BPB Reports

Regular Article

Adoptively Transferred Myeloid-Derived Suppressor Cells Decrease Influenza A Virus Infection Mortality in a Mouse Model

Zhiqi Xie,[a,b] Atsushi Kawai,[c,d,e] Nobumasa Watanabe,[f] Naoki Okada,[b] Yasuo Yoshioka,[c,d,e,g,h,i,j] and Masashi Tachibana[b,h,k,*]

[a]*Key Laboratory of Novel Targets and Drug Study for Neural Repair of Zhejiang, School of Medicine, Hangzhou City University, Hangzhou, Zhejiang, China;* [b]*Project for Vaccine and Immune Regulation, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan;* [c]*Laboratory of Nano-design for Innovative Drug Development, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan;* [d]*Vaccine Creation Group, BIKEN Innovative Vaccine Research Alliance Laboratories, Institute for Open and Transdisciplinary Research Initiatives, Osaka University, Suita, Osaka, Japan;* [e]*Vaccine Creation Group, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan;* [f]*Research Center for Genome & Medical Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan;* [g]*BIKEN Center for Innovative Vaccine Research and Development, The Research Foundation for Microbial Diseases of Osaka University, Suita, Osaka, Japan;* [h]*Global Center for Medical Engineering and Informatics, Osaka University, Osaka, Japan;* [i]*Center for Advanced Modalities and DDS, Osaka University, Suita, Osaka, Japan;* [j]*Center for Infectious Disease Education and Research, Osaka University, Suita, Osaka, Japan;* [k]*Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan*

Received December 25, 2023; Accepted February 19, 2024

In humans, influenza A virus (IAV) causes mild to severe respiratory disease, posing a major threat to public health worldwide. Increasing evidence suggests that myeloid-derived suppressor cells (MDSCs) are involved in viral infection outcomes in genetically modified mice; however, the mechanisms by which MDSCs contribute to lung pathology under normal genetic conditions remain controversial. In the present study, we intranasally infected mice with mouse-adapted IAV (A/Puerto Rico/8/1934 [PR8]) and adoptively transferred MDSCs differentiated *in vitro* intravenously to assess their functional relevance *in vivo*. After PR8 infection, the adoptive transfer of MDSCs significantly improved the survival of mice. Furthermore, MDSC transfer increased $CD4^+$ T cell and eosinophil infiltration into the lungs and decreased interleukin-6 and tumor necrosis factor-α levels in the bronchoalveolar lavage fluid. However, the viral load did not significantly decrease; this suggests that MDSCs affect virus clearance. Inducible nitric oxide synthase (iNOS) is a key factor responsible for the immunosuppressive activity of MDSCs. However, the transfer of *Nos2*-deficient MDSCs can decrease PR8 infection-induced mortality; nevertheless, the absence of iNOS in MDSCs did not affect the infiltration of inflammatory cells into the lung, suggesting that MDSCs function independently of their iNOS expression and downstream pathways. Taken together, our findings suggest that transferred MDSCs decrease IAV disease-induced mortality *in vivo* in an iNOS-independent manner. The adoptive cellular transfer of MDSCs may be an attractive therapeutic strategy for IAV infections.

Key words influenza virus, PR8, myeloid-derived suppressor cells, inducible nitric oxide synthase

INTRODUCTION

In humans, influenza is an infectious respiratory disease that is majorly caused by H1N1 and H3N2 influenza A viruses (IAVs) and influenza B viruses. According to the World Health Organization, these annual epidemics result in 3–5 million cases of severe illness and 290,000–650,000 deaths. In general, influenza is characterized by annual seasonal epidemics and sporadic pandemics involving the zoonotic strains of IAVs; in particular, the avian IAV H7N9 viruses that emerged in 2013 can infect various animal species.[1,2] IAVs primarily target and infect the airway and alveolar epithelial cells, resulting in alveolar epithelial injury that may progress to acute respiratory distress syndrome and even potential death.[3,4] IAV infection triggers the innate immune signaling pathway, producing antiviral and chemotactic molecules such as interferons (IFNs), interleukin (IL)-6, and tumor necrosis factor-alpha (TNF-α). These molecules rapidly recruit innate effector cells such as monocytes, macrophages, neutrophils, dendritic cells (DCs), and natural killer (NK) cells to the infection site to initiate immune responses. Each cell type exhibits unique mechanisms to interact with virus-infected cells, limiting viral replication and priming adaptive immune cells such as T and B cells for antigen-specific immunity and memory.[5,6] However, the regulatory mechanisms underlying these processes remain unelucidated.

