Microbial Cell Factories

RESEARCH

Open Access

Immunogenicity and protective efficacy of a recombinant *lactococcus lactis* vaccine against HSV-1 infection

Shaoju Qian[1,3,4], Ruixue Li[2], Yeqing He[1], Hexi Wang[1], Danqiong Zhang[1], Aiping Sun[1,3], Lili Yu[1,3,4], Xiangfeng Song[1,3,4], Tiesuo Zhao[1,3,4], Zhiguo Chen[1,3,4] and Zishan Yang[1,3,4*]

Abstract

Background Herpes simplex virus type 1 (HSV-1) is a major cause of viral encephalitis, genital mucosal infections, and neonatal infections. *Lactococcus lactis (L. lactis)* has been proven to be an effective vehicle for delivering protein antigens and stimulating both mucosal and systemic immune responses. In this study, we constructed a recombinant *L. lactis* system expressing the protective antigen glycoprotein D (gD) of HSV-1.

Results To improve the stability and persistence of antigen stimulation of the local mucosa, we inserted the immunologic adjuvant interleukin (IL)-2 and the Fc fragment of IgG into the expression system, and a recombinant *L. lactis* named NZ3900-gD-IL-2-Fc was constructed. By utilizing this recombinant *L. lactis* strain to elicit an immune response and evaluate the protective effect in mice, the recombinant *L. lactis* vaccine induced a significant increase in specific neutralizing antibodies, IgG, IgA, interferon-γ, and IL-4 levels in the serum of mice. Furthermore, in comparison to the mice in the control group, the vaccine also enhanced the proliferation levels of lymphocytes in response to gD. Moreover, recombinant *L. lactis* expressing gD significantly boosted nonspecific immune reactions in mice through the activation of immune-related genes. Furthermore, following the HSV-1 challenge of the murine lung mucosa, mice inoculated with the experimental vaccine exhibited less lung damage than control mice.

Conclusion Our study presents a novel method for constructing a recombinant vaccine using the food-grade, non-pathogenic, and non-commercial bacterium *L. lactis*. The findings indicate that this recombinant vaccine shows promise in preventing HSV-1 infection in mice.

Keywords Herpes simplex virus type 1, FcRn, *Lactococcus lactis*, Mucosal immunity, IL-2

*Correspondence:
Zishan Yang
yangzishan@xxmu.edu.cn
[1]Department of Immunology, School of Basic Medical Sciences, Xinxiang Medical University, Xinxiang 453003, China
[2]Department of Otolaryngology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453003, China
[3]Xinxiang Engineering Technology Research Center of Immune Checkpoint Drug for Liver-Intestinal Tumors, 453003 Henan, China
[4]Xinxiang Key Laboratory of Tumor Vaccine and Immunotherapy, School of Basic Medical Sciences, Xinxiang Medical University, Xinxiang 453003, China

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Introduction

Herpes simplex virus type I (HSV-1) is a DNA virus belonging to the α herpesvirus subfamily, which can infect the human body and cause various diseases such as oral mucosa herpes, corneal herpes, meningitis, and pneumonia. Once infected with a herpesvirus, it is carried for life. No specific treatments are currently available [1]. Therefore, it is important to develop a safe and highly efficient vaccine against HSV-1. Although there are currently no approved vaccinations against HSV-1, several investigational prophylactic vaccines are available, such as vaccines based on epitopes and recombinant, live-attenuated, and subunit cocktails. Nevertheless, extant parentally administered live attenuated or inactivated vaccines demonstrate a notable deficiency in inducing robust mucosal immunity against HSV-1 infection.

