Prolonged high-intensity exercise induces fluctuating immune responses to herpes simplex virus infection via glucocorticoids
（長時間高強度の運動は グルココルチコイドを介して 単純ヘルペスウイルス感染症に対して 変動性免疫応答を誘導する）

足立　晃正

主論文

The Journal of Allergy and Clinical Immunology 2021 年発行予定

Prolonged high-intensity exercise induces fluctuating immune responses to herpes simplex virus infection via glucocorticoids

Akimasa Adachi, MD, [1], Tetsuya Honda, MD, PhD, [1,2*], Teruki Dainichi, MD, PhD, [1], Gyohei Egawa, MD, PhD, [1], Yosuke Yamamoto, MD, PhD, [1], Takashi Nomura, MD, PhD, [1], Saeko Nakajima, MD, PhD, [1], Atsushi Otsuka, MD, PhD, [1], Masamitsu Maekawa, PhD, [3], Nariyasu Mano, PhD, [3], Naoto Koyanagi, PhD, [4,5], Yasushi Kawaguchi, DVM, PhD, [4,5], Toshiaki Ohteki, PhD, [6], Takashi Nagasawa, MD, PhD, [7], Koichi Ikuta, MD, PhD, [8], Akihiko Kitoh, MD, PhD, [1], and Kenji Kabashima, MD, PhD, [1,9,10*]

1. Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan
2. Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan
3. Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan
4. Division of Molecular Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
5. Department of Infectious Disease Control, International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
6. Department of Biodefense Research, Medical Research Institute, Tokyo Medical and Dental University (TMDU), Tokyo, Japan
7. Laboratory of Stem Cell Biology and Developmental Immunology, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University, Osaka, Japan

8. Laboratory of Immune Regulation, Department of Virus Research, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

9. Singapore Immunology Network (SIgN) and Skin Research Institute of Singapore (SRIS), Technology and Research (A*STAR), Biopolis, Singapore

10. Lead contact

*Correspondence:

Tetsuya Honda, M.D., Ph.D

Professor and Chairman,

Department of Dermatology, Hamamatsu University School of Medicine

1-20-1 Handayama, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan

Phone: +81-53-435-2303; Fax: +81-53-435-2368

E-Mail: hontetsu@hama-med.ac.jp

Kenji Kabashima, M.D., Ph.D

Professor and Chairman,

Department of Dermatology, Kyoto University

54 Shogoin, Kawahara-cho, Sakyo, Kyoto 606-8507, Japan

Phone: +81-75-751-3310; Fax: +81-75-751-4949

E-Mail: kaba@kuhp.kyoto-u.ac.jp

Conflict of interest statement

The authors have declared that no conflict of interest exists.

Keywords: prolonged high-intensity exercise, HSV-2, glucocorticoids, corticosterone, CXCR4, CXCL12, plasmacytoid dendritic cell, fluctuation, viral infection

Capsule summary

We describe that prolonged high-intensity exercise leads to impaired or enhanced immune responses against HSV-2 infection depending on the timing between infection and exercise.

Key messages

- Prolonged high-intensity exercise 17 hours after HSV-2 infection induces impaired viral clearance via glucocorticoid-induced pDC homing to the bone marrow.
- Prolonged high-intensity exercise 8 hours after HSV-2 infection induces enhanced viral clearance via glucocorticoid-induced the number of pDC in the blood.

Abbreviations

HSV, herpes simplex virus

pDC, plasmacytoid dendritic cell

IFN, interferon

CXCR4, C-X-C chemokine receptor type 4

CXCL12, C-X-C motif chemokine 12

NK, natural killer

TLR, toll like receptor

LC-MS/MS, liquid chromatography tandem mass spectrometry

HIF, hypoxia inducible factor

GR, glucocorticoid receptor

GFP, green fluorescence protein

Abstract

Background: Epidemiological studies have yielded conflicting results regarding the influence of a single bout of prolonged high-intensity exercise on viral infection.

Objective: Here, we show that prolonged high-intensity exercise induces either exacerbation or amelioration of herpes simplex virus type 2 (HSV-2) infection depending on the interval between viral exposure and exercise.

Methods: Mice were intravaginally infected with HSV-2 and exposed to run on the treadmill.

Results: Prolonged high-intensity exercise 17 h after infection impaired the clearance of HSV-2, while exercise 8 h after infection enhanced the clearance of HSV-2. These impaired or enhanced immune responses were related to a transient decrease or increase in the number of blood-circulating plasmacytoid dendritic cells (pDCs). Exercise-induced glucocorticoids transiently decreased the number of circulating pDCs by facilitating their homing to the bone marrow via the CXCL12-CXCR4 axis, which led to their subsequent increase in the blood.

Conclusion: Thus, a single bout of prolonged high-intensity exercise can be either deleterious or beneficial to antiviral immunity.

Introduction

Epidemiological studies indicate that individuals who perform regular, moderate exercise have a reduced risk of infectious diseases and cancers[1,2]. However, the effect of a single bout of exercise on immune function remains hotly debated. It was previously reported that a single bout of prolonged high-intensity exercise, such as a marathon, may increase the number of episodes of upper respiratory tract infection or herpes simplex virus (HSV) infection[3–5]. Consistently, a single bout of prolonged high-intensity exercise in mice induced high morbidity and mortality in response to several viral infections[6–8], suggesting that there is an association between a single bout of prolonged high-intensity exercise and immune system impairment against viral infection. The involvement of anti-inflammatory molecules, such as glucocorticoids, catecholamines, and interleukin-10, has been suspected[9]. On the other hand, some studies showed that prolonged high-intensity exercise improves immune surveillance, enhancing antibacterial and antiviral immunity[10,11]. Thus, the influence of a single bout of prolonged high-intensity exercise on the immune system remains controversial, and the cause of these discrepancies remains unclear.

These conflicting results might stem from the complexity of immune cell dynamics during and after prolonged high-intensity exercise. In humans, the number of circulating natural killer (NK) cells, myeloid dendritic cells (mDCs), neutrophils, monocytes and T cells transiently increased during the exercise. After the exercise, the number of circulating plasmacytoid dendritic cells (pDCs), NK cells and T cells decreased, while circulating neutrophils and monocytes increased[12–18]. Functional impairments, such as the downregulation of Toll-like receptor (TLR) expression on immune cells, have also been reported[15,19]. Redistribution of T cells in the bone marrow, Peyer's patches and the

lungs has been observed after a single bout of prolonged high-intensity exercise in a mouse study[10]. However, to date, there have been no studies that address the association between these transient changes in blood immune cells and subsequent immune responses to viruses. In addition, the underlying molecular mechanisms that cause transient changes in the number of blood cells are poorly understood.

Here, we explored the impact of a single bout of prolonged high-intensity exercise on the immune system and the underlying mechanisms using a mouse model of HSV type 2 (HSV-2) intravaginal infection. Prolonged high-intensity exercise either exacerbated or ameliorated clinical symptoms of HSV-2 infection depending on the interval between viral exposure and exercise. The number of circulating pDCs, which play important roles in protection against HSV-2, transiently decreased immediately after prolonged high-intensity exercise and increased 6-12 h after exercise. Circulating pDCs were selectively homing to the bone marrow via the CXCL12-CXCR4 pathway during exercise in a glucocorticoid-dependent manner. The blockade of glucocorticoid signaling inhibited the homing to the bone marrow of circulating pDCs and abrogated the effects of exercise on immune responses to HSV-2. Our results demonstrate that glucocorticoids play a novel role in coordinating the CXCL12-CXCR4 axis and antiviral immune regulation in response to a single bout of prolonged high-intensity exercise.

Methods

Animals. C57BL/6 (B6) female mice were purchased from Oriental Bio Services (Kyoto, Japan) and B6. Tg-(CAG-GFP) mice were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). B6.Cg-Tg(Itgax-cre)1-1Reiz/J (*Cd11c*-Cre) mice, B6.129-*Hif1α*^{tm3Rsjo}/J (*Hif1a*$^{fl/fl}$) mice, R26-CAG-tdTomato mice, and EIIa^{Cre} mice were purchased from Jackson Laboratories (Bar Harbor, ME), and B6. 129-Cxcl12^{tm2Tng} (*Cxcl12*-GFP) and B6.129-*Nr3c1*^{tm2Gsc}(*Nr3c1*$^{fl/fl}$) mice were generated as previously described[54–57]. All mice were maintained under specific pathogen-free conditions at the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University. All experiments were performed on 7- to 12-week-old female mice. All procedures were carried out under isoflurane anesthesia. Mice were housed in groups with controlled humidity, temperature, and light conditions (14-hr light/10-hr dark cycle; lights on at 7:00 a.m. and off at 9:00 p.m.). Mice were acclimated to our laboratory environment for at least 2 weeks before experiments were performed. Food and water were available *ad libitum*.

Exercise protocol. The prolonged high-intensity exercise protocol and short periods of moderate-intensity exercise protocol were performed as previously described[20,22] with some modifications. Briefly, mice were acclimated to a custom-made treadmill for 10 min at 5-7 m/min and exposed to the environment of the laboratory room for 1 h for three consecutive days. Exercise was started during the last hour of the dark cycle. The treadmill grade was set at 0%. In the prolonged high-intensity exercise group, the speed of the treadmill was initially set to 11.5 m/min and was increased by 1-1.5 m/min every 25 min. The maximum speed was 19 m/min, and the total duration of exercise was 3 h. When the speed reached over 12 m/min or 17 m/min, the intensity corresponded to

161 approximately 76% or 84% of maximal oxygen uptake (VO_2max), respectively; 50-80%
162 of VO_2max was considered to be moderate-intensity exercise, and over 80% of VO_2max
163 was considered to be high-intensity exercise[58–60]. In the short periods of moderate-
164 intensity exercise group, the speed of the treadmill was initially set to 11.5 m/min, and
165 the total duration of exercise was 30 min. Electric shock was never used in the exercise
166 session because mice readily responded to a gentle tap that encouraged them to maintain
167 the pace of the treadmill. Mice in the no-exercise group were placed in cages near the
168 treadmill, and no food or water was provided during the exercise session.
169
170 *Viral infection and cells*. HSV-2 wild-type strain 186 and Vero cells were previously
171 described[61,62]. HSV-2 stocks were prepared in Vero cells and quantified by a plaque-
172 forming assay. For HSV-2 infection, mice were pretreated by the subcutaneous injection
173 of the neck ruff with 1.67 mg of medroxyprogesterone acetate (A.N.B. Laboratories,
174 Bangkok, Thailand) per mouse in a volume of 100 μL. Five days later, mice were
175 anesthetized with isoflurane (Wako, Osaka, Japan). The vagina was washed with 100 μL
176 of phosphate-buffered saline (PBS), and mice were intravaginally infected with 3.5×10^2
177 plaque-forming units (PFU) of HSV-2 strain 186 in 10 μL volumes suspended in 199V.
178 Disease severity was assessed daily until 14 d post infection using a clinical scoring
179 system that was previously described[63]. The growth mediums used were Dulbecco's
180 modified Eagle's medium (Sigma-Aldrich, St Louis, MO) supplemented with 5% heat-
181 inactivated fetal calf serum (FCS) (Invitrogen, Carlsbad, CA), 100 U/mL penicillin, and
182 100 μg/mL streptomycin (Wako); and 199V medium (430 mL of sterilized distilled water
183 (dH_2O) supplemented with 50 mL of 199 medium (Sigma-Aldrich), 5 mL of 5% (wt/vol)
184 $NaHCO_3$ (Nacalai, Kyoto, Japan), 5 mL of 1% FCS (Invitrogen), 100 U/mL of penicillin

and 100 μg/mL streptomycin with L-glutamine (Wako)).

Viral titer measurement in the vagina. Vaginal washes were collected for 2 consecutive days after infection with HSV-2 by washing with 100 μL of 199V. Viral titers were obtained titrating vaginal wash samples with 199V (400 μL) on a Vero cell monolayer in 12-well plates for 1 h at 37°C with 5% CO_2. After aspirating all medium, 1 mL of 199O medium (199V and γ-globulins from human blood (Sigma-Aldrich)) was added, and plates were incubated for 48 h at 37°C with 5% CO_2.

Plasmacytoid dendritic cell depletion. To deplete pDCs, mice were intraperitoneally injected with 200 μg PDCA-1 Ab (clone #927; Bioxell, Lebanon, NH) three days and one day prior to HSV-2 intravaginal infection. As a control, mice were intraperitoneally injected with same amounts of isotype rat IgG2b Ab (clone#LTF-2; Bioxell). At 24 h and 48 h after injection, blood was collected by puncture with an 18G needle on the submandibular area and confirmed pDC depletion by flow cytometry.

Antibodies and flow cytometry. Single-cell suspensions of vaginal tracts were obtained as previously described[64] with some modifications. Briefly, the vagina was separated from the urethra and cervix, cut into small pieces and incubated in 4 mg/mL Dispase II (Roche, Basel, Switzerland), 0.425 mg/mL collagenase D (Roche), 30 μg/mL DNase I (Sigma-Aldrich) and 100 U/mL hyaluronidase (Sigma-Aldrich) for 45 min at 37°C. After perfusion, the lungs, liver, and gastrocnemius muscles were cut into small pieces. Lungs were digested with 150 U/mL collagenase type IV (Worthington Biochemical, Lakewood, NJ) and 150 μg/mL DNase I at 37°C for 55 min. Livers were digested with 0.05%

collagenase/Dispase (Roche) at 37°C for 30 min. Gastrocnemius muscles were digested with 250 U/mL collagenase type II (Worthington Biochemical) and 150 μg/mL DNase I at 37°C for 55 min. Cells were further digested with 0.01 M EDTA at 37°C for 5 min and were filtered through a 70-μm cell strainer. Leukocytes of liver and muscles were separated by centrifugation with a 33% Percoll solution (GE Healthcare, Fairfield, Conn). Bone marrow cell suspensions, which were flushed from femurs and tibias, lymph nodes, and spleens, were digested with 500 U/mL collagenase type II (Worthington Biochemical) and 100 μg/mL DNase I at 37°C for 25 min, further digested with 0.01 M EDTA at 37°C for 5 min and filtered through a 40-μm cell strainer. Peripheral blood was collected by puncture with an 18G needle on the submandibular area. Nonspecific antibody binding was blocked with an anti-CD16/32 antibody (BD Biosciences, San Jose, Calif) at 4°C for 10 min, and then cells were stained for surface antigens. For the intracellular staining of IFN-α, cells were fixed and permeabilized with fixation and permeabilization solution (BD Biosciences). After lysing red blood cells, cells were stained with antibodies and fixable viability dye (eBioscience, San Diego, CA) for 30 min at 4°C in Brilliant Stain Buffer (BD Biosciences) for surface antigens. Fluorescent dye- or biotin-conjugated antibodies against the following proteins were purchased from BD Bioscience, eBioscience, BioLegend (San Diego, CA), R&D Systems (Minneapolis, MN), Miltenyi Biotec (Bergisch Gladbach, Germany), and PBL Assay Science (Piscataway, NJ): B220 (RA3-6B2), CD3 (17A2), Ly6G (1A8), Ly6C (HK1.4), CXCR3 (CXCR3-173), CXCR4 (L276F12), CCR2 (475301), CCR5 (HM-CCR5), CCR7 (4B12), CCR9 (CW-1.2), CD11a/CD18 (H155-78), CD11b (M1/70), CD11c (N418), CD19 (6D5), CD45 (30-F11), CD49d (R1-2), CD115 (AF598), CD127 (A7R34), CD317 (927), ChemR23 (477806), I-A/I-E (M5/114.15.2), NK1.1 (PK136), Siglec-H (551), TLR9 (M9.D6), and IFN-α

(RMMA-1). Each cell is identified as following panel; pDC; $CD3^{-}CD19^{-}CD11b^{-}B220^{+}PDCA1^{+}$, NK cell; $CD3^{-}NK1.1^{+}$, neutrophil; $CD11b^{+}Ly6G^{+}$, inflammatory monocytes $CD11b^{+}Ly6C^{high}$, cDC; $CD11c^{+}I\text{-}A/I\text{-}E^{high}$. Multiparameter analyses were performed on an LSRFortessa cytometer (LSR II; BD Biosciences) and were analyzed using FlowJo software (Tree Star, Ashland, OR).

