Access of protective antiviral antibody to neuronal tissues requires CD4 T-cell help

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Circulating antibodies can access most tissues to mediate surveillance and elimination of invading pathogens. Immunoprivileged tissues such as the brain and the peripheral nervous system are shielded from plasma proteins by the blood–brain barrier1 and blood–nerve barrier2, respectively. Yet, circulating antibodies must somehow gain access to these tissues to mediate their antimicrobial functions. Here we examine the mechanism by which antibodies gain access to neuronal tissues to control infection. Using a mouse model of genital herpes infection, we demonstrate that both antibodies and CD4 T cells are required to protect the host after immunization at a distal site. We show that memory CD4 T cells migrate to the dorsal root ganglia and spinal cord in response to infection with herpes simplex virus type 2. Once inside these neuronal tissues, CD4 T cells secrete interferon-γ and mediate local increase in vascular permeability, enabling antibody access for viral control. A similar requirement for CD4 T cells for antibody access to the brain is observed after intranasal challenge with vesicular stomatitis virus. Our results reveal a previously unappreciated role of CD4 T cells in mobilizing antibodies to the peripheral sites of infection where they help to limit viral spread.

To investigate the mechanism of antibody-mediated protection within the barrier-protected tissues, we used a mouse model of genital herpes infection. Herpes simplex virus type 2 (HSV-2) enters the host through the mucosal epithelia, and infects the innervating neurons in the dorsal root ganglia (DRG) to establish latency3,4. Vaginal immunization by an attenuated HSV-2 with deletion of the thymidine kinase gene (TK− HSV-2) provides complete protection from lethal disease following genital challenge with wild-type (WT) HSV-2 (ref. 5) by establishing tissue-resident memory T cells (TRM)6. In vaginally immunized mice, interferon (IFN)-γ secretion by CD4 T cells, but not antibodies, are required for protection7,8. In contrast, distal immunization with the same virus fails to establish TRM and provides only partial protection6. Nevertheless, of the distal immunization routes tested, intranasal immunization with TK− HSV-2 provided the most robust protection against intravaginal challenge with WT HSV-2, whereas intraperitoneal immunization provided the least protection9,10. As shown previously9, intranasal immunization did not establish TRM in the genital mucosa (Extended Data Fig. 1a, b), despite generating a comparable circulating memory T-cell pool (Extended Data Fig. 1c, d). After vaginal HSV-2 challenge, mice that were immunized intranasally with TK− HSV-2 were unable to control viral replication within the vaginal

Figure 1 | Intranasal immunization confers B-cell-dependent neuron protection following genital HSV-2 challenge. a–d, C57BL/6 mice were immunized with TK− HSV-2 (105 plaque-forming units (p.f.u.)) via intranasal (i.n.; n = 12), intraperitoneal (i.p.; n = 5) or intravaginal (i.vag.; n = 11) routes. Five to six weeks later, these mice and naive mice (n = 4) were challenged with a lethal dose of WT HSV-2 (104 p.f.u.). Mortality (a), clinical score (b) and virus titre in vaginal wash (c) were measured on indicated days after challenge. d, Six days after challenge, virus titre in tissue homogenates including DRG and spinal cord was measured.

e–g, BALB/c mice (n = 10) or B-cell-deficient JH D mice (n = 6) were immunized intranasally with TK− HSV-2 (5 × 105 p.f.u.). Six weeks later, these mice and naive mice (n = 4) were challenged with lethal WT HSV-2 (105 p.f.u.). Mortality (e) and clinical score (f) were measured. g, Six days after challenge, virus titre in tissue homogenates including DRG and spinal cord was measured by plaque assay. Data are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (two-tailed unpaired Student’s t-test).
mucosa (Fig. 1c), but had significantly reduced viral replication in the innervating neurons of the DRG (Fig. 1d). Notably, we found that protection conferred by intranasal immunization required B cells, as IgD mice (deficient in B cells) were not protected by intranasal immunization (Fig. 1e–g). In the absence of B cells, intranasal immunization was unable to control viral replication in the DRG and spinal cord (Fig. 1g).

In mice immunized intranasally with TK−HSV-2, no evidence of vaccine virus in the DRG or the spinal cord was found (Extended Data Fig. 1e). Moreover, the intranasal route of immunization was not unique in conferring protective response, as parabiotic mice sharing circulation with intravaginally immunized partners were also partly protected from vaginal challenge with WT HSV-2 in the absence of T<sub>10.6</sub> (Extended Data Fig. 1f–h). We found that the B cells in the immunized partners were required to confer protection in the naive conjoined mice, as partners of immunized μMT mice were unprotected (Extended Data Fig. 1f–h). Moreover, antigen-specific B cells were required to confer protection, as intravaginally immunized partners whose B cells bore an irrelevant B cell receptor (against hen egg lysozyme (HEL)) were unable to confer protection in the conjoined naive partner (Extended Data Fig. 1f–h). As observed for the intranasal immunization, viral control conferred by the immunized parabiotic partner was not observed in the vaginal mucosa (Extended Data Fig. 1h), suggesting that protection occurs in the innervating neurons.