Recombinant live vector vaccines have recently emerged as the primary focus of research in the development of HSV-1 vaccines because of the advantages of avoiding the purification process of vaccine antigens and reducing the degradation of antigens in the gastrointestinal tract. Although viruses and attenuated bacteria have been widely studied as live vaccine carriers, they continue to encounter numerous challenges. Weakly virulent viruses and attenuated bacterial residues after attenuated treatment may still infect immunocompromised animals, and there may be a risk of rejuvenation [2, 3]. *Lactococcus lactis (L. lactis)* expressing exogenous antigens is an ideal live mucosal vaccine carrier. Extensive research has been conducted on the construction and application of expression systems for food-grade lactic acid bacteria, and a series of achievements have been made. The *L. lactis* IBB477 strain could exist more continuously in the gastrointestinal tract of sterile rats, while the low-adhesion strain MG1820 could not survive [4]. It has been reported that oral vaccines targeting *L. lactis* result in continuous antigen production, thereby stimulating both mucosal and systemic immune responses. Studies have shown that *L. lactis* can express various antigens, such as the S1 protein of Porcine Deltacoronavirus (PDCoV) [5], the fusion protein F1S1 [6]from *Bordetella pertussis*, and fusion antigens OmpK and FlaB [7] from *Vibrio* species. Through genetic engineering, *L. lactis* vaccines significantly enhanced the production of cytokines such as IFN-γ, IL-2, IL-4, and IL-17, indicating both Th1 and Th2 immune responses [5, 8]. Additionally, oral administration of L. lactis vaccines induced the production of specific antibodies (IgG and IgA) and enhanced T-cell responses. The study involving the F1S1 fusion protein also showed significant IgA production in lung extracts, indicating effective immune responses at distal mucosal sites following mucosal immunization [9]. These findings demonstrated that *L. lactis* was an effective mucosal vaccine delivery vehicle capable of inducing both local and systemic immune responses, including impacts on distal mucosal sites.

HSV-1 glycoprotein D (gD) is a complex antigen that contains multiple antigenic epitopes and serves as the primary immunogen that stimulates the generation of neutralizing antibodies [10]. The gD protein sequences of HSV-1 and HSV-2 are approximately 80% homologous. Despite the high homology between the gD proteins of HSV-1 and HSV-2, significant structural and functional differences exist, which may contribute to their distinct infection characteristics. The amino acid sequence variations are primarily concentrated at the N- and C-termini of the amino acid proteins. These structural disparities may lead to functional variations, such as differences in affinity for host cell receptors and mechanisms of immune evasion. While both gD proteins can induce cross-neutralizing antibodies, type-specific antigenic epitopes may result in distinct immune responses [11, 12]. The co-cytoplasmic domain of HSV-1 gD is an envelope glycoprotein necessary for viral entry, and can promote viral replication and intercellular transmission [13]. Multiple studies have shown that gD antigen immunization in animals produces a strong specific immune response. For example, Bernstein et al. showed that gD antigen protects against genital HSV-2 infection in a guinea pig model [14]. Additionally, the CJ9-gD vaccine developed by Lu et al. demonstrated significant immunogenicity in mice, eliciting high levels of neutralizing antibodies against HSV-1 and effectively preventing herpes keratitis following corneal challenge [15]. Furthermore, It was reported that a gD mutant vaccine, impaired in neural tropism, offered superior protection against HSV-2 infection compared to the gD subunit vaccine [16]. More importantly, fusion proteins that combine Fc with viral antigens, such as the HSV-2 glycoprotein used in mouse vaccination models, have shown success in augmenting antigen transport across the nasal epithelium. This approach may provide an innovative FcRn-based immunization strategy [17]. Under the mediation of FcRn, the fusion protein can effectively cross the mucosal barrier [18]. FcRn protects antigens from degradation and enables the body to produce a more durable immune response [19].

In this study, we constructed a recombinant *L. lactis* strain expressing the protective antigen gD of HSV-1. To enhance the stability and persistence of antigen-stimulated mucosa, interleukin (IL)-2, and IgG Fc fragments were inserted into the expression system for co-expression with gD. After oral immunization in mice, local mucosal and systemic immune responses were measured to assess the immunogenicity and protective efficacy of the recombinant *L. lactis* expression system.

