Differential Requirements for T Cells in Viruslike Particle- and Rotavirus-Induced Protective Immunity

Sarah E. Blutt,1,2 Kelly L. Warfield,1† Mary K. Estes,1 and Margaret E. Conner1,2*

Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas 77030,1 and Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas 770302

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Correlates of protection from rotavirus infection are controversial. We compared the roles of B and T lymphocytes in protective immunity induced either by intranasally administered nonreplicating virus particles or inactivated virus or by orally administered murine rotavirus. We found that protection induced by nonreplicating vaccines requires CD4+ T cells and CD40/CD40L. In contrast, T cells were not required for short-term protective immunity induced by infection, but both T-cell-dependent and -independent mechanisms contributed to long-term maintenance of protection. Our findings indicate that more than one marker of protective immunity exists and that these markers depend on the vaccine that is administered.

Rotavirus causes dehydrating diarrhea in children, resulting in over 600,000 deaths worldwide each year (21). Correlates of protection from rotavirus infection are controversial. Immunoglobulin A (IgA) levels moderately correlate with protection from severe disease in humans (14, 17, 24), but studies with the extensively used murine model of rotavirus infection indicate that IgA is dispensable for protection (20, 23). Both replicating homologous and heterologous rotavirus and nonreplicating protein and subunit vaccines induce protection in mice against a rotavirus challenge (7). Complete protection against reinfection is induced by replicating murine rotavirus in the absence of T cells (10), while protection induced by vaccination with the capsid protein VP6 depends solely on the presence of CD4+ T cells (16). These data suggest that although antigenically similar, replicating virus and nonreplicating protein vaccines trigger different pathways of protective immunity against rotavirus infection with differential requirements for T cells. Here, we examine the contributions of B and T lymphocytes to protective immunity induced by the intranasal administration of nonreplicating viruslike particles (VLPs) or inactivated rotavirus and the oral administration of replication-competent wild-type murine rotavirus. Understanding and comparing the requirements for the induction of protective immunity against rotavirus infection will provide critical information to ascertain the correlates of protection from rotavirus infection.

Protection induced by a live viral infection, not VLPs, is maintained long-term. CD-1 mice (Charles River, Wilmington, MA) were vaccinated intranasally on days 0 and 14 with 10 μg of VLPs plus 5 μg of mutant Escherichia coli heat-labile toxin R192G (LT-R192G); they were orally challenged after 6 weeks with the wild-type murine strain of rotavirus (ECwt), and the percentage of protection was calculated (1, 6, 8). High levels of protection (~60 to 100%) are achieved 6 weeks after the administration of rotavirus VLPs composed of proteins VP2 and VP6 (2/6-VLPs) but are significantly lower than the levels of protection induced by ECwt infection (18, 19). Orally administered ECwt induces complete protection from infection (100%) at 6 weeks that is maintained at 6 months (11). To determine if VLP-induced protection persists beyond 6 weeks, mice were vaccinated with VLPs and challenged with ECwt 6 months later. As expected, the mice vaccinated with VLPs exhibited a significantly lower level of protection than the mice that received a primary ECwt infection (Fig. 1). Unlike what has been observed with a soluble recombinant VP6 protein vaccine (16), the level of protection induced by VLPs was not maintained over time, as it was significantly lower after 6 months than it was after 6 weeks (Fig. 1). This could be attributed to inherent differences between the soluble recombinant protein vaccine and the subunit particulate vaccine or to the differences in the strains of murine rotavirus used as a challenge. Unlike VLP-induced protection, the high level of protection induced by ECwt infection was maintained over time (Fig. 1). Therefore, VLP-mediated protection results from the induction of pathways different from those induced by a live viral infection.

B cells contribute to, but are not essential for, protection from rotavirus infection. To assess the contribution of B cells to rotavirus protective immunity, JhD−/− mice [C;129(B6)-IgH-Jtm1Dhu N2] intercrossed to homozygosity on a BALB/c background (Taconic, Germantown, NY) or BALB/c mice were vaccinated or administered ECwt. Mice were also vaccinated intranasally with 10 μg of inactivated rhesus rotavirus (4) plus adjuvant to address whether replication was an important component in the induction of protective immunity. Vaccinated JhD−/− mice exhibited significantly lower levels of protection than vaccinated BALB/c mice (Fig. 2A). As reported previously for C57BL/6 mice expressing the JhD mutation (9, 15), protection established in BALB/c mice expressing the JhD−/− mutation by ECwt infection was less than that established in BALB/c mice (Fig. 2B). However, protection induced by ECwt infection in JhD−/− mice was significantly higher than that induced in VLP-treated or inactivated-virus-
treated JhD−/− mice (Fig. 2B). In agreement with previous findings (12), protection induced by ECwt was not maintained over time, as JhD−/− mice challenged at 6 months exhibited significantly lower levels of protection than mice challenged at 6 weeks (Fig. 2B). These findings indicate that B cells contribute to the induction of complete protection from rotavirus infection in BALB/c mice but that substantial protection (>60%) persists after 6 months in the absence of antibody.