Next, we investigated the basis for superior protection by antibodies following different routes of immunization. Intravaginal, intranasal and intraperitoneal routes of immunization with TK−HSV-2 resulted in comparable circulating CD4 T-cell memory responses<sup>6</sup>. While no differences were seen for other isotypes, the intranasal and intravaginal routes of immunization were superior to intraperitoneal route in generating higher levels of systemic HSV-2–specific immunoglobulin-G (IgG)2b and IgG2c responses (Extended Data Fig. 2). These results indicated that higher levels of circulating virus-specific IgG2b and IgG2c correlate with protection against vaginal HSV-2 challenge.

We next examined how antibody access to the DRG and spinal cord is mediated. Even though the peripheral nervous tissues are protected from antibody diffusion through the blood–nerve barrier, it was formally possible that secretion of antibody into the tissue occurs through transport of serum antibody by the neonatal Fc receptor for IgG (FcRn)<sup>11</sup> expressed on the endothelial cells within the infected tissues. However, we found that mice deficient in FcRn immunized intranasally with TK−HSV-2 were equally protected as the WT counterpart from vaginal HSV-2 infection (Fig. 2a, b). Thus, circulating HSV-2–specific antibodies are somehow mobilized to the neuronal tissues following local viral infection in an FcRn-independent manner, and are required for protection of the host.

If circulating antibodies are sufficient, passive transfer of HSV-2–specific antibodies alone should be able to protect the host. However, we and others<sup>12,13</sup> have found that intravenous injection of HSV-2–specific antibodies alone fails to protect naive mice against HSV-2 challenge (Fig. 2c, d). In contrast, consistent with a previous study<sup>13</sup>, we found that B-cell-deficient μMT mice immunized intranasally with TK−HSV-2 and given systemic administration of HSV-2–specific anti-serum were protected (Fig. 2c, d). Thus, these results demonstrate that it is the secreted antibodies, and not B cells themselves, in concert with non-B-cell immune cells, probably T cells induced by immunization, that seem to be required for protection. To test this possibility, we depleted CD4 T cells from mice previously immunized intranasally just before intravaginal HSV-2 challenge. In this setting, differentiation of B cells and antibody responses were allowed to occur fully in the presence of CD4 T-cell help for 6 weeks. Mice acutely depleted of CD4 T cells succumbed to challenge with HSV-2 (Fig. 2e, f), whereas depletion of CD8 T cells and natural killer (NK) cells had no effect<sup>9</sup>. Moreover, neutralization of IFN-γ before challenge, or genetic deficiency in IFN-γR, also rendered intranasally immunized mice more susceptible to intravaginal HSV-2 challenge (Fig. 2e, f). Of note, depletion of CD4 T cells from intranasally immunized mice just before the
viral challenge rendered mice incapable of viral control in the DRG, to a similar extent as the immunized B-cell-deficient μMT mice (Fig. 2g). We observed that intranasal immunization conferred near-complete protection from HSV-2 in the DRG but variable protection in the spinal cord (Figs 1d and 2g). Because HSV-2 can differentially seed the DRG and spinal cord through sensory neurons and autonomic neurons, these data suggest that the efficacy of antibody-mediated protection may depend on the route of viral entry. Further, these results indicate that circulating antibodies, CD4 T cells and IFN-γ collectively mediate neuroprotection against HSV-2.

Given that antibody-mediated protection occurs at the level of the innervating neurons and not within the vagina (Fig. 1c and Extended Data Fig. 1h), we hypothesized that CD4 T cells might control delivery of antibodies to the tissue parenchyma through secretion of IFN-γ. We detected only low levels of virus-specific and total antibodies in the DRG or spinal cord at steady state in immunized mice (Fig. 3; WT/ intranasally → D0), and undetectable levels of antibodies in these tissues in previously unimmunized mice 6 days after an acute infection with HSV-2 (Fig. 3; WT/ naive → D6). However, in mice immunized intranasally with TK− HSV-2 6 weeks earlier, increase in the levels of antibodies was detected 6 days after intravaginal HSV-2 challenge within the DRG and in the spinal cord (Fig. 3; WT/ intranasally → D6). Moreover, CD4 T cells were required for access of virus-specific antibodies to the restricted tissue such as the DRG, as depletion of CD4 T cells completely diminished antibody levels in this tissue and spinal cord (Fig. 3d; WT/ intranasally + anti-CD4 → D6). Further, similar requirement for CD4 T cells (Fig. 3b, d) and IFN-γ (Extended Data Fig. 3) was found for diffusion of total IgG2b and IgG2c isotypes into the DRG, indicating that the delivery mechanism does not discriminate virus-specificity of the antibodies. In contrast to the neuronal tissues, acute depletion of CD4 or IFN-γ blockade once antibody responses were established had no significant impact on the serum levels of anti-HSV-2 or total antibodies (Extended Data Fig. 4a, b).