Drug administration. Drugs were administered either intraperitoneally (AMD3100, RU486, eplerenone, 6-OHDA, and corticosterone) or subcutaneously (SR59230A and propranolol). All drugs were purchased from Sigma-Aldrich. AMD3100 was dissolved in PBS and administered 30 min before exercise. RU486 (30mg/kg) was dissolved in ethanol/sesame oil (1:10 vol/vol) and administered 1 h before exercise. Corticosterone (10mg/kg) was dissolved in an ethanol/sesame oil solution (1:10 vol/vol) and administered 3 h before harvesting the blood and bone marrow. SR59230A (5mg/kg), propranolol (10mg/kg) and eplerenone (1mg/kg) were dissolved in DMSO and further diluted with PBS. 6-OHDA was dissolved in 0.9% NaCl and 10^{-7} M ascorbic acid and administered as previously described [65]. All dosing was determined by previously established protocols[66–71].

Adoptive cell transfer. For adoptive transfer, the spleen and bone marrow were extracted from B6-Tg (CAG-EGFP) mice or $EIIa^{Cre}$-R26-CAG-tdTomato mice. The tissues were digested with 500 U/mL collagenase type II and 100 μg/mL DNase I at 37°C for 25 min and further digested with 0.01 M EDTA at 37°C for 5 min. After lysing red blood cells, cells were resuspended in PBS, and 4×10^{7} cells were intravenously injected into each mouse 17 h before exercise.

257

258 *Immunohistochemistry*. To prepare bone marrow sections, mice were sacrificed
259 immediately after exercise and perfused with 4 mL of 4% paraformaldehyde for 15 min.
260 Then, femurs were harvested and fixed in equivalent amounts of 4% paraformaldehyde
261 and 20% sucrose for 1 h. Fixed samples were embedded in SCEM medium (Section-Lab,
262 Hiroshima, Japan) and frozen in cooled hexane. Sections (5 μm) of undecalcified femoral
263 bone were generated by Kawamoto's film method (Cryofilm transfer kit; Section-Lab)
264 and stained with anti-PDCA1 antibody. Confocal microscopy was performed with an
265 A1RMP (Nikon, Tokyo, Japan). The distance from transferred tdTomato$^+$PDCA1$^+$ cells
266 to the nearest GFP$^+$ cells in the bone marrow of *Cxcl12*-GFP mice was analyzed with
267 NIS-Elements AR analysis 4.50.00 64 bit for Windows (Nikon) and ImageJ version 1.52p
268 (NIH, Bethesda, USA).

269

270 *Enzyme-linked immunosorbent assay (ELISA) for corticosterone, noradrenaline and*
271 *CXCL12*. Plasma corticosterone was analyzed by a competitive enzyme immunoassay
272 (Enzo Lifesciences, Farmingdale, NY), plasma noradrenaline was analyzed by a
273 sandwich ELISA (IBL International, Hamburg, Germany), and CXCL12 in extracellular
274 fluid was collected by flushing femurs and tibias with 1 mL of PBS and was analyzed by
275 a sandwich ELISA (R&D systems). All procedures were performed according to the
276 manufacturer's protocol for each ELISA kit.

277

278 *LC-MS/MS analysis*. Steroid hormones were measured with LC-MS/MS, as previously
279 described [72] with some modifications. Briefly, LC-MS/MS was performed with a 1260
280 high-performance liquid chromatographer (Agilent Technologies, Santa Clara, CA) and

6460 triple quadrupole tandem mass spectrometer (Agilent Technologies). The analytical column was a Capcell core C18 (Osaka Soda Co. Ltd., Osaka, Japan). Acetic acid/water (0.1:100, vol/vol) and acetic acid/acetonitrile/methanol (0.1:50:50, vol/vol/vol) were used as mobile phases A and B, respectively. The flow rate was set at 0.4 mL/min. Gradient elution was performed by increasing B from 50% to 100% over 12 min. The selected reaction monitoring (SRM) mode was set for MS/MS detection. All SRM transitions were optimized with the infusion analysis of each standard solution. All source parameters were optimized with the flow injection analysis of standard solutions.

In vitro stimulation of bone marrow cells. Single-cell suspensions from the bone marrow were incubated at 4×10^6 cells/well in 96-well plates in RPMI (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen), 1% sodium pyruvate, 1% nonessential amino acids (Invitrogen), 50 μM 2-mercaptoethanol (Nacalai), 100 U/mL penicillin, and 100 μg/mL streptomycin (Wako). For intracellular IFN-α staining, cells were cultured at 37°C in the presence of 0.2 μg/mL granulocyte-macrophage colony-stimulating factor (GM-CSF; Peprotech, Rocky Hill, NJ) and were stimulated with CpG-ODN 2395 (InvivoGen, San Diego, CA) (5 μM) for 9 h. GolgiStop (1 μL/mL) (BD Biosciences) and brefeldin A (1 μL/mL) (Sigma) were added for the last 6 h of the incubation. Subsequently, cells were harvested and stained with antibodies against surface markers. Fixation and permeabilization were performed with Cytofix/Cytoperm solution (BD Bioscience), followed by intracellular staining with FITC-conjugated anti-IFN-α (PBL Assay Science).

Chemotaxis assay. Whole blood cells were lysed and incubated for 3 h with corticosterone (Sigma) or vehicle. Then, cells were placed in the upper chamber of an uncoated transwell

with 5-μm pores (Corning, Corning, NY) for 1 h. CXCL12 (10 ng/mL; R&D Systems) or medium was placed in the lower chamber. Cells in the lower chamber were analyzed with flow cytometry. Cells were cultured in phenol red-free RPMI with 10% hormone-depleted FCS.

Quantitative RT-PCR analysis. Total RNA was isolated with TRIzol reagent (Invitrogen) and RNeasy kits (Qiagen, Hilden, Germany). cDNA was reverse transcribed from total RNA samples by using a Prime Script RT reagent kit (Takara Bio, Kusatsu, Japan). Quantitative RT-PCR was performed by monitoring double-stranded DNA synthesis during various PCR cycles with SYBR Green I (Roche) and a LightCycler real-time PCR apparatus (Roche) according to the manufacturer's instructions. All primers were obtained from Greiner Japan (Tokyo, Japan). The primer sequences were as follows: *Gapdh*, 5'-AGGTCGGTGTGAACGGATTTG-3' and 5'-GGGGTCGTTGATGGCAACA-3'; *Cxcl9*, 5'-GGCACGATCCACTACAAATCC-3' and 5'-GGTTTGATCTCCGTTCTTCAGT-3'; *Cxcl10*, 5'-CCAAGTGCTGCCGTC ATTTTC-3' and 5'-GGCTCGCAGGGATGATTTCAA-3'; *Cxcl11*, 5'-GGCTTCCT TATGTTCAAACAGGG-3' and 5'-GCCGTTACTCGGGTAAATTACA-3'; *Ccl3*, 5'-TGAAACCAGCAGCCTTTGCTC-3' and 5'-AGGCATTCAGTTCCAGGTCAGTG-3'; *Ccl4*, 5'-CCATGAAGCTCTGCGTGTCTG-3' and 5'-GGCTTGGAGCAAAGACTGCTG-3'; *Ccl5*, 5'-AGATCTCTGCAGCTGCCCTCA-3' and 5'-GGAGCACTTGCTGCTGGTGTAG-3'; and pan *Ifna*, 5'-CCTGAGAGAGAAGAAACACAGCC-3' and 5'-TCTGCTCTGACCACYTCCCAG-3'. For each sample, the gene expression of duplicate test reactions and a control reaction

lacking reverse transcriptase were analyzed, and the results were normalized to those of *Gapdh* mRNA.

Statistics. In the present study, we hypothesized that the clinical scores at day 14 and viral titer at day 1 and 2 might show substantial differences between CONTROL and PROLONGED groups under the respective situations with vehicle only or antagonists (AMD3100 or RU486). Also, we hypothesized that the clinical scores at day 14 and viral titer at day 1 and 2 might show substantial differences between CONTROL and PROLONGED groups in *Nr3c1*$^{fl/fl}$ and *Nr3c1*$^{fl/fl}$*Cd11c*$^{-}$Cre^{+} mice, respectively. Mann-Whitney test was performed at day 14 for clinical scores and at day 1 and day 2 for viral titers. For the analysis of survival rates, the log-rank test was performed between those groups. For the analysis of cell counts, expression levels of chemokine receptors, distances from GFP-positive cells to tdTomato-positive cells, levels of hormones and chemokines, we hypothesized that those values might show substantial differences between CONTROL and PROLONGED groups under the respective situations in each mouse genotype or drug-treated group. Mann-Whitney test was employed to compare continuous values between two groups. For nonparametric pairwise multiple comparison after Kruskal-Wallis test, Dunn's test with Holm adjustment was performed. For the analysis of the interaction effect of mouse genotype or drugs on the prolonged high-intensity exercise, the aligned rank transform (ART) procedure using analysis of variance (ANOVA) was performed to deal with non-normal distributed data[73]. All statistical analyses were performed with GraphPad Prism software ver. 7.0 for Windows and Stata SE ver. 14.2 (StataCorp LP, College Station, TX). *P*-values less than 0.05 were considered statistically significant. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, and n.s., not

significant.

Study approval. All mouse protocols were approved by the Institutional Animal Care and Use Committee of the Kyoto University Graduate School of Medicine, and all efforts were made to minimize stress.

Results

Prolonged high-intensity exercise induces impaired or enhanced immunity against HSV-2 infection depending on the interval between infection and exercise

To examine how a single bout of prolonged high-intensity exercise influences the immune system, we used a well-established mouse model of intravaginal HSV-2 infection in combination with prolonged high-intensity exercise on a treadmill that was based on previous reports with some modifications[20]. Because it has been demonstrated that HSV-2 expansion in the vagina peaks approximately 18 h after infection[21], mice were first intravaginally infected with HSV-2 (3.5×10^2 PFU), and then, 17 h later, the mice ran for 3 h at high speed (19 m/min) on a customized treadmill (late exposure to prolonged high-intensity exercise) (for details, see Material and Methods). Then, we evaluated the clinical scores and survival rates for the following two weeks and viral titers in the vagina for the following two days (Fig. 1A). With these conditions, mice in the prolonged high-intensity exercise group exhibited higher clinical scores and lower survival rates than those in the control no-exercise group (Fig. 1B). Consistently, the HSV-2 viral load in the vagina was significantly higher in the prolonged high-intensity exercise group than in the control no-exercise group (Fig. 1B). To examine whether the impaired immune response was specifically induced by prolonged high-intensity exercise, we examined the effect of short periods of moderate-intensity exercise (running for 30 min at a moderate speed (11.5 m/min))[22] on HSV-2 infection. In this condition, no significant difference was observed in the clinical symptoms of HSV-2 infection between the exercise group and the control no-exercise group (Fig. 1C).

Since it has been reported that the number of circulating immune cells changes dynamically after exercise on an hour basis[16], it is possible that the immune response may

382 be fluctuated by a small difference in interval from infection. To examine this possibility,
383 mice were infected with HSV-2 and ran at an earlier time point (8 h after infection (early
384 exposure to prolonged high-intensity exercise)). Then, the clinical symptoms of HSV-2
385 infection were evaluated. In this protocol, mice in the prolonged high-intensity exercise
386 group exhibited lower clinical scores and viral titers than those in the control no-exercise
387 group (Fig. 1D). During the exercise, a slight weight loss was observed, however, no
388 significant differences were observed between early exposure group and late exposure
389 group (see Fig. S1A in the Online Repository). Collectively, these results suggest that a
390 single bout of prolonged high-intensity exercise may either impair or enhance antiviral
391 immunity against intravaginal HSV-2 infection depending on the interval between
392 infection and exercise.
393
394 **Late exposure to prolonged high-intensity exercise impairs pDC infiltration in the**
395 **vagina after HSV-2 infection**
396 We next examined the mechanism of impaired immunity induced by late exposure to
397 prolonged high-intensity exercise. Various immune cells, such as pDCs, NK cells,
398 inflammatory monocytes and neutrophils, to infected sites is reported to contribute to
399 protection against HSV-2 intravaginal infection[23–26]. In particular, pDCs are believed to
400 play a crucial role in the initial prevention of viral spread by producing interferon (IFN)-
401 α[23,27,28], although some studies reported that pDCs are dispensable in HSV-2 infection[29].
402 Therefore, we first evaluated the influence of late exposure to prolonged high-intensity
403 exercise on the infiltration of those immune cells in the vagina 24 h after infection. While
404 the accumulation of inflammatory monocytes and neutrophils was comparable between
405 the prolonged high-intensity exercise group and the control no-exercise group, the

accumulation of pDCs and NK cells after HSV-2 infection was significantly lower in the prolonged high-intensity exercise group than in the control no-exercise group (Fig. 2A). Consistently, the mRNA expression level of *Ifna* in the vagina was lower in the prolonged high-intensity exercise group than in the control no-exercise group (Fig. 2B). Prolonged high-intensity exercise did not affect the IFN-α-producing ability or the expression level of TLR9, which is critical to produce IFN-α, in pDCs (see Fig. S1B and S1C in the Online Repository). These results suggest that late exposure to prolonged high-intensity exercise impairs anti-viral immunity by reducing the infiltration of pDCs.

To confirm the contribution of pDCs in our model, we depleted pDCs using anti-PDCA1 antibody[30] and examined its effects on clinical symptoms and viral titer after HSV-2 infection. Administration of the anti-PDCA1 antibody significantly reduced the number of circulating pDCs, but did not affect the number of NK cells, neutrophils, monocytes and conventional DCs (cDCs) (see Fig. S1D in the Online Repository). pDC-depleted mice showed exacerbated clinical symptoms to a similar extent to those of mice with prolonged high-intensity exercise, as well as increased viral titer (Fig. 2C). These results indicate that pDCs are crucial for anti-viral immunity in our model, and support our hypothesis that exercise-induced exacerbation of HSV-2 infection was through the regulation of pDC infiltration to the vagina.

It has been reported that prolonged high-intensity exercise induces transient lymphocytopenia after exercise in both humans and mice[12–18], which has been suggested as a possible cause of impaired antiviral immunity. Thus, we next measured the number of circulating pDCs after prolonged high-intensity exercise in our model. In line with previous reports, the number of pDCs significantly decreased immediately after prolonged high-intensity exercise (Fig. 2D). On the other hand, short periods of moderate-

intensity exercise did not induce a decrease in pDCs (Fig. 2E). These results suggest that a reduction in infiltrating pDCs into the vagina, which could significantly impair antiviral immunity against HSV-2 infection, might occur due to the decrease in circulating pDCs induced by late exposure to prolonged high-intensity exercise.

pDCs home to the bone marrow during the prolonged high-intensity exercise

Because a previous study suggested that circulating T cells are redistributed to several tissues after prolonged high-intensity exercise[10], we hypothesized that the decrease in circulating pDCs immediately after prolonged high-intensity exercise was caused by redistribution from the blood to other organs during the exercise. To test this hypothesis, we intravenously transferred bulk green fluorescent protein (GFP)-labeled splenocytes and bone marrow cells and examined the number of GFP-positive pDCs in other organs, such as the bone marrow, lymph nodes, spleen, lungs, liver, muscle and vagina, with or without prolonged high-intensity exercise. The number of transferred GFP-positive pDCs in the lymph node, spleen, liver, lung, muscle, and vagina were comparable between the prolonged high-intensity exercise group and the control no-exercise group. On the other hand, the number of GFP-positive pDCs in the bone marrow was significantly higher in the prolonged high-intensity exercise group than in the control no-exercise group (Fig. 3A and S1), although the increase in the bone marrow was also observed in other immune cells (see Fig. S1E in the Online Repository). In the blood, the number of transferred GFP-positive pDCs significantly decreased in the prolonged high-intensity exercise group (see Fig. S1F in the Online Repository).

