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**Recommended Citation**

Hersperger, Adam R; Siciliano, Nicholas A; DeHaven, Brian C; Snook, Adam E.; and Eisenlohr, Laurence C., "Epithelial Immunization Induces Polyfunctional CD8+ T Cells and Optimal Mousepox Protection." (2014). *Department of Microbiology and Immunology Faculty Papers*. Paper 65.  
https://jdc.jefferson.edu/mifp/65

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Published Ahead of Print 4 June 2014.


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Epithelial Immunization Induces Polyfunctional CD8⁺ T Cells and Optimal Mousepox Protection

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We assessed several routes of immunization with vaccinia virus (VACV) in protecting mice against ectromelia virus (ECTV). By a wide margin, skin scarification provided the greatest protection. Humoral immunity and resident-memory T cells notwithstanding, several approaches revealed that circulating, memory CD8⁺ T cells primed via scarification were functionally superior and conferred enhanced virus control. Immunization via the epithelial route warrants further investigation, as it may also provide enhanced defense against other infectious agents.

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nalogous to the protection of humans against smallpox, vaccinia virus (VACV) protects mice against ectromelia virus (ECTV; “mousepox”) (1–4). In this study, we examined whether the route of immunization with VACV (strain Western Reserve; 1 × 10⁶ PFU dose) influences the generation of protective immunity in BALB/c mice (females, 6 to 8 weeks old). The following vaccination routes were chosen: skin scarification (s.s.), intraperitoneal (i.p.) inoculation, or subcutaneous (s.c.) injection.

Recent studies have shown that epithelial infection elicits skin resident-memory T cells (TRM) that are highly effective at controlling a homologous, cutaneous virus challenge (5–7). Consequently, we hypothesized that the s.s. vaccination group (s.s. mice) would most effectively control a dermal (i.e., footpad [f.p.]) ECTV (Moscow strain) challenge given 30 days postimmunization. In contrast to the other groups, s.s. mice showed no signs of morbidity (Fig. 1A) or mortality (Fig. 1B) following a footpad infection with high-dose ECTV (1 × 10⁵ PFU).

To determine whether s.s. protects against ECTV infection via a heterologous route, we challenged groups of vaccinated mice (on day 30 postimmunization) via the intranasal (i.n.) route with the same dose of ECTV as described above. Although all groups experienced signs of morbidity, weight loss was significantly less severe in the s.s. group (Fig. 1C). All s.s. mice survived the i.n. challenge, but 10% of i.p. mice and 30% of s.c. mice did not (Fig. 1D). Notably, the s.s. group displayed the lowest virus titers in multiple organs at day 7 postchallenge (Fig. 1E). Additionally, none of the s.s. mice developed pox lesions, whereas some surviving animals in the i.p. and s.c. groups developed lesions on the tail or limbs (data not shown).

In general, s.s. mice were the only group of vaccinated animals in our study that failed to develop pox lesions, regardless of the route of ECTV challenge. These observations are consistent with previous reports on monkeypox infection of nonhuman primates (8–10) in which no pox lesions were observed on animals inoculated with Dryvax (Wyeth) smallpox vaccine administered by scarification. However, lesions did materialize in the context of other vaccination protocols, such as intramuscular (i.m.) injection of modified vaccinia virus Ankara (MVA) (8), which did not employ an epithelial route. Therefore, it is plausible that skin TRM, which are generated by scarification but not i.m. injection, help to prevent the appearance of lesions, which occur as a consequence of virus replication in the skin (11).

To explore the protective mechanisms provided by s.s. immunization, we assessed adaptive immune responses within each group. First, we measured VACV-specific antibody levels in each vaccination group at day 30 postimmunization. As shown in Fig. 2, vaccination via the i.p. route resulted in the greatest level of circulating antibody. Interestingly, it has been previously concluded that antibody is the sole correlate of protective immunity against secondary poxvirus challenge (10, 12–14). Given this precedent, we were surprised to observe that s.s. mice had significantly lower levels of circulating antibodies than i.p. mice. This apparent divergence from past studies (10, 12, 13) may be due to differences in dose or route of challenge. For example, it is possible that antibodies by themselves are sufficient after low-dose challenge with ECTV (12, 13), but T-cell responses become more critical as the amount of challenge inoculum increases.

To evaluate poxvirus-specific CD8⁺ T-cell (TCD8⁺) responses, we used a pool of previously identified VACV class I epitopes (15) and measured five functional parameters (CD107a, gamma interferon [IFN-γ], interleukin 2 [IL-2], MIP1α, and tumor necrosis factor alpha [TNF-α]) by using intracellular cytokine staining (ICS) assays. These ICS assays were carried out as previously described (16, 17), and anti-CD107a was included at the start of all stimulations to measure levels of degranulation (18). Our gating strategy and Boolean analysis were similar to those employed previously (16).

At day 7 postimmunization, we identified VACV-specific TCD8⁺ in the spleen and blood of all groups. Despite similar magnitudes of total response between groups (Fig. 3A), VACV-specific TCD8⁺ from s.s. mice displayed an enhanced polyfunctional profile (Fig. 3B) and higher IFN-γ expression on a per-cell basis (Fig. 3C). The two permutations that contributed most to the observed differences were cells positive for all five functions and those positive for all measured parameters except IL-2 (Fig. 3D). There was also significantly higher coexpression of TNF-α with
IFN-γ among VACV-specific T<sub>CD8</sub> from s.s. mice in both the spleen and blood (Fig. 3E). The higher multifunctional nature of T<sub>CD8</sub> from s.s. mice was maintained into the memory phase (at day 30) and also during a secondary recall response (Fig. 3F).