Increasing evidence supports the vital roles of myeloid-derived suppressor cells (MDSCs) as immunosuppressive

*To whom correspondence should be addressed. e-mail: tacci@phs.osaka-u.ac.jp

myeloid cells in inflammatory and respiratory viral infections.[7-9] Originally described in patients with cancer, MDSCs are a heterogeneous group of myeloid cells that potently suppress the activation of T cells. In mice, these cells can be categorized into two types: $CD11b^{+}Ly\text{-}6G^{-}Ly\text{-}6C^{hi}$ monocytic MDSCs and $CD11b^{+}Ly\text{-}6G^{+}Ly\text{-}6C^{int}$ polymorphonuclear MDSCs (PMN-MDSCs).[10] During a sublethal dose of A/Puerto Rico/8/1934 (PR8) IAVs in mice, the selective deficiency of invariant NKT cells leads to MDSC expansion, thereby suppressing the proliferation and activation of $CD8^{+}$ T cells. However, studies on the direct involvement of MDSCs in disease progression remain unavailable.[11] MDSCs increased after TLR7-deficient mice were infected with low-dose PR8 IAV and skewed T cell differentiation toward Th2 cells; however, the proliferation and activation of $CD8^{+}$ T cells remain unaffected.[12] Although the abovementioned studies suggest that MDSCs can exacerbate disease progression, these conclusions are based on the correlation between increased MDSCs and various indicators in genetically modified mice; nevertheless, many aspects remain undiscussed. In the present study, we transferred MDSCs and assessed the severity of PR8 IAV infection to clarify the potential cause of the lung pathology of MDSCs under normal genetic conditions and identify the possible therapeutic strategies.

MATERIALS AND METHODS

Mice Inbred male C57BL/6J mice (6–8 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and maintained under specific-pathogen-free conditions. *Nos2* knockout mice were obtained from Jackson Laboratory. All experimental procedures in mice were performed according to the institutional guidelines for animal experiments of Osaka University (Protocol Numbers: BIKEN-AP-R01-15-2 and Douyaku R03-7-2).

***In Vitro* Differentiation of MDSCs** A previously described method was used to differentiate bone marrow (BM) cells into MDSCs *in vitro*.[13] Briefly, BM cells from C57BL/6J mice were stimulated with 40 ng/mL recombinant granulocyte–macrophage colony-stimulating factor (Peprotech, NJ, USA) for 4 days.

PR8 Infection H1N1 influenza virus (strain: PR8) was kindly provided by Dr. Yasuyuki Gomi (the Research Foundation for Microbial Diseases, Osaka University, Kagawa, Japan). Under anesthesia, mice were intranasally challenged with 1.2×10^{1} $TCID_{50}$ of PR8 in 30 µL of phosphate-buffered saline (PBS). After the challenge, the body weights and survival rates of the challenged mice were monitored every two days for 14 days. The point when body weight loss reached 25% of the initial weight at the time of infection was day of death. The Institutional Review Board of the Research Institute for Microbial Diseases, Osaka University approved the virus experiments (Protocol Number: BIKEN-00006-010).

Collection of Bronchoalveolar Lavage Fluid (BALF) After 14 days of PR8 challenge, mice were sacrificed and BALF was collected. A 22-gauge catheter was inserted into the trachea, followed by the back-and-forth flushing of 0.7 and 0.5 mL of cold PBS for collection, resulting in the collection of approximately 1 mL of BALF. BALF was centrifuged at $600 \times g$ for 5 min at 4°C; the cell-free supernatants were used to measure cytokine levels and viral load. The cell pellet of BALF was treated with red cell lysis buffer and then resuspended in 1% bovine serum albumin (BSA)/PBS for flow cytometric analysis.

Flow Cytometry Cells were pelleted and washed with 1% BSA/PBS. Then, the cell suspension was blocked with anti-mouse CD16/32 antibody (BioLegend, CA, USA) for 20 min, followed by staining with the following antibodies for 20 min at 4°C: FITC-labeled Ly-6G (BioLegend), PE-labeled CD3ε (BioLegend), PerCP-labeled CD8α (BioLegend), PE/Cy7-labeled CD4 (BioLegend), APC-labeled Siglec-F (Miltenyi Biotec), APC/Cy7-labeled CD11c (BioLegend), BV421-labeled CD45 (BioLegend), and BV785-labeled CD11b (BioLegend) antibodies. Next, the cells were centrifuged at $600 \times g$ for 5 min at 4°C and resuspended in 1% BSA/PBS. The NovoCyte Flow Cytometer (ACEA Bioscience, San Diego, CA, USA) was used for analysis. Supplementary Fig. 1 illustrates the gating strategy used for flow cytometry.

Cytokine Quantification via Enzyme-Linked Immunosorbent Assay (ELISA) Commercial ELISA kits (R&D systems, MN, USA) were used to measure murine IL-6 and TNF-α levels in BALF supernatants as per the manufacturer's instructions. Cytokine levels were quantified using the standards supplied with the kits.

Virus Titer Determination Madin-Darby canine kidney cells were infected as previously described to quantify virus titers [14].

Statistical Analysis The Student's *t*-test, one-way analysis of variance, or log-rank test was used to elucidate significant differences. GraphPad Prism (GraphPad Software) was used to perform the analysis. A p-value of < 0.05 was considered statistically significant.