Next, we verified the possibility of the recruit failure from the blood to the vagina. Because both CXCR3 and CCR5 are involved in the infiltration of pDCs in the

454 mucocutaneous lesion after HSV-2 infection[23,31,32], we examined the effect of prolonged
455 high-intensity exercise on CXCR3 and CCR5 expression on circulating pDCs and the
456 mRNA expression of the ligands for these chemokine receptors (*Cxcl9*, *Cxcl10*, and
457 *Cxcl11* for CXCR3 and *Ccl3*, *Ccl4* and *Ccl5* for CCR5) in the vagina. Both the expression
458 levels of chemokine receptors and chemokines were unaffected by prolonged high-
459 intensity exercise (see Fig. S2 in the Online Repository). These results suggest that
460 impaired infiltration of pDCs into the vagina after late exposure to prolonged high-
461 intensity exercise was not associated with recruit failure from the blood to the vagina, but
462 associated with decrease of circulating pDCs which homed to the bone marrow during
463 the exercise.
464
465 **pDC homing to the bone marrow during the prolonged high-intensity exercise is**
466 **mediated by the CXCL12-CXCR4 axis**
467 We next investigated the mechanisms through which prolonged high-intensity exercise
468 induced the homing of pDCs to the bone marrow. It has been reported that the chemokine
469 receptors (CXCR4, CCR2, CCR7, CCR9, and ChemR23) and adhesion molecules (LFA1,
470 VLA4, and CD62L) may be responsible for pDC homing from the blood to lymphoid
471 organs or inflamed tissues[32–37]. Therefore, we examined the expression levels of these
472 candidates on circulating pDCs and found that the expression of CXCR4 was upregulated
473 by prolonged high-intensity exercise (Fig. 3B). The expression levels of other candidate
474 chemokine receptors were not upregulated (see Fig. S3A in the Online Repository). On
475 the other hand, the production of CXCL12, the ligand of CXCR4, in the bone marrow
476 was not changed by prolonged high-intensity exercise (see Fig. S3B in the Online
477 Repository). These findings suggest that the promotion of pDC homing to the bone

478 marrow during the prolonged high-intensity exercise might be mediated by the
479 upregulation of CXCR4 expression. In line with this hypothesis, we observed that
480 transferred tdTomato-positive, PDCA-1-positive pDCs localized closer to CXCL12-
481 producing cells in the bone marrow after prolonged high-intensity exercise (Fig. 3C).
482 To examine whether the CXCL12-CXCR4 axis was involved in the homing of
483 circulating pDCs to the bone marrow during the prolonged high-intensity exercise, we
484 administered either vehicle or AMD3100, a CXCR4 antagonist, to mice before prolonged
485 high-intensity exercise and examined the number of pDCs in the blood and bone marrow.
486 As reported previously[38], the administration of AMD3100 inhibited the homing of pDCs
487 to the bone marrow and increased the number of circulating pDCs under the steady states
488 (Fig. 3D). In addition, AMD3100 abrogated the decrease in circulating pDCs induced by
489 prolonged high-intensity exercise (Fig. 3D). Consistently, an increase in the number of
490 pDCs in the bone marrow was not observed in the prolonged high-intensity exercise group
491 treated with AMD3100 (Fig. 3D). AMD3100 did not affect the cellular contents in the
492 vagina (see Fig. S3C in the Online Repository). Collectively, these results suggest that
493 the CXCL12-CXCR4 axis plays a crucial role in the pDC homing to the bone marrow
494 during the prolonged high-intensity exercise.
495
496 **Blockade of CXCR4 signaling abolishes the exacerbation of HSV-2 intravaginal**
497 **infection induced by late exposure to prolonged high-intensity exercise**
498 We then examined whether circulating pDC homing to the bone marrow via the CXCL12-
499 CXCR4 axis is involved in the exacerbation of HSV-2 infection that is induced by late
500 exposure to prolonged high-intensity exercise. To examine this question, we administered
501 either vehicle or AMD3100 to mice in the presence or absence of prolonged high-intensity

exercise and evaluated the clinical phenotype of intravaginal HSV-2 infection. The exacerbation of HSV-2 infection (clinical scores, survival rates, and viral titers in the vagina) by late exposure to prolonged high-intensity exercise was attenuated by AMD3100 treatment (Fig. 3E). These results strongly suggest that circulating pDC homing to the bone marrow through the CXCL12-CXCR4 axis mediates the exacerbation of HSV-2 infection induced by late exposure to prolonged high-intensity exercise.

Glucocorticoids upregulate the expression of CXCR4 on pDCs

We further explored the underlying mechanisms by which prolonged high-intensity exercise upregulates CXCR4 expression on pDCs. Prolonged high-intensity exercise is known to induce hypoxemia[39]. Hypoxia inducible factor-1α (HIF-1α) is known to regulate the expression level of CXCR4[40]; therefore, we evaluated whether HIF-1α was involved in the induction of CXCR4 expression on pDCs by prolonged high-intensity exercise. We generated *Hif1a*$^{fl/fl}$*Cd11c*-Cre^{+}, in which CD11c^{+} cells, including pDCs[41], specifically lack HIF-1α. In *Hif1a*$^{fl/fl}$*Cd11c*-Cre^{+} mice, CXCR4 expression on pDCs was upregulated to a level similar to that of *Hif1a*$^{fl/fl}$ mice during prolonged high-intensity exercise (see Fig. S4 in the Online Repository). This result suggests that HIF-1α is not involved in the upregulation of CXCR4 expression on pDCs in our model.

Next, we focused on hormonal factors in the blood that are considered to be involved in CXCR4 expression, including noradrenaline, glucocorticoids, progesterone, estrogen, and aldosterone[42–46], by performing liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of steroid hormones and enzyme-linked immunosorbent assays for noradrenaline using blood samples taken immediately after prolonged high-intensity exercise. We found that the blood levels of noradrenaline,

corticosterone, and aldosterone were significantly higher in the exercise group (see Fig. S5A in the Online Repository). Then, we tested the effect of inhibitors for each candidate on CXCR4 expression on pDCs. The blockade of adrenergic receptor and aldosterone-mineralocorticoid receptor signaling did not affect the exercise-induced upregulation of CXCR4 expression on pDCs in the blood (see Fig. S5B in the Online Repository). On the other hand, the administration of RU486, a glucocorticoid receptor (GR) antagonist, abrogated the exercise-induced upregulation of CXCR4 expression on pDCs in the blood (Fig. 4A). Furthermore, the treatment of pDCs with corticosterone upregulated the expression of CXCR4 *in vivo* and *in vitro* and facilitated the migration of pDCs toward CXCL12 in a dose-dependent manner *in vitro* (Fig. 4B, 4C, and see Fig. S6A in the Online Repository). Upregulation of CXCR4 expression by the administration of corticosterone *in vivo* was also observed in cDCs and monocytes, but not in NK cells and neutrophils (see Fig. S6B in the Online Repository). The serum corticosterone level peaked approximately 3 h after the start of prolonged high-intensity exercise (see Fig. S6C in the Online Repository). On the other hand, in mice with short periods of moderate-intensity exercise, an increase in the serum corticosterone level was not observed (see Fig. S6D in the Online Repository).

These results indicate that the upregulation of CXCR4 expression on pDCs during prolonged high-intensity exercise was mediated by glucocorticoids.

Blockade of glucocorticoid signaling abrogates the effects of exercise on pDC homing and impaired antiviral immunity induced by prolonged high-intensity exercise

We next examined the effects of RU486 on the homing of circulating pDCs from the blood to the bone marrow. The administration of RU486 abolished the fluctuation of

pDCs in the blood and bone marrow in response to prolonged high-intensity exercise (see Fig. S7A in the Online Repository). RU486 also abrogated the exacerbation of HSV-2 intravaginal infection induced by prolonged high-intensity exercise (see Fig. S7B in the Online Repository). Conversely, the administration of corticosterone decreased the number of pDCs in the blood and tended to increase the number of pDCs in the bone marrow (see Fig. S7C in the Online Repository). Consistently, the administration of corticosterone exacerbated HSV-2 intravaginal infection (see Fig. S7D in the Online Repository).

To further confirm the effects of glucocorticoids on pDCs, we generated CD11c-expressing cell-specific GR-deficient (*Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+}) mice. In *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice, the exercise-induced upregulation of CXCR4 expression on pDCs and the fluctuation of pDCs in the blood and bone marrow were abrogated (Fig. 5A and 5B). Furthermore, *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice did not exhibit the exacerbation of HSV-2 intravaginal infection in response to late exposure to prolonged high-intensity exercise (Fig. 5C).

CD11c is expressed by other cells, such as mDCs, but the above findings indicate that glucocorticoids mediate the exacerbation of HSV-2 infection induced by late exposure to prolonged high-intensity exercise by upregulating CXCR4 expression on pDCs and facilitating pDC homing to the bone marrow.

Glucocorticoids are involved in enhanced antiviral immunity induced by early exposure to prolonged high-intensity exercise by an increase in circulating pDCs

Finally, we examined the underlying mechanism of enhanced immune responses against HSV-2 infection induced by early exposure to prolonged high-intensity exercise. We

monitored the number of circulating pDCs after their decrease in the blood subsequent to prolonged high-intensity exercise. We found that following the transient decrease after exercise, the number of circulating pDCs increased transiently around 6-12 h later (Fig. 6A). The inhibition of bone marrow homing by a RU486 abrogated their subsequent increase in the blood (Fig. 6B); therefore, we consider that this increase seemed to be dependent on their prior homing to the bone marrow after exercise in response to glucocorticoid activity. Furthermore, the administration of RU486 abrogated the enhanced antiviral immune responses induced by early exposure to prolonged high-intensity exercise (Fig. 6C). These results suggest that a single bout of prolonged high-intensity exercise increases the number of circulating pDCs via glucocorticoids following the transient decrease of circulating pDCs, and enhances antiviral immunity.

Discussion

Here, we demonstrated that a single bout of prolonged high-intensity exercise either impaired or enhanced antiviral immunity to HSV-2 intravaginal infection in mice depending on the interval between infection and exercise. Glucocorticoids induced by prolonged high-intensity exercise facilitated the homing of circulating pDCs to the bone marrow through the CXCL12-CXCR4 axis, which caused a transient decrease of circulating pDCs after the exercise. Following this transient decrease, the number of circulating pDCs increased 6-12 h later. These transient decreases and increases of circulating pDCs were associated with impaired and enhanced antiviral immunity, respectively (Fig. 7). Our results provide novel insights into the mechanistic link between a single bout of prolonged high-intensity exercise and the immune system.

We showed that GR signaling was involved in both the impairment and enhancement of antiviral immunity against HSV-2 intravaginal infection. Glucocorticoids are generally considered to cause immunosuppression; however, glucocorticoids may function as immunostimulants in some contexts, as we have previously discovered that glucocorticoids drive the diurnal accumulation of T cells in lymphoid organs to enhance adaptive immune responses[47]. Previous reports also showed that glucocorticoids exhibit biphasic dose-response effects in an animal model of delayed-type hypersensitivity and in macrophages activated by lipopolysaccharide and IFN-γ[48,49]. Thus, the effects of glucocorticoids on the immune system may be bidirectional and fluctuate in a context dependent manner. That may explain why the contradictory results have been reported regarding the influence of prolonged high-intensity exercise on the viral infection rate.

There are several limitations and unsolved issues in our study. First, we used *Cd11c*-Cre^{+} mice to examine the involvement of GR signaling in pDCs *in vivo*. CD11c is

611 expressed on other cells such as NK cells and mDCs, which are considered to be
612 important in the defense against HSV-2 infection[24,50]. Thus, our results using *Cd11c*-Cre^{+}
613 mice may not necessarily specific to the effects of GR signaling in pDCs. However,
614 antiviral functions of NK cells are induced by pDCs-derived IFN-α[27,28]. In addition,
615 mDCs exert their antiviral functions at much later time points (48 h after the HSV-2
616 intravaginal infection)[50,51] than our analysis timing (24 h after the infection). Furthermore,
617 mice depleted with pDCs showed similar clinical scores and viral titers to those of mice
618 with prolonged high-intensity exercise. Therefore, although we cannot exclude the
619 possibility of the contribution of GR signaling in other cells, we consider that GR
620 signaling in pDCs is, at least mainly, involved in inducing the fluctuating immune
621 responses in our model. Second, the mechanisms increasing the number of circulating
622 pDCs following the transient decrease after the exercise remain unclear. Because the
623 blockade of the pDC homing to the bone marrow by a GR antagonist diminished the
624 increase, prior homing of pDCs to the bone marrow seems to be important for the increase.
625 Homing of pDCs to the bone marrow may induce the release of progenitor cells from the
626 bone marrow into the peripheral blood, as reported in neutrophils[52]. Third, the
627 significance of pDC homing to the bone marrow during the prolonged high-intensity
628 exercise remains unclear. One hypothesis is that the bone marrow may protect pDCs from
629 the stress of prolonged high-intensity exercise. In line with this, it has recently been
630 reported that memory T cells accumulate in the bone marrow during stress from dietary
631 restriction because T cells in the bone marrow express high levels of the antiapoptotic
632 factor BCL-2, which protects them from apoptosis[53]. Thus, the positive and negative
633 effects of prolonged high-intensity exercise on antiviral immunity, as seen in our model,
634 may be secondary phenomena.

635 Together, our findings provide an important clue to elucidate the long-
636 controversial questions about the influence of a single bout of prolonged high-intensity
637 exercise on antiviral immune response. Regulation of glucocorticoid may be utilized for
638 the control of our immune responses after such strenuous exercise, although the
639 applicability of our findings in mice to humans, and in other infectious diseases such as
640 respiratory infection, are important subjects for future analysis. Furthermore,
641 understanding the biological meaning of the unique immune cell dynamics from the blood
642 to the bone marrow during the prolonged high-intensity exercise may lead to an
643 elucidation of a novel mechanism protecting us from various stress.
644

Acknowledgments

We thank G. Schütz for providing B6.129-*Nr3c1*^{tm2Gsc}(*Nr3c1*$^{fl/fl}$) mice, K. Tomari and H. Doi for technical assistance and F. Matsuda, Y. Ishihama, M. Hagiwara, H. Onogi and A. Satoh for discussing our study design. This work was supported by the Japan Society for the Promotion of Science KAKENHI (JP19K08790, JP15H05906 [T.H.], and 263395 [K.K.]), Grants-in-Aid for Scientific Research (15H05790, 15H1155, 15K15417 [K.K.], and JP15H05897), Japan Science, Japan Agency for Medical Research and Development (AMED) (19ek0410062s0201 [T.H.], 16ek0410011h0003, and 16he0902003h0002 [K.K.]), Takeda Science Foundation, and AMED-CREST under Grant Number JP19gm1210006.

Author contributions

A.A. designed and performed experiments and wrote the paper. G.E., T.D., Y.Y., T.N., S.N., A.O., A.K. discussed the data and wrote the paper. M.M. and N.M. performed LC-MS/MS analysis. N.K. and Y.K. discussed the data and lectured the protocol of virus experiments. T.O. provided *Cd11c*-Cre mice, discussed the data and wrote the paper. T.N. provided *Cxcl12*-GFP mice, discussed the data, and wrote the paper. K.I. provided *Nr3c1*$^{fl/fl}$ mice, discussed the data, and wrote the paper. T.H. and K.K. conceptualized and supervised studies, designed some experiments, discussed the data, and wrote the paper.