To examine whether antigen-specific memory CD4 T cells were required to mediate antibody access to the neuronal tissues, mice were primed intranasally with an unrelated virus, influenza A virus, and, 4 weeks later, were challenged with HSV-2 intravaginally. In contrast to mice harbouring cognate memory CD4 T cells, antibody access to neuronal tissues following intravaginal HSV-2 challenge was not observed in mice that had irrelevant memory CD4 T cells (against influenza A virus) (Extended Data Fig. 5). These data indicate that antigen-specific memory CD4 T cells are required for antibody access to the neuronal tissues.

We hypothesized that memory CD4 T cell might enter the barrier-protected tissues and mobilize antibody access through local secretion of IFN-γ. In support of this idea, we found that IFN-γ-secreting HSV-2-specific CD4 T cells entered the DRG and spinal cord around 6 days after genital HSV-2 challenge in mice that received intranasal immunization 6 weeks previously (Fig. 4a, b; WT/ intranasally → D6). Some increase in innate leukocytes bearing CD11b, Ly6G or MHCI was observed in DRG and spinal cord 6 days after challenge (Extended Data Fig. 6a). IFN-γ secretion was confined to the memory CD4 T-cell population within the DRG (Fig. 4a). Moreover, entry of effector CD4 T cells to the DRG and spinal cord at 6 days after primary vaginal HSV-2 infection was much less efficient than their memory counterpart (Fig. 4a, b; WT/ naive → D6), suggesting the intrinsic ability of T cells to migrate into these neuronal tissues is enhanced with memory development.

**Figure 3 | Memory CD4+ T cells are required for antibody access to neuronal tissues.** a–d, Naive WT mice or WT and μMT mice intranasally immunized with TK− HSV-2 (105 p.f.u.) 6 weeks earlier were challenged with a lethal dose of WT HSV-2 intravaginally. Six days after the challenge, after extensive perfusion, HSV-2-specific (a, c) and total Ig (b, d) levels in tissue homogenates of DRG and spinal cord were analysed by ELISA. To deplete CD4 T cells, CD4-specific antibody was injected on days −4, −1, 2 and 4 days after challenge. Data are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (two-tailed unpaired Student’s t-test).
Figure 4 | α4-integrin-dependent recruitment of memory CD4+ T cells is required for antibody access to neuronal tissues. WT mice immunized intranasally with TK−HSV-2 6 weeks earlier were challenged with a lethal dose of WT HSV-2. Neutralization of α4-integrin was performed on days 2 and 4 after challenge by intravenous injection of anti–α4 integrin (CD49d) antibody. a, Six days after challenge, after extensive perfusion, HSV-2-specific IFN-γ+ CD4+ T cells in DRG and spinal cord were detected by flow cytometry. b, The number of IFN-γ-secreting CD4+ T cells among 50,000 cells of CD45+ leukocytes in DRG and spinal cord is depicted. Data are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (two-tailed unpaired Student’s t-test). c, Frozen sections of DRG were stained with antibodies against CD4, VCAM-1 or CD31. Nuclei are depicted by 4',6-diamidino-2-phenylindole (DAPI) stain (blue). Images were captured using a ×10 or ×40 objective lens. Scale bars, 100 μm. Arrowhead indicates VCAM-1+ cells in parenchyma of DRG. Data are representative of at least three similar experiments. HSV-2-specific antibodies in the DRG (d) and spinal cord (e) were analysed by ELISA. Data are mean ± s.e.m. *P < 0.05 (two-tailed paired Student’s t-test). Albumin level in tissue homogenates was analysed by ELISA (f). Depletion of CD4 T cells or neutralization of IFN-γ was performed on days −4, −1, 2 and 4 days after challenge by intravenous injection of anti-CD4 (GK1.5) or anti-IFN-γ (XMG1.2), respectively. Data are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (two-tailed paired Student’s t-test).

Interaction of α4β1 (or VLA4) and VCAM-1 contributes to T-cell migration into the neuronal tissues or neutralization of IFN-γ is required for antibody access to the DRG and spinal cord. We found that the entry of memory CD4 T cells into the nervous tissue was strictly dependent on α4 subunit5. Memory CD4 T cells generated against HSV-2 expresses CD49d which is the integrin α4 subunit6. We found that the entry of memory CD4 T cells into the nervous tissue was strictly dependent on α4 integrin, as antibody blockade of α4 prevented their entry into the DRG and spinal cord (Fig. 4a, b). We observed expression of ligand for α4β1, VCAM-1, in the endothelium of DRG and spinal cord in immune-challenged mice (Fig. 4c and Extended Data Fig. 6b). Further, analysis of tissue sections revealed that the CD4 T cells were found in the parenchyma of the DRG and spinal cord, as well as within their epineurium and meninges, but not within the vasculature (Fig. 4c and Extended Data Fig. 6a, b). Notably, many CD4 T cells were found adjacent to the cell body of neurons within the DRG. Some VCAM-1 staining was found in the cytosol of neuronal cell bodies (arrowhead Fig. 4c). Additionally, intravascular staining15 with antibody to CD90.2 revealed that the vast majority of the CD4 T cells in the DRG and spinal cord are sequestered from circulation (Extended Data Fig. 7a, b). Thus, CD4 T cells recruited to the neuronal tissues access the parenchyma of the DRG and spinal cord. Notably, α4 integrin blockade of CD4 T-cell recruitment resulted in diminished access of virus-specific antibody to the DRG and spinal cord (Fig. 4d, e), with no effect on blood levels of virus-specific antibody (Extended Data Fig. 4c) or the total antibody levels of various isotypes in circulation (Extended Data Fig. 4d). Collectively, these data indicate that memory CD4 T cells enter the neuronal tissue and secrete IFN-γ to promote antibody access to the DRG and spinal cord.

