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APRIL:TACI axis is dispensable for the immune response to rabies vaccination

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ABSTRACT

There is significant need to develop a single-dose rabies vaccine to replace the current multi-dose rabies vaccine regimen and eliminate the requirement for rabies immune globulin in post-exposure settings. To accomplish this goal, rabies virus (RABV)-based vaccines must rapidly activate B cells to secrete antibodies which neutralize pathogenic RABV before it enters the CNS. Increased understanding of how B cells effectively respond to RABV-based vaccines may improve efforts to simplify post-exposure prophylaxis (PEP) regimens. Several studies have successfully employed the TNF family cytokine a proliferation-inducing ligand (APRIL) as a vaccine adjuvant. APRIL binds to the receptors TACI and B cell maturation antigen (BCMA)—expressed by B cells in various stages of maturation—with high affinity. We discovered that RABV-infected primary murine B cells upregulate APRIL *ex vivo*. Cytokines present at the time of antigen exposure affect the outcome of vaccination by influencing T and B cell activation and GC formation. Therefore, we hypothesized that the presence of APRIL at the time of RABV-based vaccine antigen exposure would support the generation of protective antibodies against RABV glycoprotein (G). In an effort to improve the response to RABV vaccination, we constructed and characterized a live recombinant RABV-based vaccine vector which expresses murine APRIL (rRABV-APRIL). Immunogenicity testing in mice demonstrated that expressing APRIL from the RABV genome does not impact the primary antibody response against RABV G compared to RABV alone. In order to evaluate the necessity of APRIL for the response to rabies vaccination, we compared the responses of APRIL-deficient and wild-type mice to immunization with rRABV. APRIL deficiency does not affect the primary antibody response to vaccination. Furthermore, APRIL expression by the vaccine did not improve the generation of long-lived antibody-secreting plasma cells (PCs) as serum antibody levels were equivalent in response to rRABV-APRIL and the vector eight weeks after immunization. Moreover, APRIL is dispensable for the long-lived antibody-secreting PC response to rRABV vaccination as anti-RABV G IgG levels were similar in APRIL-deficient and wild-type mice six months after vaccination. Mice lacking the APRIL receptor TACI demonstrated primary anti-RABV G antibody responses similar to wild-type mice following immunization with the vaccine vector indicating that this response is independent of TACI-mediated signals. Collectively, our findings demonstrate that APRIL and associated TACI signaling is dispensable for the immune response to RABV-based vaccination.

1. Introduction

Despite known methods of effective RABV PEP, over 55,000 humans are killed by RABV annually; 99% of these deaths occur in resource-poor, canine-rabies endemic countries where control of the RABV reservoir is insufficient or nonexistent and access to medical care is limited (Hampson et al., 2015; WHO Publication, 2010). RABV PEP relies on RABV neutralizing antibodies (RVNAs) to confer protection by preventing the virus from reaching the CNS, causing clinical disease (Li et al., 2011; Schnell et al., 2010). Cell-culture based inactivated RABV vaccines currently used for RABV PEP are safe and effective but they have inherent problems (Shayam

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Nomenclature

APRIL	a proliferation inducing ligand
BAFF	B cell activating factor
BAFFR	BAFF receptor
BCMA	B cell maturation antigen
CAML	calcium-modulator and cyclophilin ligand
HSPG	heparan sulfate proteoglycans
G	glycoprotein
GC	germinal center
PC	plasma cell
PEP	post-exposure prophylaxis
RABV	rabies virus
rRABV	recombinant SAD-B19 rabies vaccine
rRABV-mAPRIL	recombinant rabies vaccine expressing murine APRIL
RVNA	rabies virus neutralizing antibodies
TACI	T cell activator and CAML inhibitor
TD	T cell dependent
TI	T cell independent
TNF	tumour necrosis family

et al., 2006); multi-dose vaccination protocols and administration of costly rabies immunoglobulin at the initial clinical intervention are necessary because current vaccines fail to stimulate protective titers of RVNAs following the primary injection (Gacouin et al., 1999; Nagarajan et al., 2014; Wilde et al., 2002). Additionally, poor responders fail to mount protective responses even after repeated booster injections (Cabasso et al., 1974). Generating more immunogenic, protective vaccines against RABV would reduce the costs of prevention and save human lives (McGettigan, 2010). Increased understanding of how B cells effectively respond to RABV-based vaccines will guide the development of more effective, simplified PEP regimens.