667 **References**

668 1. Pape K, Ryttergaard L, Rotevatn TA, Nielsen BJ, Torp-pedersen C, Overgaard C,
669 et al. Leisure-time physical activity and the risk of suspected bacterial infections.
670 Med Sci Sport Exerc. 2016;48:1737–44.
671 2. Kostka T, Berthouze S, Lacour J, Bonnefoy M. The symptomatology of upper
672 respiratory tract infections and exercise in elderly people. Med Sci Sport Exerc.
673 2000;32:46–51.
674 3. Peters EM, Bateman E. Ultramarathon running and upper respiratory tract
675 infections. An epidemiological survey. S Afr Med J. 1983;64:582–4.
676 4. Nieman DC. Infectious episodes in runners before and after the Los Angeles
677 Marathon. J Sport Med Phys Fit. 1990;30:316–28.
678 5. Bonini M, Gramiccioni, Claudia Fioretti D, Ruckert B, Rinaldi M, Akdis C,
679 Todaro A, et al. Asthma, allergy and the Olympics: a 12-year survey in elite
680 athletes. Curr Opin Allergy Clin Immunol. 2015;15:184–92.
681 6. Gatmaitan B, Chason J, Lerner AM. Augmentation of the virulence of murine
682 coxackie-virus B-3 myocardiopathy by exercise. J Exp Med. 1970;131:1121–36.
683 7. Davis JM, Kohut ML, Colbert LH, Jackson DA, Ghaffar A, Mayer EP, et al.
684 Exercise , alveolar macrophage function , and susceptibility to respiratory
685 infection. J Appl Physiol. 1997;83:1461–6.
686 8. Murphy EA, Davis JM, Carmichael MD, Gangemi JD, Ghaffar A, Mayer EP.
687 Exercise stress increases susceptibility to influenza infection. Brain Behav
688 Immun. 2008;22:1152–5.
689 9. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The
690 anti-inflammatory effects of exercise: Mechanisms and implications for the

691 prevention and treatment of disease. Nat Rev Immunol [Internet]. 2011;11:607–
692 10. Available from: http://dx.doi.org/10.1038/nri3041
693 10. Krüger K, Lechtermann A, Fobker M, Völker K, Mooren FC. Exercise-induced
694 redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain
695 Behav Immun. 2008;22:324–38.
696 11. Campbell JP, Turner JE. Debunking the myth of exercise-induced immune
697 suppression: Redefining the impact of exercise on immunological health across
698 the lifespan. Front Immunol. 2018;9:1–21.
699 12. Gabriel H, Urhausen A, Kindermann W. Circulating leucocyte and lymphocyte
700 subpopulations before and after intensive endurance exercise to exhaustion. Eur J
701 Appl Physiol Occup Physiol. 1991;63:449–57.
702 13. Shephard RJ, Shek PN. Effects of exercise and training on natural killer cell
703 counts and cytolytic activity: A meta-analysis. Sport Med. 1999;28:177–95.
704 14. Steensberg A, Toft AD, Bruunsgaard H, Sandmand M, Halkjær-kristensen J,
705 Pedersen BK. Strenuous exercise decreases the percentage of type 1 T cells in the
706 circulation. J Appl Physiol. 2001;91:1708–12.
707 15. Nickel T, Emslander I, Sisic Z, David R, Schmaderer C, Marx N, et al.
708 Modulation of dendritic cells and toll-like receptors by marathon running. Eur J
709 Appl Physiol. 2012;112:1699–708.
710 16. Simpson RJ, Campbell JP, Gleeson M, Krüger K, Nieman DC, Pyne DB, et al.
711 Can exercise affect immune function to increase susceptibility to infection? Exerc
712 Immunol Rev. 2020;26:8–22.
713 17. Pedersen BK, Ullum H. NK cell response to physical activity: possible
714 mechanisms of action. Med Sci Sport Exerc. 1994;26:140–6.

715 18. Peake JM, Neubauer O, Walsh NP, Simpson RJ. Recovery of the immune system
716 after exercise. J Appl Physiol. 2017;122:1077–87.
717 19. Lancaster GI, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT, et
718 al. The physiological regulation of toll-like receptor expression and function in
719 humans. J Physiol. 2005;563:945–55.
720 20. Kohut ML, Martin AE, Senchina DS, Lee W. Glucocorticoids produced during
721 exercise may be necessary for optimal virus-induced IL-2 and cell proliferation
722 whereas both catecholamines and glucocorticoids may be required for adequate
723 immune defense to viral infection. Brain Behav Immun. 2005;19:423–35.
724 21. Schiffer JT, Swan DA, Prlic M, Lund JM. Herpes simplex virus-2 dynamics as a
725 probe to measure the extremely rapid and spatially localized tissue-resident T cell
726 response. Immunol Rev. 2018;285:113–33.
727 22. Hewitt M, Estell K, Davis IC, Schwiebert LM. Repeated bouts of moderate-
728 intensity aerobic exercise reduce airway reactivity in a murine asthma model. Am
729 J Respir Cell Mol Biol. 2010;42:243–9.
730 23. Lund JM, Linehan MM, Iijima N, Iwasaki A. Plasmacytoid dendritic cells
731 provide innate immune protection against mucosal viral infection in situ. J
732 Immunol. 2006;177:7510–4.
733 24. Nandakumar S, Woolard SN, Yuan D, Rouse BT, Kumaraguru U. Natural killer
734 cells as novel helpers in anti-herpes simplex virus immune response. J Virol.
735 2008;82:10820–31.
736 25. Iversen MB, Reinert LS, Thomsen MK, Bagdonaite I, Nandakumar R,
737 Cheshenko N, et al. An innate antiviral pathway acting before interferons at
738 epithelial surfaces. Nat Immunol. 2016;17:150–8.

739 26. Iijima N, Mattei LM, Iwasaki A. Recruited inflammatory monocytes stimulate
740 antiviral Th1 immunity in infected tissue. Proc Natl Acad Sci U S A.
741 2011;108:284–9.
742 27. Vogel K, Thomann S, Vogel B, Schuster P, Schmidt B. Both plasmacytoid
743 dendritic cells and monocytes stimulate natural killer cells early during human
744 herpes simplex virus type 1 infections. Immunology. 2014;143:588–600.
745 28. Gill N, Chenoweth MJ, Verdu EF, Ashkar AA. NK cells require type I IFN
746 receptor for antiviral responses during genital HSV-2 infection. Cell Immunol
747 [Internet]. 2011;269:29–37. Available from:
748 http://dx.doi.org/10.1016/j.cellimm.2011.03.007
749 29. Swiecki M, Wang Y, Gilfillan S, Colonna M. Plasmacytoid Dendritic Cells
750 Contribute to Systemic but Not Local Antiviral Responses to HSV Infections.
751 PLoS Pathog. 2013;9:e1003728.
752 30. Yun TJ, Lee JS, Machmach K, Shim D, Choi J, Wi YJ, et al. Indoleamine 2,3-
753 Dioxygenase-Expressing Aortic Plasmacytoid Dendritic Cells Protect against
754 Atherosclerosis by Induction of Regulatory T Cells. Cell Metab. 2016;23:852–
755 66.
756 31. Yoneyama H, Matsuno K, Zhang Y, Nishiwaki T, Kitabatake M, Ueha S, et al.
757 Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed
758 lymph nodes through high endothelial venules. Int Immunol. 2004;16:915–28.
759 32. Diacovo TG, Blasius AL, Mak TW, Cella M, Colonna M. Adhesive mechanisms
760 governing interferon- producing cell recruitment into lymph nodes. J Exp Med.
761 2005;202:687–96.
762 33. Umemoto E, Otani K, Ikeno T, Verjan Garcia N, Hayasaka H, Bai Z, et al.

763 Constitutive plasmacytoid dendritic cell migration to the splenic white pulp is
764 cooperatively regulated by CCR7- and CXCR4-mediated signaling. J Immunol.
765 2012;189:191–9.
766 34. Vermi W, Riboldi E, Wittamer V, Gentili F, Luini W, Marrelli S, et al. Role of
767 ChemR23 in directing the migration of myeloid and plasmacytoid dendritic cells
768 to lymphoid organs and inflamed skin. J Exp Med. 2005;201:509–15.
769 35. Matsutani T, Tanaka T, Tohya K, Otani K, Jang MH, Umemoto E, et al.
770 Plasmacytoid dendritic cells employ multiple cell adhesion molecules
771 sequentially to interact with high endothelial venule cells - molecular basis of
772 their trafficking to lymph nodes. Int Immunol. 2007;19:1031–7.
773 36. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et
774 al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large
775 amounts of type I interferon. Nat Med. 1999;5:919–23.
776 37. López-Cotarelo P, Gómez-Moreira C, Criado-García O, Sánchez L, Rodríguez-
777 Fernández JL. Beyond chemoattraction: multifunctionality of chemokine
778 receptors in leukocytes. Trends Immunol. 2017;38:927–41.
779 38. Chopin M, Preston SP, Lun ATLL, Tellier J, Smyth GK, Pellegrini M, et al.
780 RUNX2 mediates plasmacytoid dendritic cell egress from the bone marrow and
781 controls viral immunity. Cell Rep. 2016;15:866–78.
782 39. Dempsey JA, Wagner PD. Exercise-induced arterial hypoxemia. J Appl Physiol.
783 1999;87:1997–2006.
784 40. Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, et al.
785 Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med.
786 2003;198:1391–402.

41. Niedzielska M, Raffi FAM, Tel J, Muench S, Jozefowski K, Alati N, et al. Selective expression of the MAPK phosphatase Dusp9/MKP-4 in mouse plasmacytoid dendritic cells and regulation of IFN-β production. J Immunol. 2015;195:1753–62.

42. Nakai A, Hayano Y, Furuta F, Noda M, Suzuki K. Control of lymphocyte egress from lymph nodes through β2-adrenergic receptors. J Exp Med. 2014;211:2583–98.

43. Guo B, Huang X, Cooper S, Broxmeyer HE. Glucocorticoid hormone-induced chromatin remodeling enhances human hematopoietic stem cell homing and engraftment. Nat Med [Internet]. 2017;23:424–8. Available from: http://dx.doi.org/10.1038/nm.4298

44. Shiah Y, Tharmapalan P, Casey AE, Joshi PA, Mckee TD, Jackson HW, et al. A progesterone-CXCR4 axis controls mammary progenitor cell fate in the adult gland. Stem Cell Reports [Internet]. 2015;4:313–22. Available from: http://dx.doi.org/10.1016/j.stemcr.2015.01.011

45. Zhou WH, Wu X, Hu WD, Du MR. Co-expression of CXCR4 and CXCR7 in human endometrial stromal cells is modulated by steroid hormones. Int J Clin Exp Pathol. 2015;8:2449–60.

46. Besedovsky L, Linz B, Born J, Lange T. Mineralocorticoid receptor signaling reduces numbers of circulating human naïve T cells and increases their CD62L, CCR7, and CXCR4 expression. Eur J Immunol. 2014;44:1759–69.

47. Shimba A, Cui G, Tani-ichi S, Ogawa M, Abe S, Okazaki F, et al. Glucocorticoids drive diurnal oscillations in T cell distribution and responses by inducing interleukin-7 receptor and CXCR4. Immunity. 2018;48:1–13.

48. Lim HY, Müller N, Herold MJ, Van Den Brandt J, Reichardt HM. Glucocorticoids exert opposing effects on macrophage function dependent on their concentration. Immunology. 2007;122:47–53.
49. Dhabhar FS, Mcewen BS. Enhancing versus suppressive effects of stress hormones on skin immune function. Proc Natl Acad Sci U S A. 1999;96:1059–64.
50. Harpur CM, Kato Y, Dewi ST, Stankovic S, Johnson DN, Bedoui S, et al. Classical type 1 dendritic cells dominate priming of Th1 responses to herpes simplex virus type 1 skin infection. J Immunol. 2019;202:653–63.
51. Zhao X, Deak E, Soderberg K, Linehan M, Spezzano D, Zhu J, et al. Vaginal submucosal dendritic cells, but not langerhans cells, induce protective Th1 responses to herpes simplex virus-2. J Exp Med. 2003;197:153–62.
52. Casanova-Acebes M, Pitaval C, Weiss LA, Nombela-Arrieta C, Chèvre R, A-González N, et al. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. Cell. 2013;153:1025–35.
53. Collins N, Han SJ, Enamorado M, Link VM, Huang B, Moseman EA, et al. The bone marrow protects and optimizes immunological memory during dietary restriction. Cell [Internet]. 2019;178:1088–101. Available from: https://doi.org/10.1016/j.cell.2019.07.049
54. Ara T, Tokoyoda K, Sugiyama T, Egawa T, Kawabata K, Nagasawa T. Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny. Immunity. 2003;19:257–67.
55. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. J Exp Med. 2007;204:1653–

64.

56. Ryan HE, Poloni M, McNulty W, Elson D, Gassmann M, Arbeit JM, et al. Hypoxia-inducible factor-1α is a positive factor in solid tumor growth. Cancer Res. 2000;60:4010–5.

57. Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, et al. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat Genet. 1999;23:99–103.

58. Schefer V, Talan MI. Oxygen consumption in adult and aged C57BL/6J mice during acute treadmill exercise of different intensity. Exp Gerontol. 1996;31:387–92.

59. Plowman SA, Smith DL. Exercise physiology for health, fitness, and performance. fourth. Philadelphia: Lippincott Williams & Wilkins; 2014.

60. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. Med Sci Sports Exerc. 2011;43:1334–59.

61. Nishiyama Y, Sugiura Y, Watanabe D, Miyake Y, Goshima F, Honda T, et al. US3 protein kinase of herpes simplex virus type 2 plays a role in protecting corneal epithelial cells from apoptosis in infected mice. J Gen Virol. 1999;80:51–6.

62. Kawaguchi Y, Van Sant C, Roizman B. Eukaryotic elongation factor 1δ is hyperphosphorylated by the protein kinase encoded by the U(L)13 gene of herpes simplex virus 1. J Virol. 1998;72:1731–6.

63. Morrison LA, Da Costa XJ, Knipe DM. Influence of mucosal and parenteral immunization with a replication- defective mutant of HSV-2 on immune responses and protection from genital challenge. Virology. 1998;243:178–87.
64. Iijima N, Linehan MM, Saeland S, Iwasaki A. Vaginal epithelial dendritic cells renew from bone marrow precursors. Proc Natl Acad Sci U S A [Internet]. 2007;104:19061–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2141908&tool=pmcentrez&rendertype=abstract
65. Grebe KM, Takeda K, Hickman HD, Bailey AM, Embry AC, Bennink JR, et al. Sympathetic nervous system increases proinflammatory cytokines and exacerbates influenza A virus pathogenesis. J Immunol. 2010;184:540–4.
66. Liu Q, Li Z, Gao J, Wan W, Ganesan S, McDermott DH, et al. CXCR4 antagonist AMD3100 redistributes leukocytes from primary immune organs to secondary immune organs, lung, and blood in mice. Eur J Immunol. 2015;45:1855–67.
67. Prüss H, Tedeschi A, Thiriot A, Lynch L, Loughhead SM, Stutte S, et al. Spinal cord injury-induced immunodeficiency is mediated by a sympathetic-neuroendocrine adrenal reflex. Nat Neurosci. 2017;20:1549–59.
68. Quattrocelli M, Barefield DY, Warner JL, Vo AH, Hadhazy M, Earley JU, et al. Intermittent glucocorticoid steroid dosing enhances muscle repair without eliciting muscle atrophy. J Clin Invest. 2017;127:2418–32.
69. Markey KA, Towle AC, Sze PY. Glucocorticoid influence on tyrosine hydroxylase activity in mouse locus coeruleus during postnatal development. Endocrinology. 1982;111:1519–23.

70. Bexis S, Docherty JR. Role of α1- and β3-adrenoceptors in the modulation by SR59230A of the effects of MDMA on body temperature in the mouse. Br J Pharmacol. 2009;158:259–66.

71. Spear PG, Manoj S, Yoon M, Taylor JM, Lin E, Susmarski N. Alternative entry receptors for herpes simplex virus and their roles in infection and disease. Vol. 2, Future Virology. 2007. p. 509–17.

72. Koal T, Schmiederer D, Pham-tuan H, Röhring C, Rauh M. Standardized LC–MS/MS based steroid hormone profile-analysis. J Steroid Biochem Mol Biol [Internet]. 2012;129:129–38. Available from: http://dx.doi.org/10.1016/j.jsbmb.2011.12.001

73. Wobbrock J, Findlater L, Gergle D, Higgins J. The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. In: Proceedings of the ACM Conference on Human Factors in Computing Systems (CHI '11). 2011. p. 143–6.

Figure legends

Figure 1. Prolonged high-intensity exercise induces impaired or enhanced immunity against HSV-2 infection depending on the interval between infection and exercise.

(A) Experimental design. Mice were intravaginally infected with HSV-2 and ran on a treadmill 8 or 17 h after infection. Then, the indicated experiments were performed. Created with BioRender.com. (B-D) Mean clinical scores, survival rates and viral titers in vaginal wash. (B) Prolonged high-intensity exercise (PROLONGED) was performed 17 h after HSV-2 intravaginal infection (CONTROL: n=21; PROLONGED: n=22), (C) short periods of moderate-intensity exercise (SHORT) was performed 17 h after HSV-2 intravaginal infection (n=22 per group), and (D) prolonged high-intensity exercise was performed 8 h after HSV-2 intravaginal infection (n=26 per group). The results were pooled from three independent experiments. Error bars represent the mean ± the standard error of the mean (SEM). *P<0.05, **P<0.01, and n.s., not significant (Mann-Whitney test for clinical scores and viral titers and log rank test for survival rates (B-D)).

Figure 2. Late exposure to prolonged high-intensity exercise impairs pDC infiltration in the vagina after HSV-2 infection.