How might IFN-γ secreted by CD4 T cells enable circulating antibody to access the neuronal tissues? It is well known that IFN-γ acts on the endothelial cells to remodel tight junctions and increase permeability16. We observed that recombinant IFN-γ injected intravaginally was sufficient to enable antibody access to the vaginal lumen, suggesting that IFN-γ is sufficient to induce both vascular and epithelial permeability in peripheral tissues (Extended Data Fig. 8a) and to enhance VCAM-1 expression on endothelial cells (Extended Data Fig. 8b). To assess whether antibody access to the neuronal tissues mediated by CD4 T cells and IFN-γ is through increased vascular permeability, we measured release of blood albumin into the neuronal tissue following genital HSV-2 challenge in intranasally immunized mice. Notably, we observed that vascular permeability occurred in the DRG and spinal cord in a CD4 T-cell- and IFN-γ-dependent manner, as measured by leakage of blood albumin to the neuronal tissues by ELISA and immunohistochemical analysis (Fig. 4f and Extended Data Fig. 9a). We confirmed CD4-dependent vascular permeability to the DRG and the spinal cord using intravenous injection of 70kDa fluorescein isothiocyanate (FITC)-dextran, which has a similar size to IgG (Extended Data Fig. 9b). Collectively, our results support the notion that CD4 T cells enable antibody delivery to the sites of infection by secreting IFN-γ and enhancing microvascular permeability. This mechanism of antibody delivery is crucial for host immune protection, as depletion of CD4 T cells, inhibition of CD4 T-cell migration into the neuronal tissues or neutralization of IFN-γ renders immune mice susceptible to infection.

To determine whether our findings extend beyond HSV-2, we examined antibody access to the neuronal tissue following a different neurotropic virus, vesicular stomatitis virus (VSV), a negative sense RNA virus of the Rhabdoviridae family. Upon intranasal inoculation, VSV infects olfactory sensory neurons in the nasal mucosa and enters the CNS through the olfactory bulb17. In contrast, intravenous infection with VSV is well tolerated, and generates robust T- and B-cell responses (Extended Data Fig. 10)18. To determine whether antibody access to the brain requires memory CD4 T cells, we immunized mice with VSV intravenously. Five weeks later, immunized mice were challenged with VSV intranasally. Entry of VSV-specific antibodies was monitored in the brain 6 days after intranasal challenge. Consistent with the data obtained from HSV-2 infection, we observed a striking dependence on CD4 T cells of antibody access to the brain (Extended Data Fig. 10b). Further, anti-α4 antibody treatment of mice immediately before intranasal VSV challenge also diminished antibody access to the brain,
without impacting VSV-specific antibodies in circulation (Extended Data Fig. 10c). Furthermore, we observed that vascular permeability to the brain was dependent on α4 integrin, as antibody blockade of α4 integrin resulted in diminished albumin leakage to the brain (Extended Data Fig. 10d). Taken together, these results indicate that the requirement for α4-integrin and memory CD4 T cells for antibody access applies to two distinct neurotropic viruses, HSV-2 and VSV, and suggest a general mechanism of antibody access to the immunoprivileged tissues protected by the blood–nerve barriers.

We have demonstrated a role of CD4 T cells in controlling antibody access to neuronal tissues through local migration and secretion of IFN-γ. Circulating CD4 memory T cells effectively target antibody delivery to the sites of infection through their secretion of IFN-γ, presumably upon recognition of cognate antigenic peptides presented by local antigen-presenting cells.20 These results indicate the requirement for CD4 T-cell help at the effector phase of the antibody response, and add to the growing appreciation of CD4 T cells in paving the way to other effector cell types such as CD8 T cells.21–23 We believe that the requirement for CD4 T cells for antibody access in neuronal tissue reflects an additional layer of control imposed by the immunoprivileged sites. In accessible tissues, inflammatory leukocytes can migrate and, in response to PAMPs, secrete cytokines such as TNF-α that are sufficient to trigger vascular permeability independently of CD4 T cells. However, after neurotropic viral infections, the infected neurons are expected to be poor at producing inflammatory cytokines that remodel vascular tight junctions. At the same time, recruitment of innate leukocytes is blocked by shutdown of specific chemokines in the ganglia of HSV-1-infected mice.24 Curiously, expression of T-cell-trophic chemokines CXCL9 and CXCL10 was preserved in the DRG of infected mice,24 suggesting that access by lymphocytes is permitted. Thus, in neuronal tissues, the entry of viral-specific CD4 T cells is crucial to provide cytokines that permit antibodies through the induction of vascular permeability.