RESULTS

The PR8 virus is a highly adapted influenza virus strain that can result in disease symptoms and mortality in mice. To determine the role of MDSCs in IAV infection outcomes, *in vitro* differentiated MDSCs were adoptively transferred into mice before 5 and 1 day after PR8 infection; mortality and weight loss were monitored for 2 weeks (Fig. 1A). The intranasal administration of PR8 virus considerably decreased the weight and killed all mice without MDSC transfer; however, the adoptive transfer of MDSCs significantly improved mice survival, with no significant delay in weight loss (Fig. 1B, C). Flow cytometry of BALF samples revealed that there was no significant difference between total $CD45^{+}$ cell infiltration into the lung of the control and MDSC transfer groups. However, detailed analysis of each cell type revealed a significant increase in $CD4^{+}$ T cell and eosinophil infiltration in the MDSC transfer group; in contrast, macrophage and DC infiltration decreased (Fig. 1D). Furthermore, the adoptive transfer of MDSCs decreased IL-6 and TNF-α levels in the BALF (Fig. 1E); this suggests that they suppress lung inflammation. However, after MDSC transfer, viral load did not significantly change in the BALF; this suggests that MDSCs have a limited effect on virus clearance (Fig. 1F).

Inducible nitric oxide synthase (iNOS) is a key factor responsible for the immunosuppressive activity of MDSCs; therefore, we determined whether MDSC function in PR8 infection is associated with iNOS. First, we observed that a single adoptive transfer of MDSCs yielded effects comparable with double MDSC transfer (Supplementary Fig. 2). Subsequently, the MDSCs differentiated from $Nos2^{+/+}$ or $Nos2^{-/-}$