**T cells are essential for protection induced by vaccines and long-term maintenance of protection.** Although T cells are not required for protection induced by rotavirus infection (10), T cells are essential for protection induced by a soluble VP6 vaccine (5, 16). To further characterize the contribution of T cells to protection from rotavirus infection, β/δ T-cell-receptor knockout (TCRKO) mice (B6.129P2-Tcrbtm1Mom Tcrdtm1Mom/J; Jackson Laboratory, Bar Harbor, ME) or the Jackson Laboratory-recommended control C57BL/6 mice were vaccinated with VLPs or inactivated virus or were orally administered ECwt. Vaccinated TCRKO mice failed to develop detectable fecal or serum antibody, while infected TCRKO mice had detectable but significantly lower titers of both fecal and serum rotavirus-specific antibody than the C57BL/6 mice (data not shown). Vaccinated TCRKO mice exhibited limited protection from ECwt 6 weeks after vaccination compared to that exhibited by the C57BL/6 mice (Fig. 3A). Virus-infected TCRKO mice were highly protected against a challenge at 6 weeks, but protection waned significantly by 6 months (Fig. 3B). Antibody titers were similar at 6 weeks and 6 months, indicating that the difference in protection was not due to waning antibody levels (data not shown). These results suggest that T cells are essential for protection induced by nonreplicating vaccines and contribute to the maintenance of long-term protection through a non-antibody-mediated process induced by rotavirus infection in C57BL/6 mice.

**CD4+ T cells and the expression of CD40/CD40L are essential for protection induced by nonreplicating vaccines.** We investigated whether protection could be attributed to ei-
that the CD4 or CD8 T-cell subsets. CD4\(^{−/−}\) (B6.129S2-CD4\(^{m1Mak}\)/J) and \(\beta_{2m}^{-/-}\) (B6.129P2-B2\(^{m1Unc}\)/J) mice (both on a congenic C57BL/6 background; Jackson Laboratory) were vaccinated with 2/6-VLPs or orally administered EC\(_{wt}\). The CD4\(^{−/−}\) and \(\beta_{2m}^{-/-}\) mice infected with EC\(_{wt}\) were completely protected from a rotavirus challenge at 6 weeks (Fig. 4). The vaccinated \(\beta_{2m}^{-/-}\) mice exhibited high levels of protection (~89%); however, these levels of protection were significantly lower than those induced by EC\(_{wt}\) infection (Fig. 4). The vaccinated CD4\(^{−/−}\) mice had significantly lower levels of protection and geometric mean titers of fecal antibody than the vaccinated wild-type CD4\(^{+/+}\) mice and the EC\(_{wt}\)-infected CD4\(^{+/+}\) mice (Fig. 4 and data not shown). Therefore, protection induced by 2/6-VLPs appears to be modulated by CD4 T cells, with a small contribution from CD8 T cells.

T cells mediate specific antibody production through CD40L signaling (13). To determine if CD40/CD40L expression is critical to VLP- or infection-induced protective immunity, CD40\(^{−/−}\) (B6.129S2-CD40\(^{lgtm1Imx}\)) and CD40L\(^{−/−}\) (B6.129P2-CD40\(^{tm1Kik}\)/J) mice (both congenic on a C57BL/6 background; Jackson Laboratory) were vaccinated with 2/6-VLPs or administered EC\(_{wt}\). The vaccinated CD40\(^{−/−}\) or CD40L\(^{−/−}\) mice failed to develop fecal or serum antibody (data not shown) and had much lower levels of protection against rotavirus challenge at 6 weeks (Fig. 4) than the C57BL/6 mice (Fig. 3B) and the CD40\(^{+/+}\) and CD40L\(^{+/+}\) mice receiving the EC\(_{wt}\) infection (Fig. 4). In contrast, all infected mice developed serum and fecal antibody titers similar to those of TCRKO and CD4\(^{+/+}\) mice, which were significantly reduced compared to those of the wild-type mice (data not shown), and were completely protected after 6 weeks (Fig. 3B and 4). These data indicate that CD40/CD40L signaling is an important component of nonreplicating-vaccine-induced protective immunity to rotavirus infection. Although CD40/
CD40L plays an essential role in antibody production, the interaction also mediates antigen-presenting cell activation and influences CD4 or CD8 cellular immunity (22). More studies are necessary to determine whether one or both of the CD40/CD40L signaling functions are critical to VLP-induced immunity.

The contribution of T cells to rotavirus protective immunity. Clearly, subunit vaccines induce protection that requires CD4⁺ T cells. Neither the VLPs or inactivated virus examined above nor a soluble VP6 vaccine (16) induce protection in the absence of CD4⁺ T cells. In contrast, CD4⁺ T cells contribute little to short-term protective immunity induced by replicating virus, which supports previous work indicating that rotavirus infection induces a strong T-cell-independent response (2, 10). However, after examination of the maintenance of protection in the absence of T cells, we find that T cells support and enhance long-term immunity against rotavirus infection. Interestingly, although the lack of T cells causes a decrease in protective immunity, substantial protective immunity is still present after 6 months, indicating that T-cell-independent immunity persists longer than anticipated. Therefore, rotavirus immunity is induced through both T-cell-dependent and -independent pathways. One caveat to examining the effect that lymphocyte subsets have on the induction of rotavirus protective immunity is the influence of the genetic background on the mutation phenotype. The ability of SCID mice to clear rotavirus infection differs based on whether the defect is present in BALB/c or C57BL/6 mice (10). Our own work has indicated significant differences between the two strains in susceptibility to rotavirus infection, as well as in protection, induced by VLPs (3). More studies are necessary to tease out the specific features that are unique to the inbred mouse strains that influence rotavirus immunity.

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