(A) Flow cytometric analysis of the number of indicated cells in the vagina 24 h after HSV-2 infection. MOCK: mock-infected with Vero cell lysate. (B) Quantitative PCR analysis of *Ifna* mRNA expression in the vagina 24 h after HSV-2 infection. (C) Mean clinical scores, survival rates and viral titers in vaginal wash. (C) Mice were injected with either isotype antibodies or anti-PDCA-1 antibodies and prolonged high-intensity exercise was performed 17 h after HSV-2 intravaginal infection (Isotype CONTROL: n=23; Isotype PROLONGED: n=21; Ab CONTROL: n=21; Ab PROLONGED: n=18).

(D) Representative flow cytometric panels of pDCs and the number of pDCs in the blood immediately after late exposure to prolonged high-intensity exercise. (E) Flow cytometric analysis of the number of pDCs in the blood immediately after late exposure to short periods of moderate-intensity exercise. Data are representative of three independent experiments (A, D, E) and pooled from two to three independent experiments (B, C). Error bars represent the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 and n.s., not significant (Dunn's test with Holm adjustment after Kruskal-Wallis test (A, B) or Mann-Whitney test (C, D)).

Figure 3. Exacerbation of HSV-2 infection induced by prolonged high-intensity exercise is mediated through pDC homing to the bone marrow via the CXCL12-CXCR4 axis

(A) The number of transferred pDCs in the bone marrow immediately after late exposure to prolonged high-intensity exercise. (B) A representative histogram (left panel) and the mean fluorescence intensity (MFI) (right panel) of CXCR4 expression on pDCs in the blood 2 h after the start of prolonged high-intensity exercise. (C) Immunohistochemical analysis of pDCs in the bone marrow (left panel) and the mean distance from the transferred tdTomato$^+$PDCA1$^+$ cells to the nearest GFP$^+$ cells in the bone marrow of *Cxcl12*-GFP mice (right panel). Scale bar = 50 μm. (D) The number of transferred pDCs immediately after prolonged high-intensity exercise. (E) Mean clinical scores, viral titers in vaginal washes, and survival rates in mice that did or did not receive AMD3100 (CONTROL Vehicle: n=22; PROLONGED Vehicle: n=21; CONTROL AMD3100: n=22; PROLONGED AMD3100: n=21). Error bars represent the mean ± SD (A, B, D) and mean ± SEM (E). The results were representative of two to three independent experiments

(A-D) or were pooled from three independent experiments (E). *P<0.05, **P<0.01, ***P<0.001, and n.s., not significant (Mann-Whitney test (A-E)).

Figure 4. GR signaling regulates the expression of CXCR4 on pDCs.

(A) Flow cytometric analysis of CXCR4 expression on pDCs in the blood 2 h after the start of prolonged high-intensity exercise (PROLONGED) with or without RU486 (30 mg/kg). Representative histograms (left panel) and MFI of CXCR4 expression (right panel) are shown. (B) Representative histograms (left panel) and MFI (right panel) of CXCR4 expression on pDCs in the blood 3 h after the administration of vehicle or corticosterone (10 mg/kg), as analyzed by flow cytometry. (C) Transwell migration assay. Whole blood cells were lysed and pretreated with vehicle or corticosterone at the indicated concentration for 3 h and were placed in the upper chamber of the transwell for 1 h. The number of pDCs in the lower chamber containing CXCL12 (10 ng/mL) was evaluated by flow cytometry. Each dot represents a single mouse. Error bars represent the mean ± SD. The results are representative of two to three independent experiments. *P<0.05, **P<0.01, ***P<0.001, and n.s., not significant (Mann-Whitney test (A-C) and the aligned rank transform (ART) procedure using analysis of variance (ANOVA) for the interaction effect (A)).

Figure 5. Loss of GR signaling in CD11c-expressing cells abrogates the effects of late exposure to prolonged high-intensity exercise on HSV-2 infection.

(A) Representative histograms (left panel) and MFI (right panel) of CXCR4 expression on pDCs in the blood 2 h after the start of prolonged high-intensity exercise (PROLONGED) in *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice and *Nr3c1*$^{fl/fl}$ mice. (B) The number of

pDCs in the blood and in the bone marrow in *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice and *Nr3c1*$^{fl/fl}$ mice immediately after prolonged high-intensity exercise. Each dot represents a single mouse. (C) Mean clinical scores, viral titers in vaginal washes, and survival rates in the intravaginal HSV-2 infection model with *Nr3c1*$^{fl/fl}$ mice and *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice (CONTROL *Nr3c1*$^{fl/fl}$ mice: n=27; PROLONGED *Nr3c1*$^{fl/fl}$ mice: n=25; CONTROL *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice: n=27; PROLONGED *Nr3c1*$^{fl/fl}$*Cd11c*-Cre^{+} mice: n=27). The results are representative of three independent experiments (A, B) or were pooled from three independent experiments (C). Error bars represent the mean ± SD (A, B) and the mean ± SEM (C). **P<0.01, ***P<0.001, ****P<0.0001, and n.s., not significant (Mann-Whitney test (A-C), ART procedure using ANOVA for the interaction effect (A) and log rank test for survival rates (C)).

Figure 6. Glucocorticoids are involved in enhanced antiviral immunity induced by early exposure to prolonged high-intensity exercise by an increase in circulating pDCs

(A) Flow cytometric analysis of the number of pDCs in the blood before (-3 h) and after prolonged high-intensity exercise at the indicated time points (n=5). (B) Flow cytometric analysis of the number of pDCs in the blood 12 h after the start of exercise. Mice were administered vehicle or RU486 (30 mg/kg) 1 h before exercise. (C) Mean clinical scores, survival rates, and viral titers in vaginal washes. Mice were administered vehicle or RU486 (30 mg/kg) 1 h before prolonged high-intensity exercise (PROLONGED) (CONTROL Vehicle: n=16; PROLONGED Vehicle: n=14; CONTROL RU486: n=16; PROLONGED RU486: n=14). Each dot represents a single mouse (A, B). Error bars represent the mean ± SD (A, B) and the mean ± SEM (C). The results are representative

of two independent experiments (A**)** or were pooled from two to three (B, C) independent experiments. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, and n.s., not significant (Mann-Whitney test (A-C) and log rank test for survival rates (C)).

Figure 7. A graphical summary.

Prolonged high-intensity exercise increases the level of glucocorticoids in the blood. Glucocorticoids upregulate CXCR4 expression on pDCs in the blood, which facilitates their homing to the bone marrow and their decrease in the blood. The decrease in pDCs in the blood leads to the impaired infiltration of pDCs to infected sites (vagina), causing the exacerbation of HSV-2 infection. The glucocorticoid-induced pDC homing to the bone marrow, on the other hand, drives the recovery of pDCs in the blood approximately 6-12 h after exercise, leading to enhanced immune responses against HSV-2 infection. Created with BioRender.com.

Legend to Supplementary Figures

Figure S1. Interferon production and Toll-like receptor (TLR) 9 expression in pDCs and the number of pDCs in the blood, spleen, liver, vagina, muscle, lymph node, and lung after prolonged high-intensity exercise.

(A) Weight loss during the exercise in early and late exposure group (n=15). (B) Flow cytometric analysis of intracellular IFN-α in pDCs. (C) Representative histogram (left panel) and MFI (right panel) of TLR9 expression in pDCs in the blood immediately after prolonged high-intensity exercise. (D) Flow cytometric analysis of the number of pDCs, cDCs, NK cells, neutrophils and monocytes in the blood (day 0) of mice. (E) Flow cytometric analysis of the number of the number of transferred NK cells, cDCs, neutrophils and monocytes in the bone marrow immediately after late exposure to prolonged high-intensity exercise. (F) Flow cytometric analysis of the number of transferred pDCs in the indicated tissues immediately after prolonged high-intensity exercise. Each dot represents a single mouse (B-F). The results were pool of three independent experiments (A) or were representative of two to three independent experiments (B-F). Error bars represent the mean ± standard deviation (SD) (A-F). *P<0.05, **P<0.01, ***P<0.001, and n.s., not significant (Mann-Whitney test (A-F)).

Figure S2. The expression levels of CCR5, CXCR3 in the blood and their ligands in the vagina after prolonged high-intensity exercise.

(A) Representative histograms (upper panels) and MFI (lower panels) of CCR5 and CXCR3 on pDCs in the blood, as analyzed by flow cytometry. (B) Quantitative PCR analysis of *Ccl3, Ccl4, Ccl5, Cxcl9, Cxcl10*, and *Cxcl11* in the vagina 24 h after HSV-2 infection. Each dot represents a single mouse. Error bars represent the mean ± SD. Data are representative of two to three independent experiments. Mann-Whitney test (A) and Dunn's test with Holm adjustment after Kruskal-Wallis test (B).

Figure S3. The expression of chemokine receptors and adhesion molecules on pDCs after prolonged high-intensity exercise and the concentration of CXCL12 in the bone marrow.

(A) Flow cytometric analysis of the expression levels of CCR2, CCR7, CCR9, ChemR23, LFA1, VLA4, and CD62L on pDCs in the blood immediately after prolonged high-intensity exercise. MFIs are shown. (B) The concentration of CXCL12 in the bone marrow. Immediately after prolonged high-intensity exercise, femurs and tibias were flushed with PBS, and the concentration of CXCL12 in extracellular fluids was analyzed by ELISA. (C) Flow cytometric analysis of the number of pDCs, NK cells, cDCs,

neutrophils and inflammatory monocytes in the vagina 24 h after mock infection in mice treated with vehicle or AMD3100 17 h after mock infection. Each dot represents a single mouse. Error bars represent the mean ± SD. These results are representative of two to three independent experiments. n.s., not significant (Mann-Whitney test).

Figure S4. HIF-1α is not involved in the upregulation of CXCR4 expression on pDCs after prolonged high-intensity exercise.

Flow cytometric analysis of CXCR4 expression on pDCs in the blood 2 h after the start of prolonged high-intensity exercise (PROLONGED) in *Hif1a*$^{fl/fl}$ or *Hif1a*$^{fl/fl}$-*Cd11c*-Cre^{+} mice. Each dot represents a single mouse. Error bars represent the mean ± SD. Data are representative of three independent experiments. *P<0.05, and n.s., not significant (Mann-Whitney test and ART procedure using ANOVA for the interaction effect).

Figure S5. Blood levels of noradrenaline and steroid hormones, and the effects of antagonists for adrenergic receptor and mineralocorticoid receptor on CXCR4 expression on pDCs after prolonged high-intensity exercise.

(A) Immediately after prolonged high-intensity exercise, whole blood was harvested, and serum or plasma was isolated by centrifugation. Then, serum was subjected to LC-

MS/MS analysis to analyze steroid hormones, and plasma was subjected to ELISA to analyze noradrenaline. (B) Flow cytometric analysis of CXCR4 expression on pDCs in the blood 2 h after the start of prolonged high-intensity exercise. Mice were administered either vehicle, SR59230A (β3-adrenergic receptor (AR) antagonist) (5 mg/kg), propranolol (β1- and β2-AR antagonist) (10 mg/kg), 6-OHDA (sympathectomy), or eplerenone (mineralocorticoid receptor antagonist) (1 mg/kg). Each dot represents a single mouse. Error bars represent the mean ± SD. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, and n.s., not significant (Mann-Whitney test (A, B), and ART procedure using ANOVA for the interaction effect (B).

Figure S6. Upregulation of CXCR4 expression on pDCs by corticosterone, and blood levels of corticosterone over time during and after prolonged high-intensity exercise or short periods of moderate-intensity exercise.

(A) Representative histogram and flow cytometric panels of CXCR4 expression on pDCs treated with corticosterone. Whole blood cells from naïve wild-type mice were lysed and incubated with vehicle or corticosterone for 3 h at the indicated concentration. The cells were then subjected to flow cytometric analysis. (B) MFI of CXCR4 expression on NK cells, cDCs, neutrophils and monocytes in the blood 3 h after the administration of vehicle

or corticosterone (10 mg/kg), as analyzed by flow cytometry. Each dot represents a single mouse. (C) Time course of the blood level of corticosterone in mice after prolonged high-intensity exercise (PROLONGED) (n=4-5 per group). The time after the start of exercise is shown. (D) Time course of the blood level of corticosterone in mice with short periods of moderate-intensity exercise (SHORT) (n=4-5 per group). Time after the start of exercise is shown. Data are representative of two-three independent experiments. Error bars represent the mean ± SD. *P<0.05 and **P<0.01 (Mann-Whitney test).

Figure S7. GR signaling mediates the effects of late exposure to prolonged high-intensity exercise on the clinical symptoms of intravaginal HSV-2 infection.

(A) Flow cytometric analysis of the number of transferred pDCs immediately after prolonged high-intensity exercise. (B) Mean clinical scores, viral titers in vaginal washes, and survival rates in the intravaginal HSV-2 infection model in mice administered with vehicle or RU486 (n=21 per group). (C) Flow cytometric analysis of the number of transferred pDCs in the blood and in the bone marrow 3 h after the administration of vehicle or corticosterone (10 mg/kg). (D) Mean clinical scores, viral titers in vaginal washes, and survival rates in mice treated with vehicle or corticosterone (10 mg/kg) 17 and 20 h after HSV-2 infection (VEHICLE: n=28; CORTICOSTERONE: n=26). Each

dot represents a single mouse (A, C). Error bars represent the mean ± SD (A, C) and the mean ± SEM (B, D). These results are representative of two independent experiments (A, C) or were pooled from three independent experiments (B, D). *P<0.05, **P<0.01, and n.s., not significant (Mann-Whitney test (A-D) and log rank test for survival rates (B, D)).

Figure 1

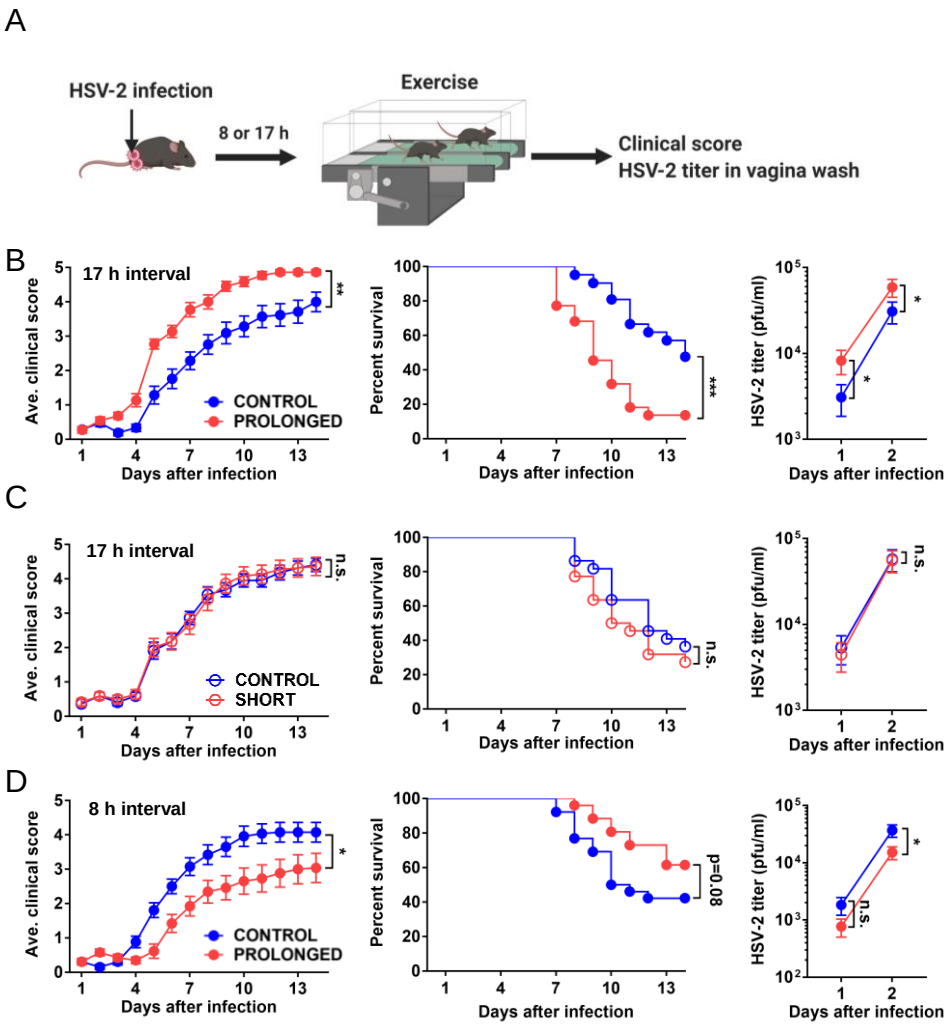

A
HSV-2 infection
8 or 17 h
Exercise
Clinical score
HSV-2 titer in vagina wash
B
17 h interval
Ave. clinical score
Days after infection
CONTROL
PROLONGED
**
Percent survival
***
HSV-2 titer (pfu/ml)
*
C
17 h interval
CONTROL
SHORT
n.s.
D
8 h interval
p=0.08