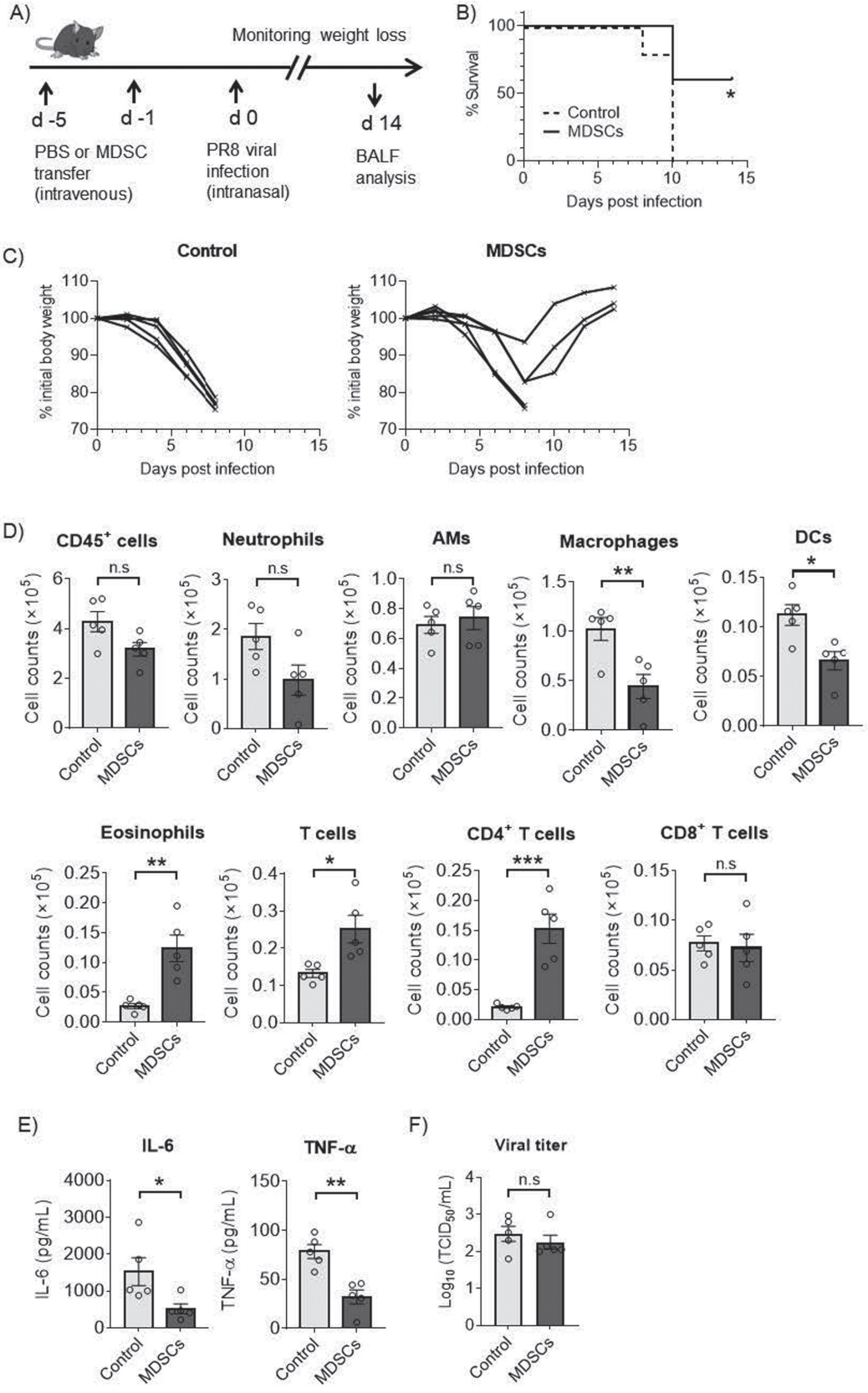

Fig. 1. Decreased Mortality of PR8 Virus by Adoptively Transferred Myeloid-Derived Suppressor Cells (MDSCs)

(A) C57BL/6J male mice (n = 5 per group) were intranasally infected with PR8 viruses, followed by the adoptive transfer of MDSCs. Then, mice were monitored daily for 2 weeks for (B) survival and (C) body weight loss. The log-rank (Mantel–Cox) test was used to perform statistical analysis (*$p < 0.05$). (D) Total number of $CD45^+$ cells, neutrophils, alveolar macrophages (AMs), macrophages, dendritic cells (DCs), eosinophils, T cells, $CD4^+$ T cells, and $CD8^+$ T cells in the bronchoalveolar lavage fluid (BALF) were assessed via flow cytometry (means ± S.E.M.; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ via one-way analysis of variance). (E) Cytokines in BALF were analyzed via ELISA (means ± S.E.M.; *$p < 0.05$ and **$p < 0.01$ via Student's *t*-test). (F) Viral titer was assessed in BALF (means ± S.E.M., statistical analysis was performed using Student's *t*-test).