On the other hand, aberrant entry and activation of CD4 T cells predispose immunoprivileged tissues for access to autoantibodies and tissue damage.25,26 Thus, this mode of targeted release of circulating antibodies not only provides a rapid and efficient mechanism of pathogen control, but may also restrict antibody release to irrelevant sites to limit immunopathology. Our results implicate that antibody-based vaccines or treatment against neurotropic viruses would benefit from generating robust circulating CD4 T-cell memory responses. Conversely, treatment of autoantibody-mediated neuropathies including chronic inflammatory demyelinating polynuropathy and Guillain–Barré syndrome might benefit from preventing the accessibility of autoantibodies to target neurons enabled by CD4 T cells.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.I. (akiko.iwasaki@yale.edu).
METHODS

Mice. Six- to eight-week-old female C57BL/6 (CD45.2+) and congenic C57BL/6 B6.SJL-PtprcaPep3bBoyJ (B6.SJL-Pep3b) mice, B6.129-Ifgμ−/−(Thy1.1) B6.129 Swiss-2Hg-Ifgμ−/−(Thy1.1) B6.129X1-FcγR1−/−(Dcr1) (FcRn−/−) mice were purchased from the National Cancer Institute and Jackson Laboratory. J1D mice (B-cell deficient on BALB/c background) were obtained from Taconic Animal Models. All procedures used in this study complied with federal guidelines and institutional policies by the Yale School of Medicine Animal Care and Use Committee.

Viruses. HSV-2 strains 186yn−: TK− and 186yn− were gifts from D. Knipe. These viruses were propagated and titrated on Vero cells (ATCC CCL-81) as previously described20. Influenza virus A/Puerto Rico/3334 (A/PR8: H1N1) and WT/HSV were propagated as previously described21,22.

Virus infection. Six- to eight-week-old female mice injected subcutaneously with Depo Provera (Pharmacia Upjohn, 2 mg per mouse) were immunized intravaginally, intraperitoneally or intranasally with 10^6 p.f.u. of HSV-2 (186yn− TK−) as previously described20. For secondary challenge, immunized mice were challenged vaginally with 10^6 p.f.u. of WT HSV-2 (186yn− TK−) (100% lethal dose for naïve mice). In the case of BALB/c and J1D mice, these mice were immunized with 5 × 10^6 to 10^7 p.f.u. of HSV-2. For secondary challenge, immunized mice were challenged with 10^6 p.f.u. of WT HSV-2 (100% lethal dose for naïve mice). The severity of disease was scored as follows: 0, no sign; 1, slight genital erythema and oedema; 2, moderate genital inflammation; 3, purulent genital lesions; 4, hind-limb paralysis; 5, pre-moribund. Owing to humane concerns, the animals were euthanized before reaching moribund state. To measure virus titre in peripheral tissues, vaginal tissues, DRG and spinal cord were harvested in ABC buffer (0.5 mM MgCl_2·6H_2O, 1% glucose, 5% HI FBS and penicillin–streptomycin) including 1% amphotericin B (Sigma). Thereafter, these tissues were homogenized by lysing matrix D (MP Biomedicals), followed by clarification by centrifugation. Viral titres were obtained by titration of tissue samples on a Vero cell monolayer. Protein concentration in tissue homogenates was measured by a DC protein assay kit (Bio-Rad Laboratories). C57BL/6 mice were intravenously inoculated with WT/HSV (2 × 10^6 p.f.u. per mouse) or intranasally with influenza A/PR8 (10^6 p.f.u. per mouse). For secondary challenge, HSV-immunized mice were re-infected intranasally with WT/HSV (1 × 10^6 p.f.u. per mouse).

Antibodies. Anti-Cd90.2 (30-H12), anti-Cd90.1 (OX-7), anti-Cd45.2 (104), anti-Cd45.1 (A20), anti-Cd4 (GK1.5, RM4.5 and RM4-4), anti-Cd19 (6D3), anti-Cd45R/B220 (RA3-6B2), anti-Mhc class II (I-A/I-E, M5/114.15.2), anti-Cd69 (H1.2F3), anti-Cd44 (IM7), anti-Cd49 (R1-2), anti-Nk4 (29A1.4) and anti-Ifnγ (XM1.2 and R4-6A2) were purchased from e-Bioscience or Bioclegen.

Isolation of leucocytes from peripheral tissues. The genital tracts of vaginal tissues treated with Depo-Provera were dissected from the urethra and cervix. Before collection of neuronal tissues, mice were perfused extensively using transcardiac perfusion and perfusion through inferior vena cava and great saphenous vein with more than 30 ml of PBS. The DRG and the adjacent region of the spinal cord were harvested in PBS for flow cytometry or ABC buffer for tissue homogenization. The tissues in PBS were then incubated with 0.5 mg/ml 1% Proteinase K (Roche) for 15 min at 37°C. Thereafter, vaginal tissues were digested with 1 mg/ml collagenase D (Roche) and 30 μg/ml DNase I (Sigma-Aldrich) at 37°C for 25 min. The resulting cells were filtered through a 70-μm filter.23,24

Flow cytometry. Preparation of single-cell suspensions from spleen, draining lymph node (inguinal lymph node and iliac lymph nodes), vagina and neuronal tissues were described previously. Multiparameter analyses were performed on an LSR II flow cytometer (Becton Dickinson) and analysed using FlowJo software.