Figure 2

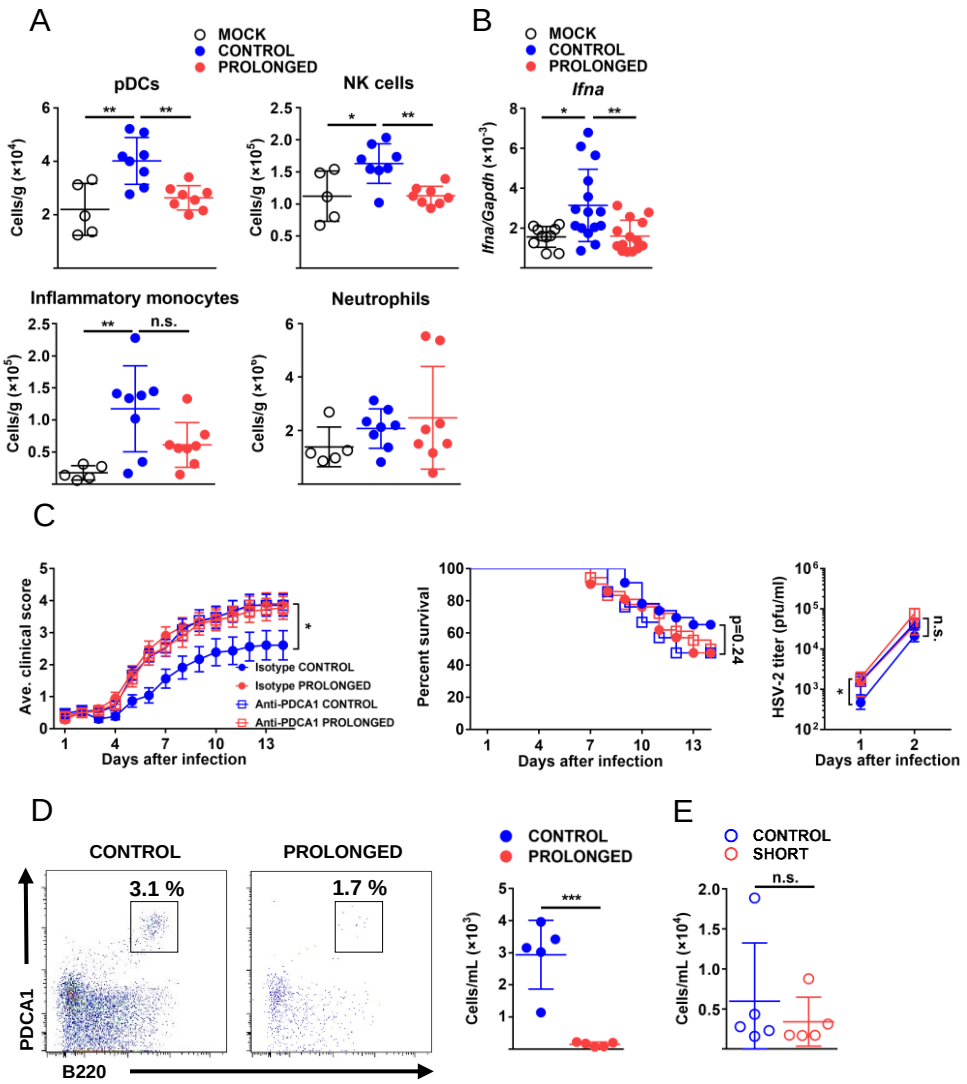

A
MOCK
CONTROL
PROLONGED
pDCs
Cells/g (×10⁴)
NK cells
Cells/g (×10⁵)
Inflammatory monocytes
Cells/g (×10⁵)
Neutrophils
Cells/g (×10⁶)
B
Ifna
Ifna/Gapdh (×10⁻³)
C
Ave. clinical score
Days after infection
Isotype CONTROL
Isotype PROLONGED
Anti-PDCA1 CONTROL
Anti-PDCA1 PROLONGED
Percent survival
p=0.24
HSV-2 titer (pfu/ml)
n.s.
D
CONTROL
PROLONGED
3.1 %
1.7 %
PDCA1
B220
Cells/mL (×10³)
E
CONTROL
SHORT
n.s.
Cells/mL (×10⁴)

Figure 3

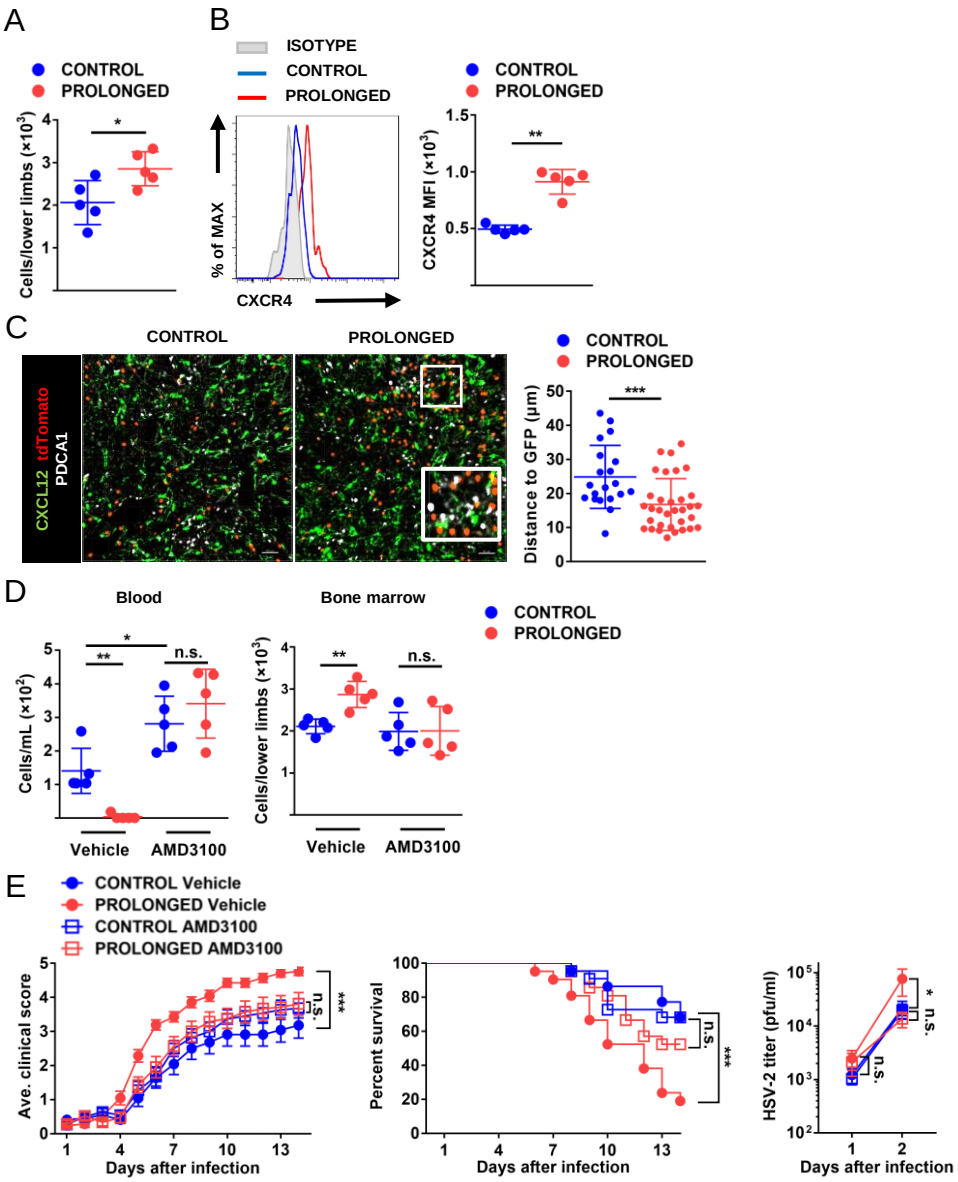

A
CONTROL
PROLONGED
Cells/lower limbs (×10³)
*
B
ISOTYPE
CONTROL
PROLONGED
% of MAX
CXCR4
CONTROL
PROLONGED
CXCR4 MFI (×10³)
**
C
CONTROL
PROLONGED
CXCL12 tdTomato
PDCA1
CONTROL
PROLONGED
Distance to GFP (μm)
***
D
Blood
Bone marrow
CONTROL
PROLONGED
Cells/mL (×10²)
*
**
n.s.
Vehicle
AMD3100
Cells/lower limbs (×10³)
**
n.s.
Vehicle
AMD3100
E
CONTROL Vehicle
PROLONGED Vehicle
CONTROL AMD3100
PROLONGED AMD3100
Ave. clinical score
n.s.
***
Days after infection
Percent survival
n.s.
***
Days after infection
HSV-2 titer (pfu/ml)
n.s.
*
n.s.
Days after infection

Figure 4

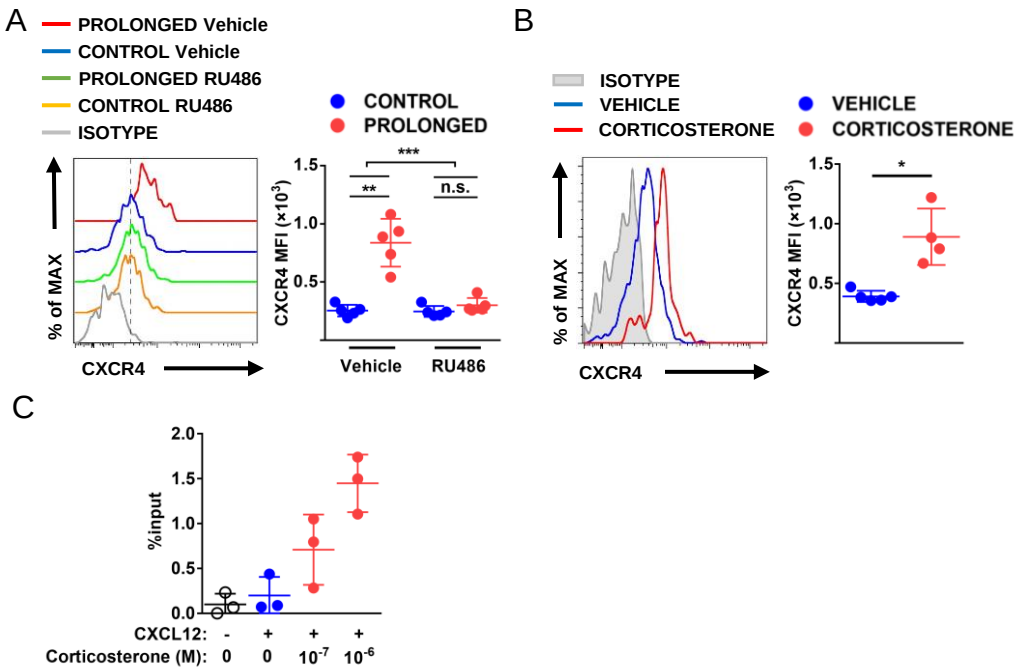

A
PROLONGED Vehicle
CONTROL Vehicle
PROLONGED RU486
CONTROL RU486
ISOTYPE
% of MAX
CXCR4
CONTROL
PROLONGED
CXCR4 MFI (×10³)
***
**
n.s.
Vehicle
RU486
B
ISOTYPE
VEHICLE
CORTICOSTERONE
% of MAX
CXCR4
VEHICLE
CORTICOSTERONE
CXCR4 MFI (×10³)
*
C
%input
CXCL12: - + + +
Corticosterone (M): 0 0 10⁻⁷ 10⁻⁶

Figure 5

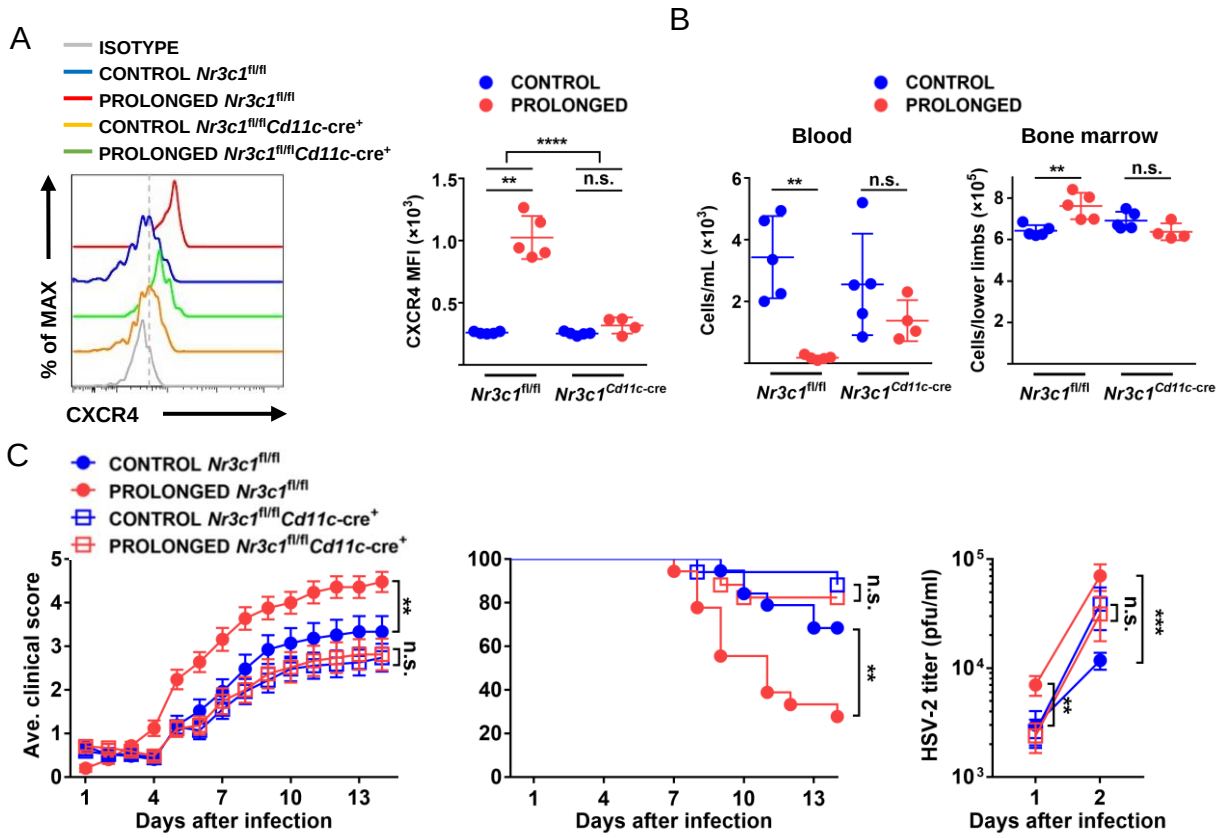

A
ISOTYPE
CONTROL Nr3c1fl/fl
PROLONGED Nr3c1fl/fl
CONTROL Nr3c1fl/flCd11c-cre+
PROLONGED Nr3c1fl/flCd11c-cre+
% of MAX
CXCR4
CONTROL
PROLONGED
****
**
n.s.
CXCR4 MFI (×10³)
Nr3c1fl/fl
Nr3c1Cd11c-cre
B
CONTROL
PROLONGED
Blood
Bone marrow
Cells/mL (×10³)
Cells/lower limbs (×10⁵)
C
CONTROL Nr3c1fl/fl
PROLONGED Nr3c1fl/fl
CONTROL Nr3c1fl/flCd11c-cre+
PROLONGED Nr3c1fl/flCd11c-cre+
Ave. clinical score
Days after infection
HSV-2 titer (pfu/ml)
***