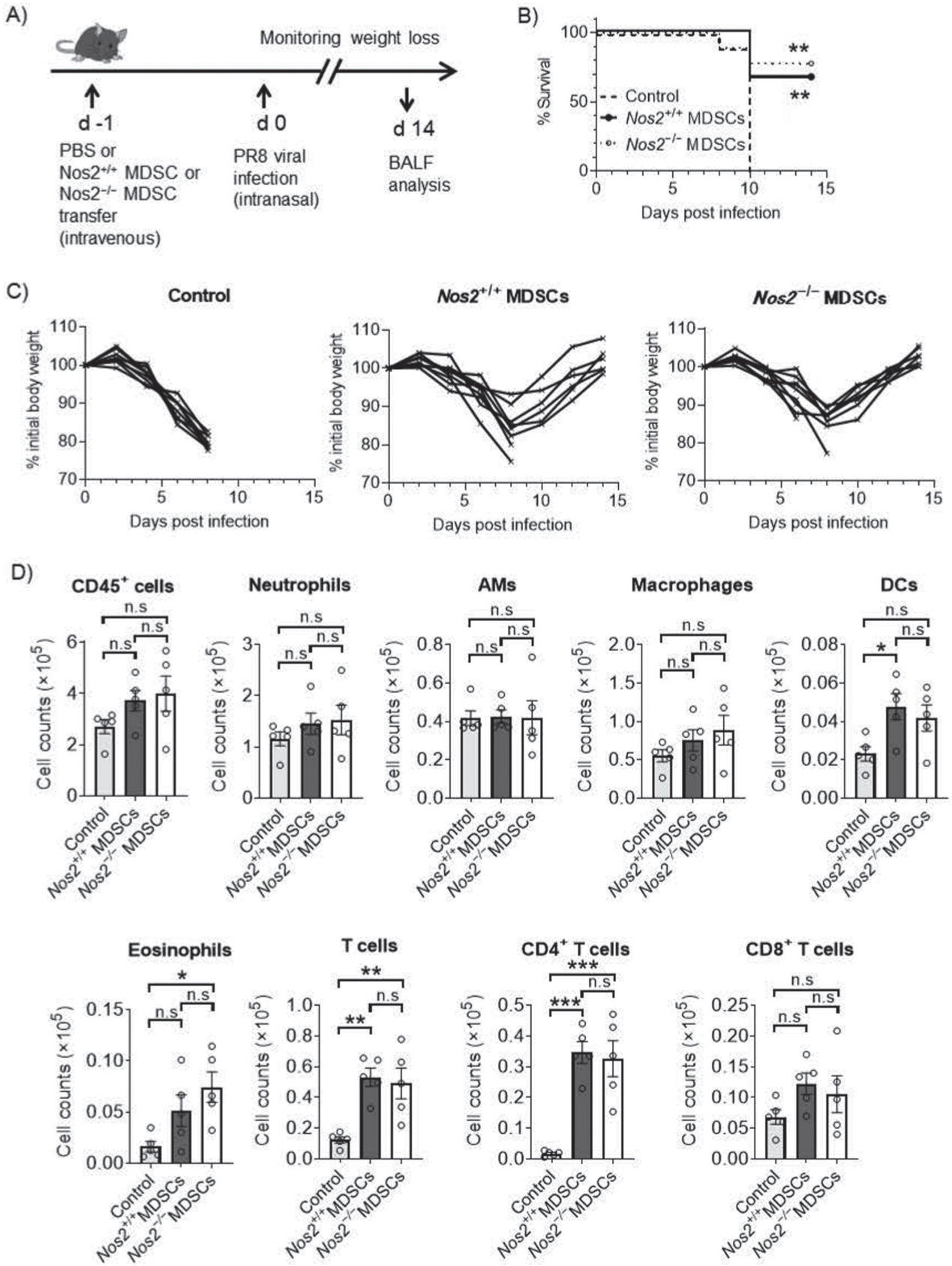

Fig. 2. Myeloid-Derived Suppressor Cells (MDSCs) Function Independently of iNOS Expression

(A) C57BL/6J male mice (n = 9 per group) were intranasally infected with PR8 viruses and adoptively transferred with MDSCs. Then, mice were monitored daily for 2 weeks for (B) survival and (C) body weight loss. The log-rank (Mantel–Cox) test was used to perform statistical analysis (**$p < 0.01$). (D) Total number of CD45$^+$ cells, neutrophils, alveolar macrophages (AMs), macrophages, dendritic cells (DCs), eosinophils, T cells, CD4$^+$ T cells, and CD8$^+$ T cells were assessed in the bronchoalveolar lavage fluid (BALF) via flow cytometry (means ± S.E.M.; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ via one-way analysis of variance).

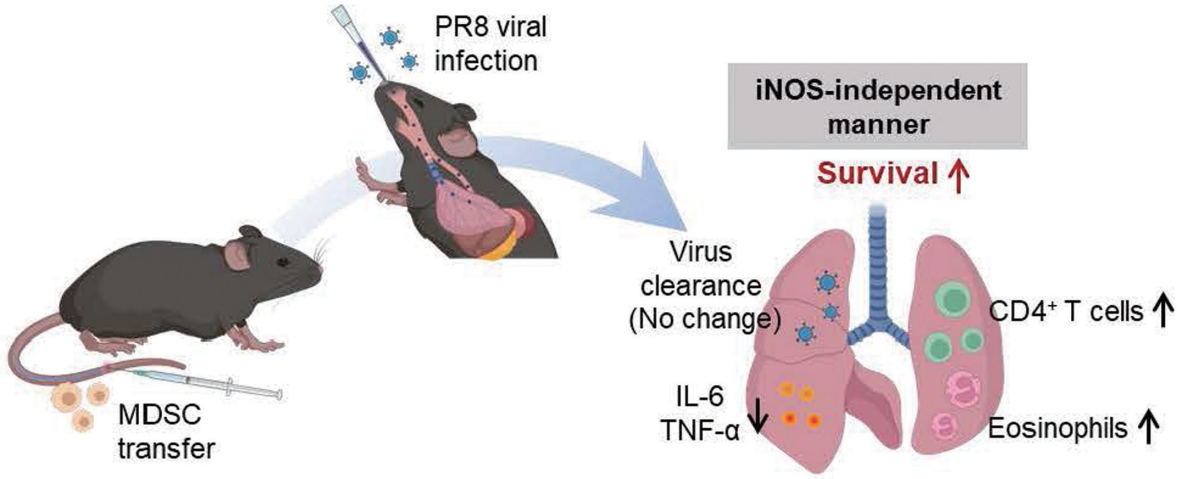

Fig. 3. Schematic Illustration to Show That Transferred Myeloid-Derived Suppressor Cells (MDSCs) Decrease Influenza A Virus (IAV) Disease-Induced Mortality *In Vivo* in an iNOS-Independent Manner

BM cells *in vitro* were singularly transferred into mice (Fig. 2A). The transfer of *Nos2*$^{-/-}$ MDSCs decreased PR8 infection-induced mortality and weight loss, with no difference with the transfer of *Nos2*$^{+/+}$ MDSCs (Fig. 2B, C). Consistently, the infiltration of inflammatory cells into the lung remained unaffected in the absence of iNOS in MDSCs (Fig. 2D). Collectively, our findings suggest that the adoptive transfer of MDSCs decreases IAV infection-induced mortality *in vivo*, which is iNOS-independent (Fig. 3).

DISCUSSION

In general, MDSCs play a dual role in viral infections, conferring protective functions by mitigating inflammation and T cell hyperactivation as well as potentially suppressing beneficial immune responses.[15] Factors such as infectious disease pathology, pathogen virulence mechanisms, and disease stage can affect the roles of MDSCs.[16] In the present study, we observed that the adoptive transfer of MDSCs before PR8 infection can alleviate weight loss and mortality, possibly owing to the anti-inflammatory effects of MDSCs. This finding was supported by the observed decrease in inflammatory factors such as IL-6 and TNF-α after MDSC transfer. Consistent with our findings, gentamicin-induced gut microbiota disruption in mice decreased CD11b$^+$Ly-6G$^+$ cells (identified as PMN-MDSCs), thereby exacerbating PR8 viral infection severity. Notably, the transplantation of spleen-derived CD11b$^+$Ly-6G$^+$ cells conferred significant protective effects against PR8 viral infection.[17] Collectively, these findings suggest that the adoptive cellular transfer of MDSCs can be a promising therapeutic strategy for IAV infections.