Prior work has demonstrated a correlation between control of HIV replication and the presence of polyfunctional T<sub>CD8</sub> (17, 19–21). Since T<sub>CD8</sub> primed by scarification displayed higher functionality, we hypothesized that T<sub>CD8</sub> from s.s. mice would confer greater control of ECTV in vivo. To test this, we purified total splenic T<sub>CD8</sub> from vaccinated mice (n = 5, pooled), transferred 5 × 10<sup>6</sup> cells (22) into naive mice, and subsequently infected them with ECTV (3 × 10<sup>4</sup> PFU; f.p. route). Stimulation of cells using the major histocompatibility complex (MHC) class I peptide pool revealed that equivalent numbers of VACV-specific T<sub>CD8</sub> were transferred across all groups (data not shown). As shown in Fig. 3G, mice that received T<sub>CD8</sub> primed by s.s. achieved the highest degree of control over ECTV, demonstrating a direct relationship between virus control and the functional capacity of antiviral T<sub>CD8</sub>.

**FIG 1** Scarification of VACV elicits optimal control of ECTV regardless of challenge route. (A and B) Groups of naive or vaccinated mice (5 per group) were challenged with 1 × 10<sup>5</sup> PFU of ECTV in the left hind footpad and subsequently monitored for weight loss (A) and mortality (B). (C and D) Groups of naive or vaccinated mice (10 per group) were challenged with 1 × 10<sup>5</sup> PFU of ECTV via the i.n. route and subsequently monitored for weight loss (C) and mortality (D). (E) Separate cohorts of vaccinated mice (5 per group) were infected with 1 × 10<sup>5</sup> PFU of ECTV via the i.n. route. On day 7 postchallenge, the indicated organs were isolated and levels of ECTV were quantified using standard plaque assays. These data are representative of two independent experiments. UND, undetectable. *, P value < 0.05; **, P value < 0.01. Statistical analysis was performed using GraphPad Prism. Error bars represent the mean and standard error of the mean.

(A to E) For i.p. and s.c. injections, the virus inoculum was given in a total volume of 100 µl of 1× PBS. Scarification was performed at the base of the tail using a 27-gauge needle and a 10-µl drop of VACV in 1× PBS.

**FIG 2** Immunization via the i.p. route yields the highest levels of circulating antipoxvirus antibodies. Plasma was isolated by retro-orbital bleeding from mice that had been immunized with VACV 30 days earlier via the indicated routes. Levels of circulating antibodies were quantified using plates coated with VACV at 1 × 10<sup>6</sup> PFU per well. Antibody titers were determined by calculating the 50% effective concentration (EC<sub>50</sub>) using nonlinear regression in GraphPad Prism (version 5.0a). Values calculated above or below the dilution range were set to 1/30 and 1/10,000, respectively. ***, P value = 0.001. The solid black lines represent the mean value within each group. Statistical analysis was performed using GraphPad Prism. Each data point represents one individual mouse. Black data points are from one individual experiment, and blue data points are from a second independent trial.

IFN-γ among VACV-specific T<sub>CD8</sub> from s.s. mice in both the spleen and blood (Fig. 3E). The higher multifunctional nature of T<sub>CD8</sub> from s.s. mice was maintained into the memory phase (at day 30) and also during a secondary recall response (Fig. 3F).

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The ability of s.s. vaccination to generate skin TRM cells has been of recent interest. As expected, we found in this study that mice vaccinated by scarification of VACV, which induces poxvirus-specific TRM cells in the skin (5, 7, 23), most effectively controlled a challenge dose of ECTV given via cutaneous inoculation. However, perhaps less predictably, s.s. mice also demonstrated a better outcome following heterologous i.n. challenge and most effectively controlled ECTV in the lungs. Therefore, it appears that circulating T-cell responses elicited by epithelial infection deserve consideration in addition to skin-resident populations. Since this study employed a somewhat virulent strain of VACV, future work will determine if these results hold true in the context of attenuated vaccine strains, such as MVA or Lister.

In summary, we found that in comparison with other routes, epithelial inoculation generated circulating TCD8+ with superior ability to secrete multiple cytokines and better control over ECTV replication in vivo. Importantly, antibody levels alone did not dictate the degree of protection or correlate with full virus control. Instead, it appears that both antibodies and TCD8+ cooperate to bring about optimal control of a high-dose ECTV challenge. These findings point toward the importance of investigating the protective effects of scarification in the context of other pathogens.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant U19AI083008. This study includes work carried out within the Kimmel Cancer Center Flow Cytometry Facility, which is supported in part by NCI Cancer Center support grant P30 CA56036. MHC class I peptides for TCD8+ stimulation were obtained through the NIH Biodefense and Emerging Infections Research Resources Repository (H-2 Peptide Arrays, Epitopes of Vaccinia Virus Proteins, NR-4058).
REFERENCES