Figure 6

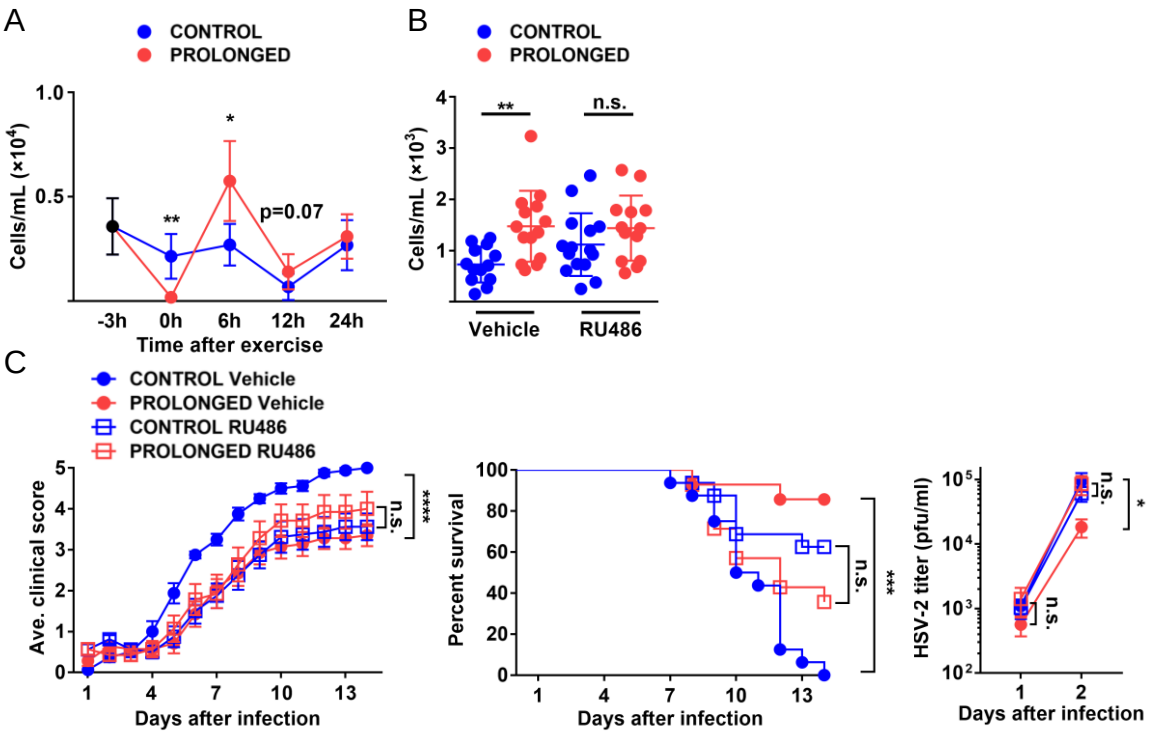

A
CONTROL
PROLONGED
Cells/mL (×10^4)
1.0
0.5
**
*
p=0.07
-3h
0h
6h
12h
24h
Time after exercise
B
CONTROL
PROLONGED
Cells/mL (×10^3)
4
3
2
1
**
n.s.
Vehicle
RU486
C
CONTROL Vehicle
PROLONGED Vehicle
CONTROL RU486
PROLONGED RU486
Ave. clinical score
n.s.
****
Days after infection
Percent survival
n.s.
***
Days after infection
HSV-2 titer (pfu/ml)
n.s.
n.s.
*
Days after infection

Figure 7

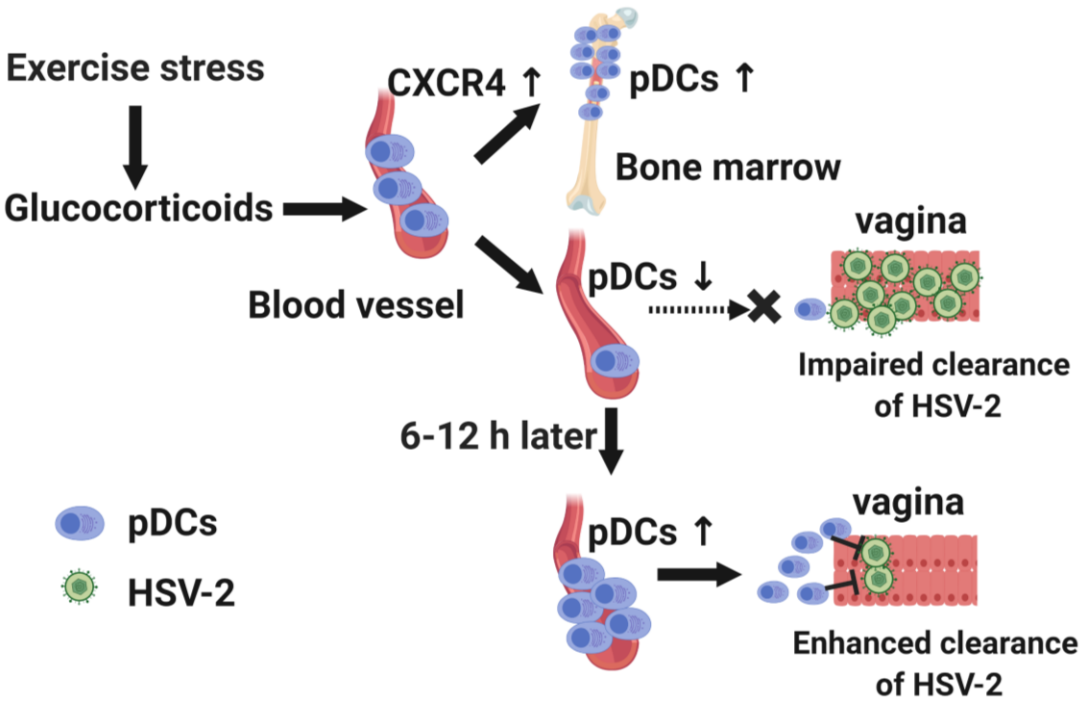

Exercise stress
Glucocorticoids
CXCR4 ↑
pDCs ↑
Bone marrow
Blood vessel
pDCs ↓
vagina
Impaired clearance of HSV-2
6-12 h later
pDCs
HSV-2
pDCs ↑
vagina
Enhanced clearance of HSV-2

Figure S1

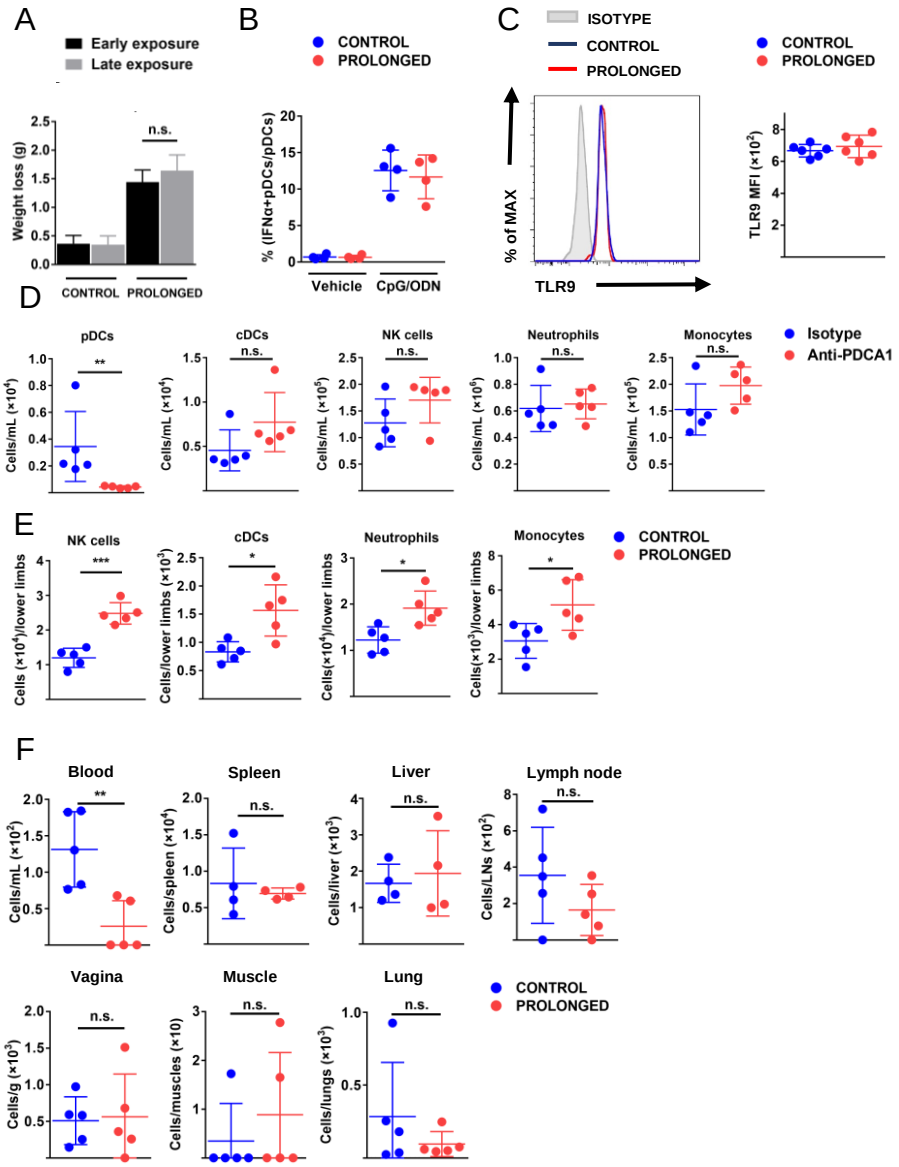

A
Early exposure
Late exposure
Weight loss (g)
n.s.
CONTROL
PROLONGED
B
CONTROL
PROLONGED
% (IFNα+pDCs/pDCs)
Vehicle
CpG/ODN
C
ISOTYPE
CONTROL
PROLONGED
% of MAX
TLR9
CONTROL
PROLONGED
TLR9 MFI (×10²)
D
pDCs
cDCs
NK cells
Neutrophils
Monocytes
Cells/mL
n.s.
Isotype
Anti-PDCA1
E
NK cells
cDCs
Neutrophils
Monocytes
Cells/lower limbs
CONTROL
PROLONGED
F
Blood
Spleen
Liver
Lymph node
Vagina
Muscle
Lung
Cells/mL (×10²)
Cells/spleen (×10⁴)
Cells/liver (×10³)
Cells/LNs (×10²)
Cells/g (×10³)
Cells/muscles (×10)
Cells/lungs (×10³)
CONTROL
PROLONGED

Figure S2

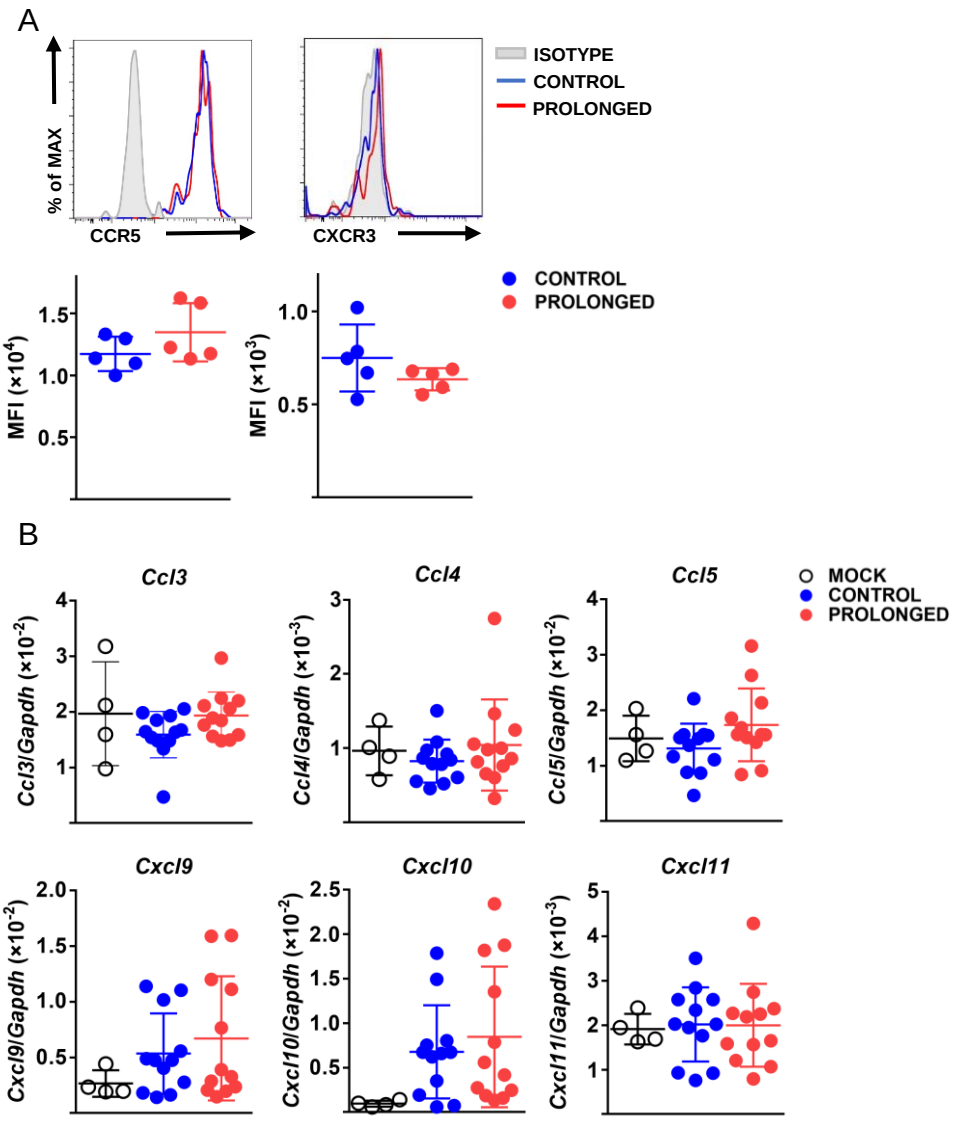

A
% of MAX
CCR5
CXCR3
ISOTYPE
CONTROL
PROLONGED
MFI (×10⁴)
MFI (×10³)
CONTROL
PROLONGED
B
Ccl3
Ccl4
Ccl5
Ccl3/Gapdh (×10⁻²)
Ccl4/Gapdh (×10⁻³)
Ccl5/Gapdh (×10⁻²)
MOCK
CONTROL
PROLONGED
Cxcl9
Cxcl10
Cxcl11
Cxcl9/Gapdh (×10⁻²)
Cxcl10/Gapdh (×10⁻²)
Cxcl11/Gapdh (×10⁻³)