Single or double transfers of MDSC significantly reduced mouse mortality in response to PR8 infection; however, they also induced variations in the composition of infiltrating cells in the BALF. Considering the divergent results observed for macrophages and DCs, it suggests that these cells may not play a primary role in mediating mouse mortality in response to PR8 infection. Interestingly, both experiments demonstrated a substantial increase in CD4$^+$ T cells, indicating a potential key role alongside eosinophils. Although additional studies are warranted to identify the exact cause, increased CD4$^+$ T cell and eosinophil infiltration could have contributed to positive outcomes after MDSC transfer. CD4$^+$ T cells comprise various subgroups, including Th1, Th2, Th9, and Th17, with a complex balance. In particular, Th1 cells are vital for clearing IAV infections; suppressing Th2 cells via IFN-γ, IL-2, and IL-12 secretion; and promoting the production of neutralizing antibodies along with B cells.[18,19] In contrast, Th2 and Th9 cells play detrimental roles in IAV infections by secreting cytokines such as IL-4, IL-5, IL-6, IL-10, IL-13, and IL-9, exacerbating lung damage and delaying viral clearance.[12,19] An in-depth analysis of the changes in the phenotypic ratios of CD4$^+$ T cells after MDSC transfer will provide insights into its mechanisms. Eosinophils are multifunctional cells that confer immediate protection to the epithelial barrier in the early influenza stages, neutralize viruses, and promote cellular immunity. Furthermore, they exert protective effects by secreting antiviral factors, providing B cell assistance, and improving defense against exogenous pathogens such as bacteria.[20-22] In addition, there is a correlation between CD8$^+$ T cell proportion and activation and CD4$^+$ T cell or eosinophil infiltration.[22, 23] However, in the present study, CD8$^+$ T cells did not increase, suggesting that MDSCs inhibit CD8$^+$ T cell proliferation and/or infiltration. These observations suggest that the infiltration of CD4$^+$ T cells and/or eosinophils prevents weight loss and that the decrease in mortality and CD8$^+$ T cells will eliminate virus-infected cells.

Nitric oxide (NO), a key inflammatory signaling molecule, is significantly produced in IAV-infected hosts, thereby suggesting its role as a potent pathogenic agent.[24-26] The interaction between NO and superoxide anions, resulting in peroxynitrite formation, plays a role in the pathogenic mechanism of influenza virus pneumonia.[27] Studies have demonstrated that the morbidity, mortality, and cytokine production in the lung tissues of *Nos2*-deficient mice decrease after they are challenged with a highly pathogenic IAV.[28, 29] In our MDSC transfer model, iNOS remains intact in the host; however, the iNOS-induced deterioration mechanism can function, which is inhibited by MDSC transfer. In contrast, NO secretion from MDSCs is independent of the decreased mortality observed in IAV infection after adoptive MDSC transfer. This observation prompts intriguing questions about the multifaceted nature of

the immune response during IAV infection. It suggests that mitigating effects of MDSCs on mortality might function through mechanisms extending beyond the direct inhibition of iNOS.

In the present study, adoptively transferred MDSCs did not increase $CD8^+$ T cells and failed to sufficiently exclude IAV; this suggests that they result in chronic infections. Hemophagocytosis is observed during chronic infections, and hemophagocytic syndrome is characterized by this condition and hepatitis-like findings. If not appropriately treated, individuals with this condition can die. Hemophagocytic syndrome is also observed in individuals with IAV infection and COVID-19.[30-32] During hemophagocytosis, the production of IL-10, an immunosuppressive factor, is induced, thereby inhibiting immune cell responses to viruses.[33,34] Consistently, in the present study, MDSCs, as immune inhibitory cells, may experience similar immune cell responses, leading to insufficient viral clearance.

In conclusion, we demonstrated that MDSC transfer can attenuate the lethal effects of influenza; however, MDSCs may suppress $CD8^+$ T cells, resulting in insufficient viral clearance. Nevertheless, the adoptive cellular transfer of MDSCs may represent an attractive therapeutic strategy for future pandemics.

Funding This work was supported in part by JSPS KAKENHI [grant numbers JP22H03533 (M.T.) and JP20H03404 (Y.Y.), Grants-in-aid for Scientific Research (B)], a grant from The Drug Discovery Science Division, Open and Transdisciplinary Research Initiatives, Osaka University, and Takeda Science Foundation (M.T.). This research was also partially supported by the National Natural Science Foundation of China [grant number 82303730], the Zhejiang Provincial Natural Science Foundation of China [grant number LQ23H160001 (Z.X.)], and the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research) from AMED [grant numbers JP23ama121052 and JP23ama121054].