In vivo treatment with neutralizing/depleting antibodies. C57BL/6 mice or BALB/c mice were immunized with TK− HSV-2 virus. Five to eight weeks later, these mice were injected intravenously (tail vain) with 300 μg of anti-Cd4 (GK1.5, BioXcell), anti-Ifnγ (XM1.2, BioXcell) antibody at days 4, 2, 4 after HSV-2 challenge. In vivo depletion for CD4 was confirmed by fluorescence-activated cell sorting analysis of the cell suspension from spleen. For the neutralization of α4-integrin, purified anti-mouse α4 integrin/CD49d (PS2; SouthernBiotech) was given a tail vain injection of 300 μg antibody at days 2 and 4 after challenge.

Parabiosis. Parabiosis was performed as previously described with slight modifications25. Naive or immunized C57BL/6 mice, HELTg and μMT mice were anaesthetized with a mixture of ketamine/xylazine (100 mg/kg and 10 mg/kg body weight respectively). After shaving the corresponding lateral aspects of each mouse, matching skin incisions were made from behind the ear to hip and sutured together with Chromic Gut (4-0, Ethicon). With a 7-mm stainless steel wound clips (Roboz). Measurement of virus-specific Ig and total Ig in serum and tissue homogenates. Ninety-six-well ELISA plates were filled with 100 μl of heat-inactivated purified HSV-2 (10^6–10^7 p.f.u. equivalent per 100 μl) or heat-inactivated purified VSV (5 × 10^6 p.f.u. equivalent per 100 μl) for virus-specific Ig measurement or goat anti-mouse Ig (1:10,000; SouthernBiotech, 1010-01) for total Ig measurement in carbonate buffer (pH 9.5) and then incubated overnight at 4°C. On the following day, these plates were washed with PBS-Tween 20 and blocked for 2 h with 5% PBS in PBS. Tissue samples and serum samples in ABC buffer were then plated in the wells and incubated for at least 4 h at ambient temperature (20–25°C). After washing in PBS-Tween 20, HRP-conjugated anti-mouse IgG1, IgG2, IgM, IgA, IgG2a, IgG2b or IgG2c (SouthernBiotech) was added to the wells for 1 h, followed by washing and adding TMB solution (e Bioscience). Reactions were stopped with 1 N H_2SO_4 and absorbance was measured at 450 nm. The sample antibody titres were defined by using Ig standard (C57BL/6 Mouse Immunoglobulin Panel; SouthernBiotech) or mouse IgG2a (HOPC-1; SouthernBiotech).

Albumin ELISA. Using tissue homogenates (DRG and spinal cord) prepared after extensive perfusion, albumin ELISA (Genway) was performed according to the instruction.

Immunofluorescence staining. Frozen sections (8 μm in thickness) were cut, fixed and left to dry at ambient temperature. These tissues were stained with the antibodies (anti-Cd4 (H129.19), anti-MHC class II (M5/114.15.2 anti-VCAM-1 (429/MVCAM.A), anti-Cd31 (390 and MEC13.3), anti-Lyt-2 (IA/18), anti-Cd11b (M1/70) and anti-mouse albumin (Goat pAb/Bethyl Laboratories) as previously described26. These slides were washed and incubated with DAPI and mounted with Fluoromount-G (SouthernBiotech). They were analysed by fluorescence microscopy (BX51; Olympus).

Vascular permeability assays. Spinal column was harvested from intranasal TK− HSV-2-immunized mice 45 min after tail vein injection with 200 μl of 5 mg/ml Orange Green 488-conjugated dextran (70 kDa, D7173, Thermo Fisher Scientific) in PBS. Spine was then fixed with 4% paraformaldehyde in PBS overnight, and frozen sections cut (8 μm in thickness) for immunohistochemical analysis27. DNA isolation from tissues. C57BL/6 mice were immunized intranasally with TK− HSV-2. Six weeks later, vaginal tissues, DRG and spinal cord of these mice were lysed in 10 mg/ml Proteinase K (Roche) to isolate DNA at 55°C overnight. After removing these tubes, phenol equilibrated with Tris pH 8.0 was added. Thereafter, upper aqueous phase was added to phenol/chloroform (1:1). The upper aqueous phase was re-suspended with sodium acetate, pH 6.0, and 100% ethanol at room temperature. After washing and centrifuging, the concentration of isolated DNA pellet was measured. The level of HSV-2 genomic DNA in peripheral tissues on the basis of HSV-2 gpD (forward primer: AGGGAGGAATACCTGGGATT; reverse primer: GGCTGGAACCCCCGTAACT) was analysed by quantitative PCR using purified viral DNA genome as standard.