Figure S3

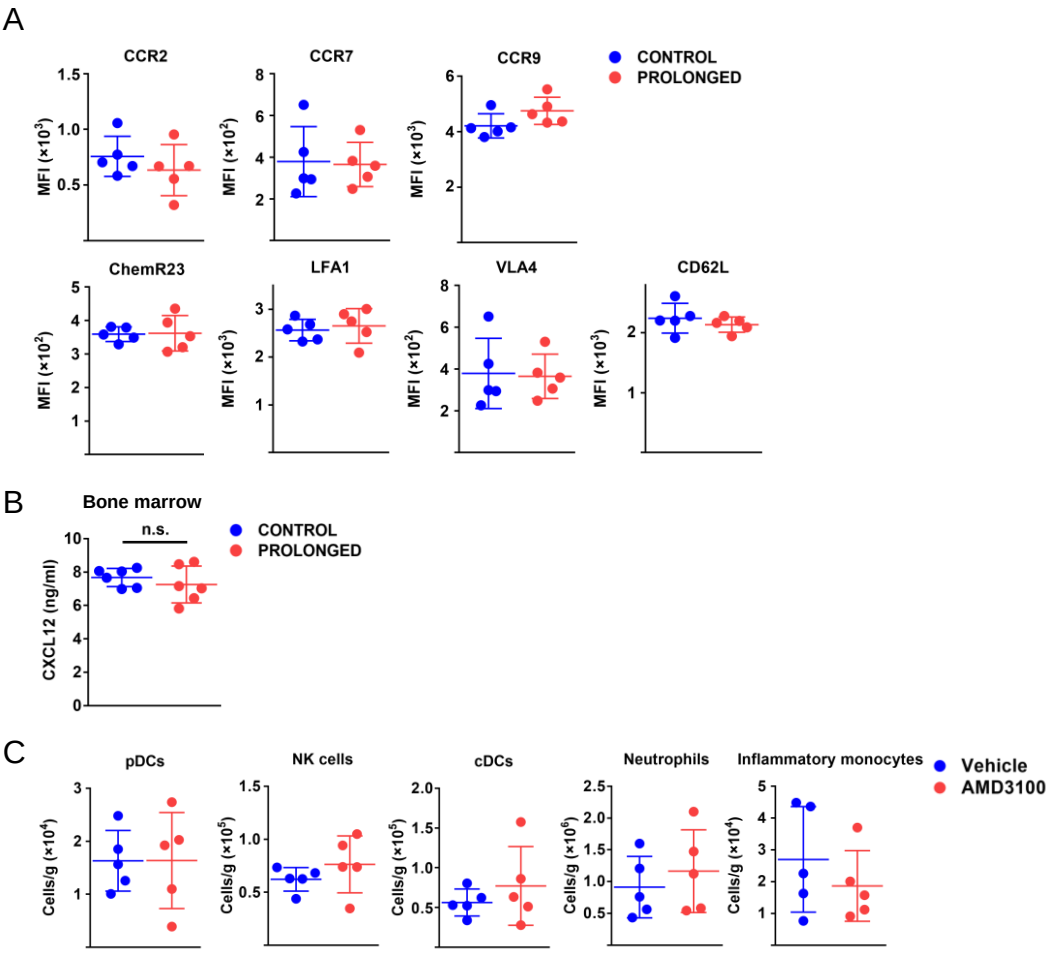

A
CCR2
CCR7
CCR9
CONTROL
PROLONGED
MFI (×10³)
MFI (×10²)
ChemR23
LFA1
VLA4
CD62L
B
Bone marrow
n.s.
CONTROL
PROLONGED
CXCL12 (ng/ml)
C
pDCs
NK cells
cDCs
Neutrophils
Inflammatory monocytes
Vehicle
AMD3100
Cells/g (×10⁴)
Cells/g (×10⁵)
Cells/g (×10⁶)

Figure S4

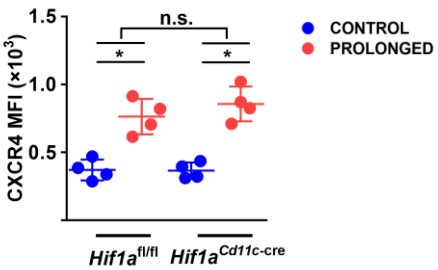

1.5
1.0
0.5
CXCR4 MFI (×10³)
n.s.
*
*
CONTROL
PROLONGED
Hif1a^fl/fl
Hif1a^Cd11c-cre

Figure S5

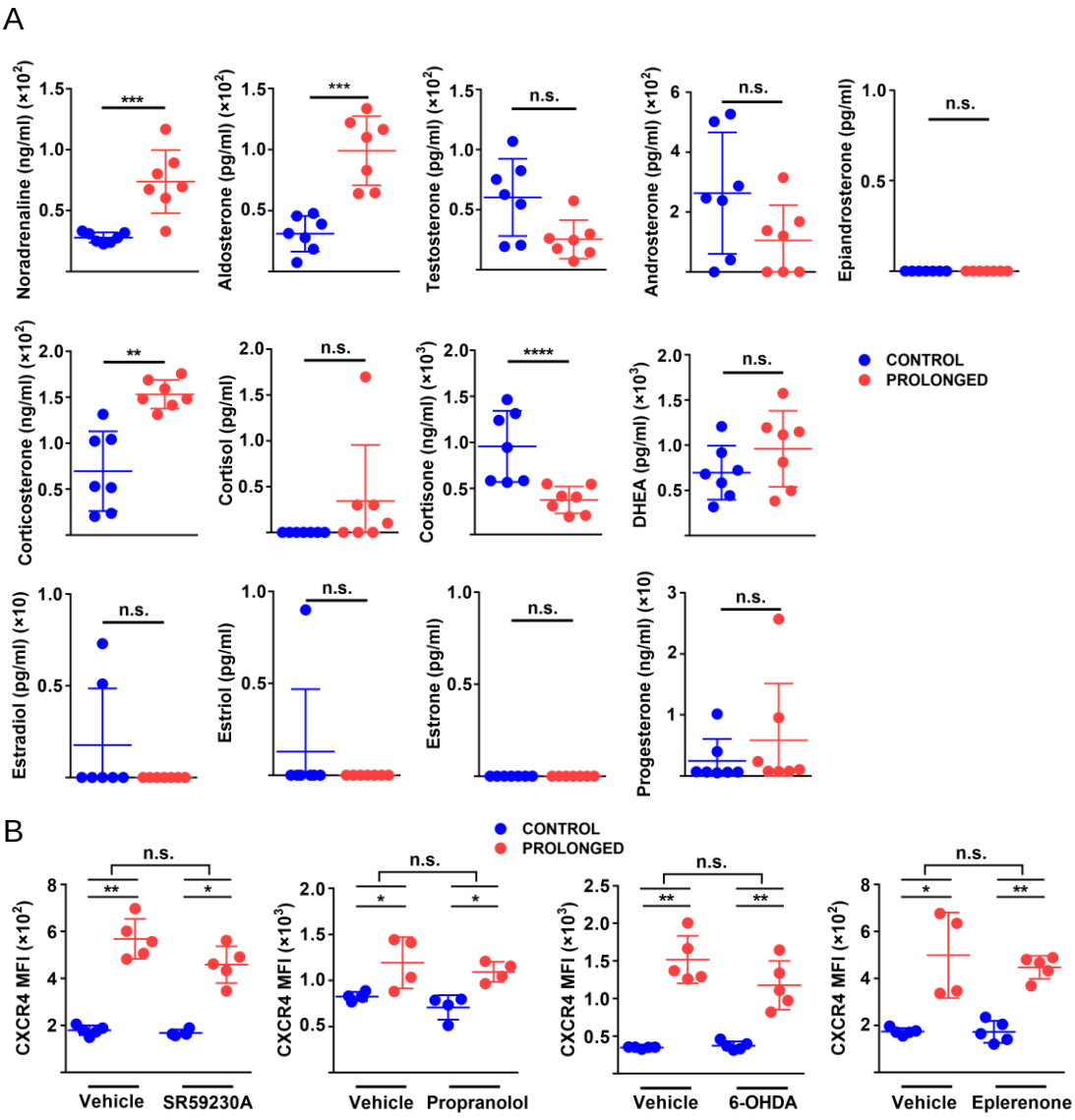

A
Noradrenaline (ng/ml) (×10²)
Aldosterone (pg/ml) (×10²)
Testosterone (pg/ml) (×10²)
Androsterone (pg/ml) (×10²)
Epiandrosterone (pg/ml)
Corticosterone (ng/ml) (×10²)
Cortisol (pg/ml)
Cortisone (ng/ml) (×10³)
DHEA (pg/ml) (×10³)
Estradiol (pg/ml) (×10)
Estriol (pg/ml)
Estrone (pg/ml)
Progesterone (ng/ml) (×10)
n.s.
CONTROL
PROLONGED
B
CXCR4 MFI (×10²)
CXCR4 MFI (×10³)
Vehicle
SR59230A
Propranolol
6-OHDA
Eplerenone

Figure S6

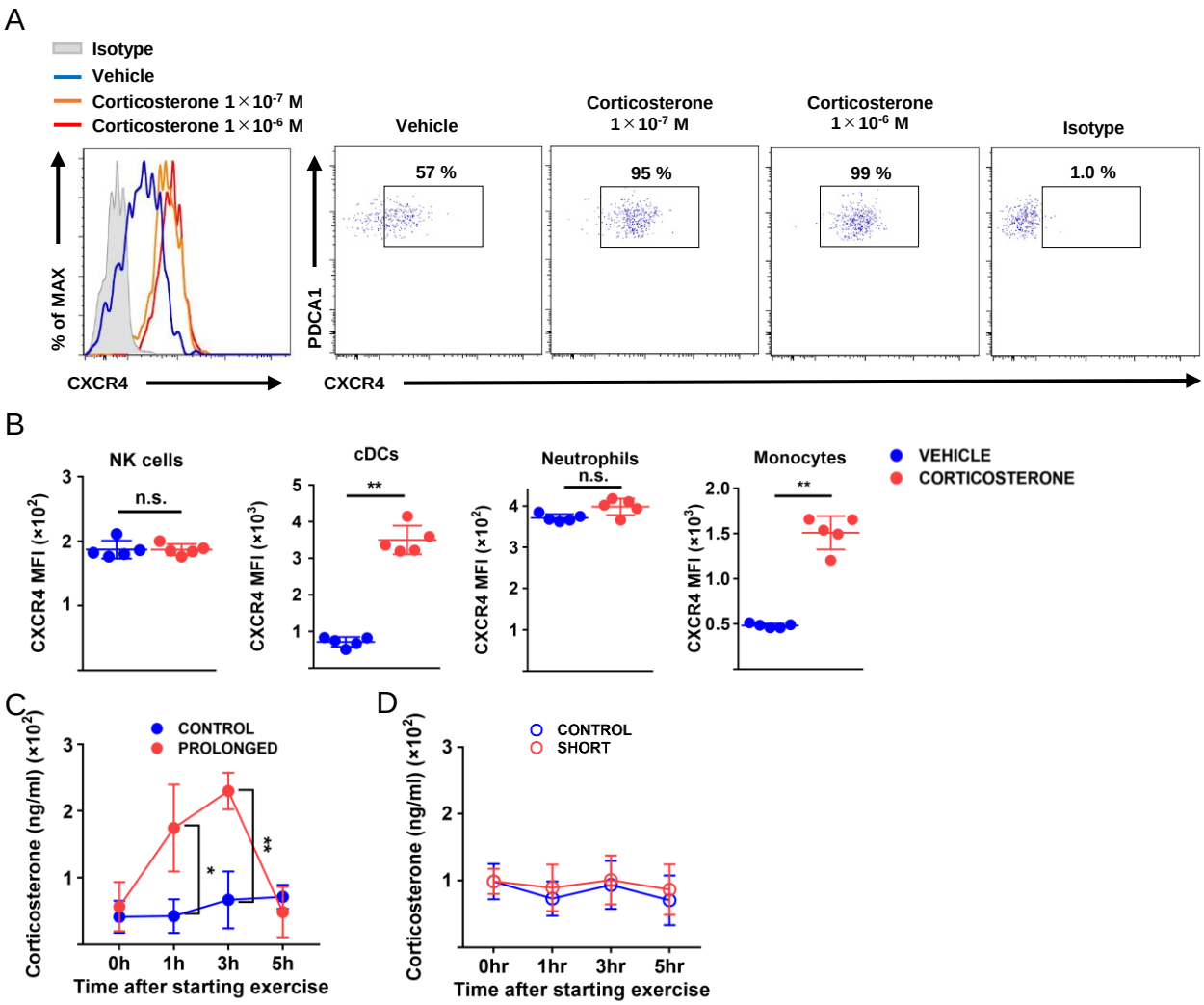
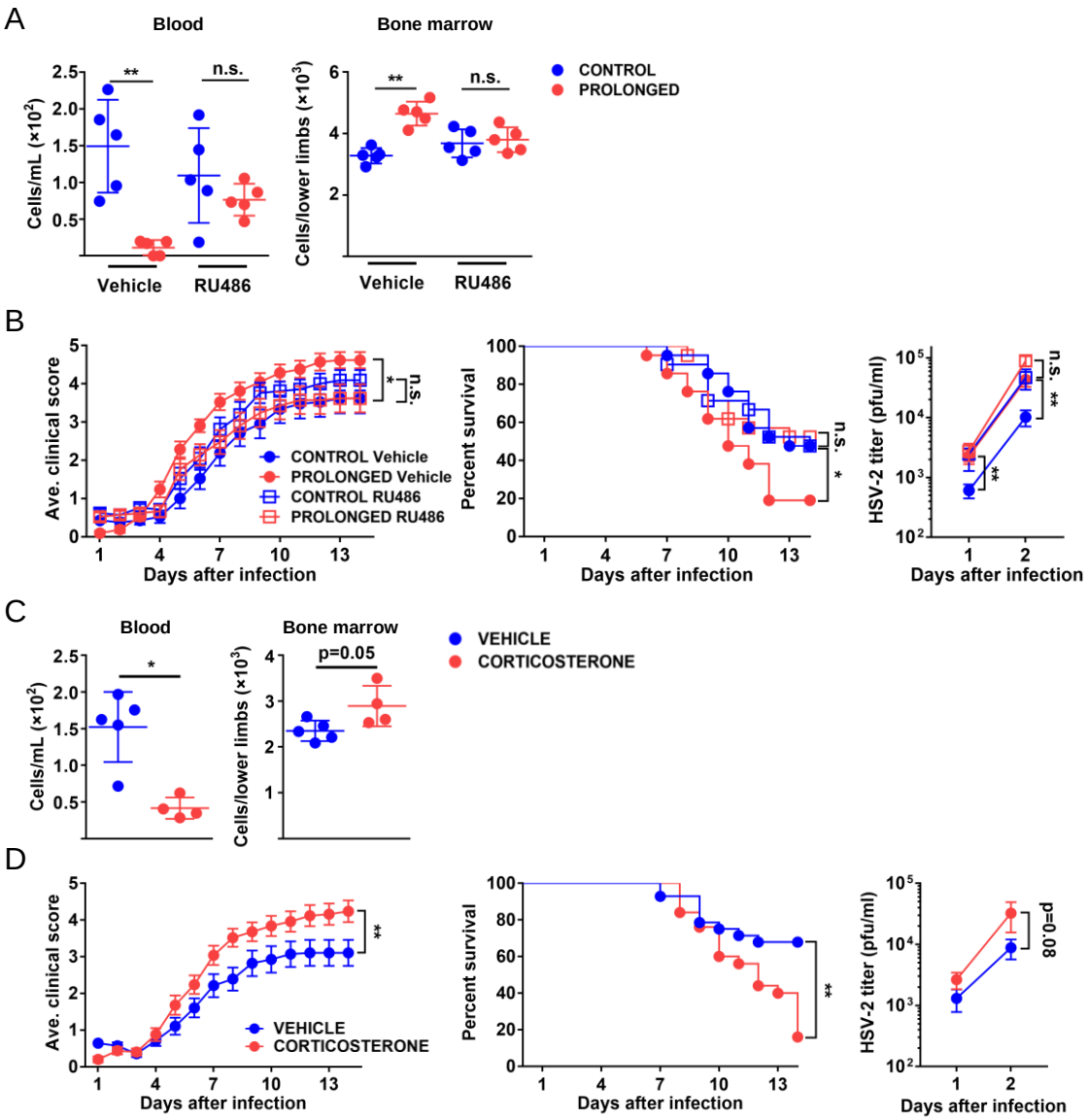

A
Isotype
Vehicle
Corticosterone 1×10-7 M
Corticosterone 1×10-6 M
% of MAX
CXCR4
Vehicle
Corticosterone 1×10-7 M
Corticosterone 1×10-6 M
Isotype
57 %
95 %
99 %
1.0 %
PDCA1
CXCR4
B
NK cells
n.s.
cDCs
**
Neutrophils
n.s.
Monocytes
**
CXCR4 MFI (×102)
CXCR4 MFI (×103)
VEHICLE
CORTICOSTERONE
C
CONTROL
PROLONGED
Corticosterone (ng/ml) (×102)
0h
1h
3h
5h
Time after starting exercise
D
CONTROL
SHORT
Corticosterone (ng/ml) (×102)
0hr
1hr
3hr
5hr
Time after starting exercise

Figure S7


A
Blood
Bone marrow
Cells/mL (×10²)
Cells/lower limbs (×10³)
**
n.s.
Vehicle
RU486
CONTROL
PROLONGED
B
Ave. clinical score
Days after infection
CONTROL Vehicle
PROLONGED Vehicle
CONTROL RU486
PROLONGED RU486
Percent survival
HSV-2 titer (pfu/ml)
C
Blood
Bone marrow
p=0.05
VEHICLE
CORTICOSTERONE
D
p=0.08