Table 1 Primers used in this study

Primer name	Sequence (5′- 3′)
HSV-1 gD-F	CATGCCATGGCCATGCATCACCATCATCATCATAAGTACGCTCTGGC
HSV-1 gD-R	CGAGCCACCTCCTCCGGACCCACCCCCGCCGAGAAAGGGCTGGTCGGTACT
IL-2-F	GGCGGGGGTGGGTCCGGAGGAGGTGGCTCGGCATGCGCTCCGACTTCTAG
IL-2-R1	AATGGTACCAGTCAGAGTAGAGATGATAGACTGAGAGAA
IL-2-R2	AGATCCCGAGCCACCTCCTCCGGACCCACCCCCGCCTGATCCAGTCAGAGTAGAGATGATAGACTGAGAGA
IgG Fc-F	GGATCAGGCGGGGGTGGGTCCGGAGGAGGTGGCTCGGGATCTGAGCCCAGAGGGCCCACA
IgG Fc-R	AATGGTACCAATGGTACCCATTTACCCGGAGTCCGGG

Materials and methods

Strains, plasmids, and culture media

The HSV-1 strain KOS (accession no. KT899744.1) was stored in the Basic Medical Sciences Department at Xinxiang Medical University. HSV-1 cells were propagated in Vero cells. Plasmid pNZ8148 and *L. lactis* NZ3900 strain were purchased from Zoman Biotechnology (Beijing, China). *L. lactis* NZ3900 was grown at 30 °C without vibration in an M17 medium containing 0.5% glucose. Transformed *L. lactis* cells were screened and cultured in an M17 medium without glucose.

Construction of *L. lactis* pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc

The fusion of the HSV-1 KOS strain glycoprotein gD at 852 bp (GenBank: JQ320083.1), linker1 at 30 bp (sequence: ggcgggggtg ggtccggagg aggtggctcg), and mouse IL-2 at 456 bp (GenBank: X07256.1) was referred to as gD-IL-2. This fused gene fragment, gD-IL-2, was further fused with mouse IgG Fc at 599 bp (corresponding to the IgG2a heavy chain gene) using linker2 at 42 bp (sequence: ggatcaggcg ggggtgggtc cggaggaggt ggctcgggat ct) to generate the construct named gD-IL-2-Fc. The recombinant plasmids pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc were constructed by inserting the digested pNZ8148 vector (3167 bp) into the NcoI/KpnI restriction endonuclease site.

The L. lactis NZ3900 competent cells were pre-cultured. The competent cells were electroporated with the pNZ8148-usp45-gD-IL-2 and pNZ8148-usp45-gD-IL-2-Fc positive plasmids and then grown in M17 media with 100 μg/mL of chloramphenicol. The usp45 (81 bp) was used to facilitate the secretion of the fusion proteins in *L. lactis*, which is a signal peptide derived from *L. lactis*. The plasmids were then ready for further expression and functional studies in *L. lactis.* PCR and enzyme digestion were used to extract and identify recombinant plasmids. For the second time, the positive plasmid was converted into *L. lactis* NZ3900. The positive recombinant NZ3900 strain containing pNZ8148-usp45-gD-IL-2 or pNZ8148-usp45-gD-IL-2-Fc has been rechristened as pNZ8148-gD-IL-2 or pNZ8148-gD-IL-2-Fc. As a negative control, *L. lactis* NZ3900 (renamed pNZ8148) was electroporated with the empty vector pNZ8148. The primers used for PCR are listed in Table 1.

Identification of proteins expressed by the recombinant strain

The recombinant *L. lactis* strains pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc were cultivated for 8–10 h at 30 °C in an M17 medium containing 0.5% glucose. After dilution to 1:25 in fresh M17 medium, the recombinant *L. lactis* cells were cultured until their OD600 reached 0.3 ~ 0.4. Subsequently, a final concentration of 5 ng/mL nisin was used to activate the expression of the recombinant genes. The culture supernatants were separated by centrifugation after 20 h of induction at 30 °C. His-labeled mouse monoclonal antibody, with the His tag located at the N-terminus, was then used for Western blot identification. Following correct identification, the supernatant was concentrated with 80% saturated ammonium sulfate, and after 2 days of dialysis in the refrigerator at 4 °C, the supernatant was filtered with a 0.45 μm filter membrane. Finally, the supernatant was purified