Statistical analysis. No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Survival curves were analysed using a log-rank test. For other data, normally distributed continuous variable comparisons used a two-tailed unpaired Student’s t-test or paired Student’s t-test with Prism software. To compare two non-parametric data sets, a Mann–Whitney U-test was used.

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Extended Data Figure 1 | In the absence of TREM, B cells are required for the protection of the host against genital HSV-2 challenge. a, C57BL/6 mice and μMT mice were immunized intravaginally or intranasally with TK− HSV-2. Five weeks later, vaginal tissue sections were stained for CD4+ cells (red) and MHC class II+ cells (green). Blue labelling depicts nuclear staining with DAPI (blue). Images were captured using a ×10 or ×40 objective lens. Scale bars, 100 μm. Data are representative of three similar experiments. b–d, BALB/c mice and JHD mice were immunized with TK− HSV-2 (10^5 p.f.u.) intranasally or intravaginally. Six weeks later, the number of total CD4+ T cells and HSV-2-specific IFN-γ+ CD4+ T cells in the vagina (b), spleen (c) and peripheral blood (d) were analysed by flow cytometry. Percentages and total number of IFN-γ+ cells among CD4+CD90.2+ cells are shown. Data are mean ± s.e.m. *P < 0.05; **P < 0.001; ***P < 0.001 (two-tailed unpaired Student’s t-test). e, C57BL/6 mice were immunized intravaginally (naive → D7) or intranasally (WT/i.n. → D0) with TK− HSV-2 virus. At the indicated time points (D7: 7 days after immunization; WT/i.n. → D0: 6 weeks after immunization), total viral genomic DNA in the vaginal tissues, DRG and spinal cord were measured by quantitative PCR. f–h, Intravaginally immunized C57BL/6 (WT), μMT and HEL-BCR Tg mice (left partner) were surgically joined with naive WT mice (right partner). Three weeks after parabiosis, the naive partner was challenged with a lethal dose of WT HSV-2 intravaginally. Mortality (e), clinical score (f) and virus titre in vaginal wash (g) following viral challenge are depicted.
Extended Data Figure 2 | Mucosal TK− HSV-2 immunization generates higher levels of virus-specific IgG2b and IgG2c compared with intraperitoneal immunization. WT mice were immunized with TK− HSV-2 (10^5 p.f.u. per mouse) via intravaginal, intraperitoneal or intranasal routes. Six weeks later, these mice were challenged with a lethal dose of WT HSV-2 intravaginally. At the indicated days after challenge, HSV-2-specific Ig (a) and total Ig (b) in serum were analysed by ELISA. Data are mean ± s.e.m. *P < 0.05 (Mann–Whitney U-test).
Extended Data Figure 3 | IFN-γ enhances antibody access to the DRG. WT mice immunized with TK− HSV-2 (10⁵ p.f.u. per mouse) intranasally 6 weeks earlier were challenged with a lethal dose of WT HSV-2 intravaginally. Six days after challenge, after extensive perfusion, HSV-2-specific (a) and total Ig (b) in DRG homogenates were analysed by ELISA. Depletion of CD4 T cells or neutralization of IFN-γ was performed on days −4, and −1, 2 and 4 days after challenge by intravenous injection of anti-CD4 (GK1.5) or anti-IFN-γ (XMG1.2), respectively. Data are mean ± s.e.m. *P < 0.05; **P < 0.001 (two-tailed unpaired Student’s t-test).
Extended Data Figure 4 | Neutralization of IFN-γ, α4-integrin or depletion of CD4 T cells has no impact on circulating immunoglobulin levels. a, b, WT mice immunized intranasally with TK− HSV-2 6–8 weeks earlier were challenged with a lethal dose of WT HSV-2. Depletion of CD4 T cells or neutralization of IFN-γ was performed on days −4, and −1, 2 and 4 days after challenge by intravenous injection of anti-CD4 (GK1.5) or anti-IFN-γ (XMG1.2), respectively. At time points indicated, HSV-2-specific Ig in the blood (n = 4) (a) and total Ig in the blood (n = 4) (b) were measured. c, d, WT mice immunized intranasally with TK− HSV-2 6 weeks earlier were challenged with a lethal dose of WT HSV-2. Neutralization of α4-integrin was performed on days 2 and 4 after challenge by intravenous injection of anti-α4-integrin/CD49b antibody. Six days later, HSV-2-specific antibody (c) and total antibody (d) in the blood were measured. Data are representative of three similar experiments.
Extended Data Figure 5 | An irrelevant immunization fails to increase the levels of total antibodies in neuronal tissues. a, C57BL/6 mice were immunized with a sublethal dose of influenza A/PR8 virus (10 p.f.u. per mouse) intranasally. Three weeks later, Flu-specific IFN-γ+ CD4+ T cells in spleen and neuronal tissues (DRG and spinal cord) (CD45.2+) following co-culture with HI-Flu/PR8 loaded splenocytes (CD45.1+) were analysed by flow cytometry. As a control, lymphocytes isolated from spleen of TK− HSV-2 intranasally immunized mice 6 weeks after vaccination were used for co-culture. (**P < 0.001; two-tailed unpaired Student’s t-test). b–d, C57BL/6 mice were immunized with a sublethal dose of influenza A/PR8 virus (10 p.f.u. per mouse). Four weeks later, these mice were challenged with a lethal dose of WT HSV-2 (10⁴ p.f.u. per mouse) intravaginally. Six days after challenge, total antibodies in lysate in DRG (b), spinal cord (c) and blood (d) were measured by ELISA.
Extended Data Figure 6 | Most CD4 T cells recruited to the DRG and spinal cord of immunized mice are localized in the parenchyma of neuronal tissues. 

