heterologous antigen [20]. Following VRP infection, the replicon RNA encoding the transgene is expressed at very high levels in the first infected cells; however, progeny virions are not produced. VRP stimulate potent systemic and mucosal antibody responses directed against both the antigen carried in the viral genome and soluble antigens simultaneously delivered during a concomitant VRP infection [21–25].

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A specific role for type I IFN signaling in alphavirus-induced adaptive immunity has previously been established. The activation of B and T lymphocytes (as measured by upregulated CD69 expression) was significantly impaired in IFNα/β RKO mice, suggesting that alphavirus-induced lymphocyte activation requires IFN signaling [26]. Additionally, Leitner et al. [27] demonstrated a role for type I IFN signaling with an alphavirus replicase-based vaccine, as this vaccine induced protective anti-tumor immunity in wildtype animals, but not in IFNα/β RKO mice. An additional study performed by Restifo and co-workers suggested that the ability of replicase-based vaccines to break immunological tolerance was dependent upon a single interferon stimulated gene involved in the viral dsRNA response, RNaseL [28]. Moreover, Hidmark et al. recently demonstrated that the systemic IgG adjuvant effect of SFV replicon particles is dependent upon type I IFN signaling, as SFV replicons failed to augment serum IgG responses directed against co-delivered antigen in IFNα/β RKO mice.

In this report we have evaluated the role of type I IFN signaling in the stimulation of systemic and mucosal antibody responses by VRP as expression vectors, expressing the hemagglutinin (HA) gene from influenza (flu) virus (HA-VRP), and as adjuvants, following co-delivery of null VRP with soluble ovalbumin (OVA) protein. HA-VRP induced equivalent flu-specific systemic IgG and IgA antibody responses in both wildtype (wt) and IFNα/β RKO mice. In contrast, while HA-VRP vaccinated, wt mice produced strong flu-specific IgA responses at several mucosal surfaces, mucosal IgA responses were essentially undetectable in vaccinated IFNα/β RKO mice. Interestingly, null VRP significantly augmented OVA-specific serum IgG and fecal IgA antibodies in both wt and IFNα/β RKO mice. These results suggest that type I IFN signaling is critical for VRP expression-vector-induced mucosal IgA responses; however, plays a less significant role in the VRP adjuvant effect. This analysis offers a new perspective on the precise role of IFN signaling in alphavirus immunity.

2. Materials and methods

2.1. VEE replicon constructs

The construction and packaging of VRP was performed as previously described [20,29]. Briefly, confluent monolayers of BHK-21 cells were co-electroporated with the *in-vitro*-transcribed replicon RNA and two defective helper RNAs which express the viral structural genes *in trans*. In this study, two different replicon constructs were utilized: (1) VRP expressing the HA gene from the A/PR/8/34 strain of influenza virus (HA-VRP); and (2) VRP which lack a functional transgene downstream of the 26S promoter (null VRP) [23]. HA-VRP and null VRP were quantitated by immunocytochemistry of infected BHK cells with anti-sera against HA [20] and null VRP [23], respectively. All replicon particles utilized in this study were packaged in the wildtype (V3000) envelope.

2.2. Animals and immunizations

Groups of 8–16-week-old 129 Sv/Ev and 129 Sv/Ev IFN α/β RKO mice were immunized in a 0.01 ml volume in the rear footpad as previously described [23]. Breeder pairs of 129 Sv/Ev animals were obtained from Dr. Barbara Sherry, North Carolina State University, or were purchased from Taconic Laboratories and breeder pairs of the RKO animals were obtained from Dr. Herbert Virgin, Washington University. Animals were immunized at week 0 and week 4 with either HA-VRP or ovalbumin (OVA, Sigma) in the presence or absence of null VRP as an adjuvant in low endotoxin, filter-sterilized PBS as described [23].

2.3. Antibody-secreting-cell (ASC) enzyme-linked immunospot (ELISPOT)

Splenocytes and nasal lymphocytes were prepared from immunized animals as previously described [23] and evaluated in an ASC ELISPOT assay, modified from Thompson et al. [23]. Briefly, purified influenza virus antigen (500 ng/well, Charles River Spafas) was used to coat 96-well nitrocellulose membrane plates (Millipore) overnight at 4 °C. Plates were blocked for 2 h with complete media (10% serum) and 2-fold dilutions of single cell suspensions were then added to plates in duplicate and incubated overnight. Plates were washed, and bound spots were detected by the addition of HRP-conjugated goat anti-mouse γ or α chain-specific antibodies (Southern Biotechnology Associates), followed by addition of 3-amino-9-ethylcarazole (AEC, Sigma). ASCs were enumerated with a computerized ELISPOT plate reader (Immunospot) and data are presented as the number of antigen-specific ASCs per 10^6 cells plated.

2.4. Sera, fecal extracts, and vaginal washes

All sample collection was prepared as previously described [23]. Blood was harvested from individual animals either from the tail vein, following cardiac puncture, or from the submandibular plexus, and sera collected following centrifugation in microtainer serum separator tubes (Becton Dickinson). Fecal extracts and vaginal lavage fluids were prepared from individual animals as previously described [23]. Samples were analyzed for the presence of antigen-specific IgG and IgA antibodies via ELISA (see below).

2.5. Enzyme-linked immunosorbant assay (ELISA)

ELISAs for influenza- and OVA-specific antibodies were performed on serum, fecal extracts, and vaginal washes as previously described [23]. Briefly, antigen solutions were incubated in 96-well plates (Costar) overnight at 4 °C to allow antigens to bind to the plate and plates were blocked for 2 h for flu or overnight for OVA, at RT. Following removal of blocking solution, plates were incubated at room temperature for 2 h (flu) or overnight (OVA) with serial dilutions of individual samples diluted in the appropriate blocking buffer. Plates were washed and incubated for 1 h with HRP-conjugated goat anti-mouse γ or α chain-specific antibodies (Southern Biotechnology Associates or Sigma). Finally, plates were washed, and developed with *O*-phenylenediamine dihydrochloride substrate for 30 min. Antibody endpoint titers are reported as the reciprocal of the highest dilution that resulted in an $OD_{450} \geq 0.2$. Data are presented as the geometric mean ± standard error of the mean (SEM).

2.6. Statistical analysis

Antibody titers and ASC values were evaluated for statistically significant differences by the Mann–Whitney non-parametric test (GraphPad INSTAT). A p value of ≤ 0.05 was considered significant. The Bonferroni correction for multiple comparisons was applied to the data presented in Fig. 3, as appropriate.

3. Results

3.1. Role of type I IFN signaling in VRP expression-vector-induced systemic immunity

Systemic and mucosal immunity can be induced by VRP used in two different modalities. In the first instance, VRP express an