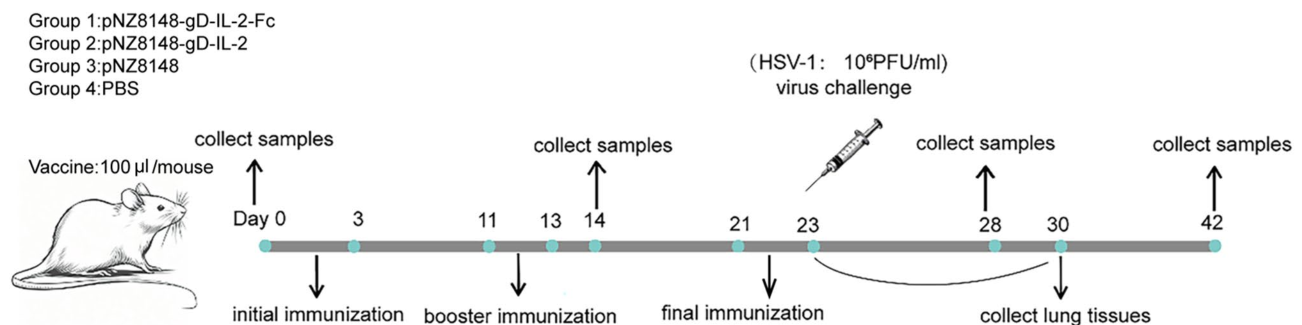

Fig. 1 Strategy for the animal experiment. Mice were randomly divided into four groups and orally vaccinated with initial immunization (day 1–3), booster immunization (day 11–13), and final immunization (day 21–23). Samples were collected on days 0, 14, 28, and 42 for ELISA and RT-qPCR analysis. Histology and virus titers were assessed using lung samples taken 7 d following viral challenge

using a nickel ion-affinity chromatography column with a His label. The purified proteins were identified by SDS-PAGE and Western blot electrophoresis, and then preserved at -80 °C for subsequent applications.

Mice immunization and viral challenge

Six to eight-week-old female C57BL/6 mice of specific-pathogen-free grade were obtained from Henan Skbex Biotechnology Co., Ltd. and raised in air-filtered containers under pathogen-free conditions. All animal experiments were performed in accordance with the guidelines of the Ethics Committee of Xinxiang Medical University, China (approval number XYLL-20230271). To evaluate the immunogenicity of the recombinant strain pNZ8148-gD-IL-2-Fc used as an oral vaccine, mice were randomly assigned to one of four groups of mice, with two experimental groups (pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc groups) and two control groups (PBS and pNZ8148 groups). The experimental groups were gavaged with 100 μg of recombinant *L. lactis* expressing pNZ8148-gD-IL-2 or pNZ8148-gD-IL-2-Fc per mouse, which were induced by nisin for 20 h and collected from the culture supernatants, while the control group was gavaged with pNZ8148 or PBS. The immunization program was as follows: initial immunization (day 1–3), booster immunization (day 11–13), and final immunization (day 21–23).

Each immunization was administered continuously for 3 days, and blood, spleen, and intestinal samples (3 cm in length) were collected on days 0, 14, 28, and 42 for enzyme-linked immunosorbent assay (ELISA) and RT-qPCR. After the completion of immunization, the mice were subjected to anesthesia and challenge, and the four groups of mice were infected with HSV-1 (10^6 PFU/mL) via nasal drop for 100 μL, followed by sacrifice after 7 days of infection. The infection of each group of mice was evaluated, and lung tissues were collected and homogenized to extract DNA and determine the copy number of HSV-1. Moreover, pathological sections of lung tissue were prepared to evaluate infection and damage. Figure 1 shows the experimental schedule.