**a**, C57BL/6 mice were immunized intranasally with TK− HSV-2. Six days after challenge of immunized mice 6 weeks prior, neuronal tissue sections (DRG and spinal cord) were stained for CD4+ cells and VCAM-1+ cells or CD31+ cells (red or green). Blue labelling depicts nuclear staining with DAPI (blue). Images were captured using a ×10 or ×40 objective lens. Scale bars, 100 μm.

**b**, C57BL/6 mice were immunized intranasally with TK− HSV-2. Six weeks later, mice were challenged with WT HSV-2 intravaginal and neuronal tissues were collected 6 days later. DRG and spinal cord were stained for CD4+ cells (red) and MHC class II+ cells, CD11b+ cells or Ly6G+ cells (green). Blue labelling depicts nuclear staining with DAPI (blue). Images were captured using a ×10 or ×40 objective lens. Scale bars, 100 μm. Data are representative of at least three similar experiments.
Extended Data Figure 7 | Intravascular staining reveals localization of CD4 T cells in the parenchyma of neuronal tissues. a, b, C57BL/6 mice immunized intranasally with TK− HSV-2 6 weeks previously were challenged with lethal WT HSV-2. Six days after challenge, Alexa Fluor 700-conjugated anti-CD90.2 antibody (3 μg per mouse) was injected intravenously (tail vein) into immunized mice. Five minutes later, these mice were killed for fluorescence-activated cell sorting analysis of intravascular versus extravascular lymphocytes. Data are representative of at least two similar experiments.
Extended Data Figure 8 | Recombinant IFN-γ is sufficient to increase epithelial and vascular permeability in vaginal tissues. a, WT mice immunized with TK− HSV-2 (10⁶ p.f.u.) intranasally 6 weeks earlier were injected intravaginally with recombinant mouse IFN-γ (10 μg per mouse) (n = 3) or PBS (n = 3). At the indicated time points, HSV-2-specific Ig (a) and total Ig (b) in vaginal wash were measured by ELISA. c, Two days after rIFN-γ treatment, vaginal tissue sections were stained for VCAM-1⁺ cells (red) or CD4⁺ cells (green) and CD31⁺ cells (green). Blue labelling depicts nuclear staining with DAPI (blue). Images were captured using a ×10 or ×40 objective lens. Scale bars, 100 μm. Data are representative of at least three similar experiments.
Extended Data Figure 9 | Vascular permeability in DRG and spinal cord is augmented following WT HSV-2 challenge.  

a, C57BL/6 mice were immunized intranasally with TK− HSV-2. Six days after challenge of mice immunized 6 weeks previously, neuronal tissue sections (DRG and spinal cord) were stained for CD4+ cells (red) and mouse albumin (green). Blue labelling depicts nuclear staining with DAPI (blue).  

b, C57BL/6 mice were immunized intranasally with TK− HSV-2. Six weeks later, these mice were challenged with lethal WT HSV-2. Six days after challenge, Oregon green 488-conjugated dextran (70 kDa) (5 mg ml−1, 200 μl per mouse) was injected intravenously into intranasally immunized mice. Forty-five minutes later, these mice were killed for immunohistochemical analysis. GM, grey matter; WM, white matter. Data are representative of three similar experiments.
Extended Data Figure 10 | Memory CD4+ T cells are required for the increase in antibody levels and vascular permeability in the brain following VSV immunization and challenge. a, C57BL/6 mice were immunized intravenously with WT VSV \( (2 \times 10^6 \text{ p.f.u. per mouse}) \). Five weeks later, these mice were challenged intranasally with WT VSV \( (1 \times 10^7 \text{ p.f.u. per mouse}) \). Six days after challenge, VSV-specific IFN-γ+ CD4+ T cells in spleen (CD45.2+) following co-culture with HI-VSV loaded splenocytes (CD45.1+) or HI HSV-2 loaded splenocytes were analysed by flow cytometry. Data are mean ± s.e.m. *P < 0.05; **P < 0.001 (two-tailed unpaired Student’s t-test). b, c, Five weeks after VSV immunization, these mice were challenged intranasally with WT VSV \( (1 \times 10^7 \text{ p.f.u. per mouse}) \). Six days after challenge, VSV-specific antibodies in lysate of brain (b) and serum (c) were measured by ELISA. Depletion of CD4 T cells was performed on −4, −1, 2 and 4 days after challenge by intravenous injection of anti-CD4 (GK1.5). d, Albumin levels in tissue homogenates were analysed by ELISA. Data are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney U-test).