Acknowledgments The authors thank Dr. Takachika Hiroi for providing the *Nos2* knockout mice.

Conflict of interest Y.Y. was employed by the Research Foundation for Microbial Diseases, Osaka University. All other authors declare no conflicts of interest.

REFERENCES

1) Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, Palese P, Shaw ML, Treanor J, Webster RG, Garcia-Sastre A. Influenza. *Nat. Rev. Dis. Primers*, **4**, 3 (2018).
2) Subbarao K. Avian influenza H7N9 viruses: a rare second warning. *Cell Res.*, **28**, 1–2 (2018).
3) van Riel D, den Bakker MA, Leijten LM, Chutinimitkul S, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T. Seasonal and pandemic human influenza viruses attach better to human upper respiratory tract epithelium than avian influenza viruses. *Am. J. Pathol.*, **176**, 1614–1618 (2010).
4) Hogan BL, Barkauskas CE, Chapman HA, Epstein JA, Jain R, Hsia CC, Niklason L, Calle E, Le A, Randell SH, Rock J, Snitow M, Krummel M, Stripp BR, Vu T, White ES, Whitsett JA, Morrisey EE. Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell*, **15**, 123–138 (2014).
5) Chen X, Liu S, Goraya MU, Maarouf M, Huang S, Chen JL. Host Immune Response to Influenza A Virus Infection. *Front. Immunol.*, **9**, 320 (2018).
6) Krammer F. The human antibody response to influenza A virus infection and vaccination. *Nat. Rev. Immunol.*, **19**, 383–397 (2019).
7) Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat. Rev. Immunol.*, **21**, 485–498 (2021).
8) Cassetta L, Bruderek K, Skrzeczynska-Moncznik J, Osiecka O, Hu X, Rundgren IM, Lin A, Santegoets K, Horzum U, Godinho-Santos A, Zelinskyy G, Garcia-Tellez T, Bjelica S, Taciak B, Kittang AO, Hoing B, Lang S, Dixon M, Muller V, Utikal JS, Karakoc D, Yilmaz KB, Gorka E, Bodnar L, Anastasiou OE, Bourgeois C, Badura R, Kapinska-Mrowiecka M, Gotic M, Ter Laan M, Kers-Rebel E, Krol M, Santibanez JF, Muller-Trutwin M, Dittmer U, de Sousa AE, Esendagli G, Adema G, Lore K, Ersvaer E, Umansky V, Pollard JW, Cichy J, Brandau S. Differential expansion of circulating human MDSC subsets in patients with cancer, infection and inflammation. *J. Immunother. Cancer*, **8** (2020).
9) Tcyganov EN, Hanabuchi S, Hashimoto A, Campbell D, Kar G, Slidel TW, Cayatte C, Landry A, Pilataxi F, Hayes S, Dougherty B, Hicks KC, Mulgrew K, Tang CA, Hu CA, Guo W, Grivennikov S, Ali MA, Beltra JC, Wherry EJ, Nefedova Y, Gabrilovich DI. Distinct mechanisms govern populations of myeloid-derived suppressor cells in chronic viral infection and cancer. *J. Clin. Invest.*, **131** (2021).
10) Hegde S, Leader AM, Merad M. MDSC: Markers, development, states, and unaddressed complexity. *Immunity*, **54**, 875–884 (2021).
11) De Santo C, Salio M, Masri SH, Lee LY, Dong T, Speak AO, Porubsky S, Booth S, Veerapen N, Besra GS, Grone HJ, Platt FM, Zambon M, Cerundolo V. Invariant NKT cells reduce the immunosuppressive activity of influenza A virus-induced myeloid-derived suppressor cells in mice and humans. *J. Clin. Invest.*, **118**, 4036–4048 (2008).
12) Jeisy-Scott V, Davis WG, Patel JR, Bowzard JB, Shieh WJ, Zaki SR, Katz JM, Sambhara S. Increased MDSC accumulation and Th2 biased response to influenza A virus infection in the absence of TLR7 in mice. *PLoS One*, **6**, e25242 (2011).
13) Xie Z, Ago Y, Okada N, Tachibana M. Valproic acid attenuates immunosuppressive function of myeloid-derived suppressor cells. *J. Pharmacol. Sci.*, **137**, 359–365 (2018).
14) Shibuya M, Tamiya S, Kawai A, Hirai T, Cragg MS, Yoshioka Y. Synergistic effect of non-neutralizing antibodies and interferon-gamma for cross-protection against influenza. *iScience*, **24**, 103131 (2021).
15) Grassi G, Notari S, Gili S, Bordoni V, Casetti R, Cimini E, Tartaglia E, Mariotti D, Agrati C, Sacchi A. Myeloid-Derived Suppressor Cells in COVID-19: The Paradox of Good. *Front. Immunol.*, **13**, 842949 (2022).
16) Koushki K, Salemi M, Miri SM, Arjeini Y, Keshavarz M, Ghaemi A: Role of myeloid-derived suppressor cells in viral respiratory infections; Hints for discovering therapeutic targets for COVID-19. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie,* 144, 112346 (2021).
17) Sun Y, He Z, Li J, Gong S, Yuan S, Li T, Ning N, Xing L, Zhang L, Chen F, Li Z, Wang J, Luo D, Wang H. Gentamicin Induced Microbiome Adaptations Associate With Increased BCAA Levels and Enhance Severity of Influenza Infection. *Front. Immunol.*, **11**, 608895 (2020).
18) Krueger PD, Goldberg MF, Hong SW, Osum KC, Langlois RA, Kotov DI, Dileepan T, Jenkins MK. Two sequential activation modules control the differentiation of protective T helper-1 (Th1) cells. *Immunity*, **54**, 687–701 e684 (2021).
19) Benova K, Hanckova M, Koci K, Kudelova M, Betakova T. T cells and their function in the immune response to viruses. *Acta Virol.*, **64**, 131–143 (2020).
20) Macchia I, La Sorsa V, Urbani F, Moretti S, Antonucci C, Afferni C, Schiavoni G. Eosinophils as potential biomarkers in respiratory viral infections. *Front. Immunol.*, **14**, 1170035 (2023).
21) Bonjour K, Palazzi C, Silva TP, Malta KK, Neves VH, Oliveira-Barros EG, Neves I, Kersten VA, Fortuna BT, Samarasinghe AE, Weller PF, Bandeira-Melo C, Melo RCN. Mitochondrial Population in Mouse Eosinophils: Ultrastructural Dynamics in Cell Differentiation and