In an effort to understand and potentially augment protective B cell responses to RABV-based vaccines, we have evaluated the effects of the TNF superfamily cytokine, APRIL, on the antibody response to RABV vaccination. APRIL is expressed by myeloid-derived cells including monocytes, macrophages, and dendritic cells. APRIL, like most TNF superfamily cytokines, forms soluble trimers which can bind to TNF receptors (reviewed in Mackay et al., 2003). APRIL trimers promiscuously bind to the TNF receptors transmembrane activator and calcium-modulator and cyclophilin ligand (CAML)-interactor (TACI), and B cell maturation antigen (BCMA) (Yu et al., 2000). APRIL competes with its sister molecule, B-cell activating factor (BAFF), for receptor binding sites; APRIL and BAFF have diverse interactions, forming functional heterotrimers and regulating signaling through dynamic stoichiometric interactions with each other and receptors (Roschke et al., 2002; Schuepbach-Mallepell et al., 2015).

APRIL plays a role in lymphoid development and activation, influencing humoral immune responses; recombinant APRIL is a costimulator of B and T cells *in vitro* and leads to increased B cell numbers and T cell activation *in vivo* (Yu et al., 2000). APRIL transgenic mice exhibit improved T-cell independent (TI) type 2 responses and demonstrate that APRIL boosts antigen-specific antiviral IgM responses to T-cell dependent (TD) antigens (Stein et al., 2002). Live RABV-based vaccine induces neutralizing IgM antibodies that are protective against pathogenic RABV challenge (Dorfmeier et al., 2013a); expression of APRIL in the context of RABV vaccination could improve this early protective response. Importantly, APRIL has also been shown to support antibody-secreting PC survival, suggesting that APRIL in the context of RABV vaccination might improve long-term antibody responses important in sustaining the protective effects of vaccination (Benson et al., 2008; Jourdan et al., 2014).

In this report, we evaluated the hypothesis that APRIL expression in temporospatial association with RABV antigen exposure during vaccination would augment and improve anti-RABV antibody responses. Interestingly, mice immunized with rRABV-mAPRIL demonstrated similar antibody responses to mice immunized with rRABV. In an effort to ascertain the role of APRIL in the response to RABV vaccination, we show that APRIL is dispensable for the anti-RABV G antibody response. Importantly, the antibody response to RABV vaccination does not depend on TACI-mediated signals. Collectively, our work provides new insight into the role of APRIL and TACI signaling in the context of antiviral responses. These results can guide future vaccine development, particularly those which rely on early antibody responses for protection.

2. Materials and methods

2.1. In vitro splenocyte infection with rRABV and flow cytometry

Spleens were collected from 6 to 10 week old C57BL/6 mice (The Jackson Laboratory), homogenized, and red blood cells were lysed. Splenocytes were plated at 10^7 cells/mL, infected with rRABV at a multiplicity of infection (MOI) of 5 and cultured for 48 h. Splenocytes were harvested, washed with FACS buffer (PBS containing 2% heat-inactivated FBS), blocked with anti-mouse CD16/32 antibody and surface stained with B220 (APC-Cy7, RA3-6B2, BD Biosciences) and CD4 (APC, RM4.5, BD Biosciences). Cells were fixed in 2% paraformaldehyde and stained intracellularly using PermWash buffer (BD Biosciences) for RABV nucleoprotein (N) (FITC, Fujirebio Diagnostics, Inc.) and murine APRIL (PE, A3D8, Biolegend). Cells were resuspended in FACS buffer and analyzed on a BD LSR II flow cytometer. RABV N-positive gates were set so that mock-infected cells analyzed in parallel showed <1% RABV N-positive cells (Lytle et al., 2013; Norton et al., 2014). Data were analyzed using FlowJo (FlowJo, LLC) and Prism 5 (Graphpad).

2.2. Recombinant RABV-based vaccine construction and recovery

rRABV is a molecular clone of the SAD-B19 vaccine strain of RABV (Conzelmann et al., 1990; Schnell et al., 2000). To construct rRABV expressing APRIL, the murine APRIL gene was amplified from pCMV6-Kan/Neo-APRIL (OriGene, MC203367) by RT-PCR with Taq Polymerase (Invitrogen) using forward primer JPMRP-52 (5′-TTT CGT ACG ATT ATG CCA GCC TCA TCT CCA GG -3′) (BsiWI underlined) and reverse primer JPMRP-53 (5′- AAA GCT AGC TCA TAG TTT CAC AAA CCC CAG G -3′) (NheI underlined). Digestion and insertion of this PCR product into the rRABV plasmid using BsiWI and NheI (New England Biolabs) resulted in recombinant RABV plasmid encoding murine APRIL. Infectious virus was recovered, concentrated and purified as described previously (McGettigan et al., 2001; Norton et al., 2014; Schnell et al., 2000) yielding rRABV-mAPRIL.

2.3. One- and multi-step growth curves

Growth kinetics of rRABV and rRABV-mAPRIL were determined as previously described (McGettigan et al., 2003).

2.4. Western blotting

BSR cells were infected with rRABV or rRABV-APRIL at a MOI of