Inflammatory Diseases. *Front. Cell Dev. Biol.*, **10**, 836755 (2022).
22) Samarasinghe AE, Melo RC, Duan S, LeMessurier KS, Liedmann S, Surman SL, Lee JJ, Hurwitz JL, Thomas PG, McCullers JA. Eosinophils Promote Antiviral Immunity in Mice Infected with Influenza A Virus. *J. Immunol.*, **198**, 3214–3226 (2017).
23) Riberdy JM, Christensen JP, Branum K, Doherty PC. Diminished primary and secondary influenza virus-specific CD8(+) T-cell responses in CD4-depleted Ig(-/-) mice. *J. Virol.*, **74**, 9762–9765 (2000).
24) Rimmelzwaan GF, Baars MM, de Lijster P, Fouchier RA, Osterhaus AD. Inhibition of influenza virus replication by nitric oxide. *J. Virol.*, **73**, 8880–8883 (1999).
25) Keshavarz M, Solaymani-Mohammadi F, Namdari H, Arjeini Y, Mousavi MJ, Rezaei F. Metabolic host response and therapeutic approaches to influenza infection. *Cell. Mol. Biol. Lett.*, **25**, 15 (2020).
26) Zaki MH, Akuta T, Akaike T. Nitric oxide-induced nitrative stress involved in microbial pathogenesis. *J. Pharmacol. Sci.*, **98**, 117–129 (2005).
27) Babizhayev MA, Deyev AI, Yegorov YE. Non-hydrolyzed in digestive tract and blood natural L-carnosine peptide ("bioactivated Jewish penicillin") as a panacea of tomorrow for various flu ailments: signaling activity attenuating nitric oxide (NO) production, cytostasis, and NO-dependent inhibition of influenza virus replication in macrophages in the human body infected with the virulent swine influenza A (H1N1) virus. *J. Basic Clin. Physiol. Pharmacol.*, **24**, 1–26 (2013).
28) Perrone LA, Belser JA, Wadford DA, Katz JM, Tumpey TM. Inducible nitric oxide contributes to viral pathogenesis following highly pathogenic influenza virus infection in mice. *J. Infect. Dis.*, **207**, 1576–1584 (2013).
29) Jayasekera JP, Vinuesa CG, Karupiah G, King NJC. Enhanced antiviral antibody secretion and attenuated immunopathology during influenza virus infection in nitric oxide synthase-2-deficient mice. *J. Gen. Virol.*, **87**, 3361–3371 (2006).
30) Larroche C, Mouthon L. Pathogenesis of hemophagocytic syndrome (HPS). *Autoimmun. Rev.*, **3**, 69–75 (2004).
31) Janka GE. Hemophagocytic syndromes. *Blood Rev.*, **21**, 245–253 (2007).
32) Tang LV, Hu Y. Hemophagocytic lymphohistiocytosis after COVID-19 vaccination. *J. Hematol. Oncol.*, **14**, 87 (2021).
33) Maakaroun NR, Moanna A, Jacob JT, Albrecht H. Viral infections associated with haemophagocytic syndrome. *Rev. Med. Virol.*, **20**, 93–105 (2010).
34) Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence *in vivo*. *Nat. Med.*, **12**, 1301–1309 (2006).