Detection of cytokines and specific antibody in mice

Using commercial ELISA kits (Excell Bio., China), the levels of cytokines interferon (IFN)-γ and IL-4 in blood-serum and spleen samples were determined on day 42 post-immunization following oral vaccination of the mice. An indirect ELISA was employed to measure HSV-1-specific IgA and IgG antibodies in the serum on days 0, 14, 28, and 42 post-immunization, in the intestine on day 42, and in the lung before and after the HSV-1 challenge(days 0 and 7), following published protocols with slight modifications [6]. Specifically, we diluted the mouse serum 1:20 before detection. After adding 100 μL per well and coating a 96-well plate with purified HSV-1 gD protein at a concentration of 2.0 μg/mL, the plate underwent overnight incubation at 4 °C. For detection, horseradish peroxidase(HRP)-conjugated goat anti-mouse IgG (Sigma, diluted 1:4000) or IgA (Abcam, diluted 1:5000) was used as a secondary antibody to measure specific antibody levels against gD in mice.

Antigen-Induced Proliferation of lymphocytes in Vitro

Blood and spleen samples were obtained from three mice from each group on days 0, 14, 28, and 42 following vaccination to prepare them for a lymphocyte proliferation experiment. Briefly, splenocytes (5×10^7 cells/mL) and 100 μL of blood were suspended in DMEM media containing 10% fetal calf serum and then transferred to a 96-well flat-bottom plate. Following a 72-hour HSV-1 restimulation, the cells were treated with culture media for control purposes. The positive control wells contained 50μL of DMEM complete culture medium with 20 μg/mL ConA, while the negative control wells contained only DMEM complete culture medium. The plates were incubated for an additional 4 h after adding 10 μL of MTT per well. Proliferation was then assessed using OD490 values.

Real-time quantitative PCR

The relative expression of immune-related genes in mouse intestinal mucosal tissues was determined using RT-qPCR. Relative quantitative evaluation of mouse cDNA derived from intestinal mucosal tissues was performed using RT-qPCR. GenScript Corporation (Nanjing, China) synthesized the immune-related genes TLR2, MyD88, TRAF6, p65, IRF7, IL-1β, and GAPDH following previous instructions [20]. The SYBR Green Real-Time PCR Mix (Takara, Dalian, China) was used for RT-qPCR, which was performed on an ABI 7500 fluorescence quantifier (Applied Biosystems, Foster City, CA, USA). Moreover, the expression of TLR2, MyD88, and TRAF6 in the lungs was also assessed using RT-qPCR both before and after the challenge(day 0 and day 7), following the same procedure to ensure consistency in the detection and quantification of these genes across different tissues. Mouse lung tissue was ground to extract DNA, followed by amplification and cloning of the gD gene of the HSV-1 KOS strain into the pMD18-T vector (Takara). The quantity of HSV-1 viral DNA in the samples was determined using the standard curve cycle threshold (Ct) value. Gene expression levels were calculated relative to GAPDH expression employing the delta-delta cycles to threshold ($2-\Delta\Delta Ct$) method.

Preparation of pathological sections

At necropsy, the lungs of the mice from the challenged and control groups were dissected and routinely fixed for 36 h in 10% formalin solution. After fixation, the lungs

were dehydrated, embedded, sectioned, and mounted on glass slides. Hematoxylin and eosin (HE) staining was performed on the prepared slides, which were then analyzed using a standard microscope.

Statistical analysis

Data were analyzed as mean ± SEM. Group comparisons were conducted using one-way ANOVA and executed using Prism (GraphPad Software Inc., San Diego, CA, USA). Pearson's test was used to evaluate the correlations between the mRNA levels of various genes. Significance was determined at levels of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Results

Expression and identification of recombinant L. Lactis pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc

The glycoprotein gD of HSV-1 is composed of 369 amino acid residues, and its structural framework is characterized by an immunoglobulin-like core flanked by an elongated C-terminus and an N-terminal hairpin loop. The N-terminal region, which contains 14 amino acid receptor-binding ligands for herpesvirus entry mediator A, is particularly critical. In this study, we engineered a gD-Fc fusion protein, in which the extracellular domain of HSV-1 gD was fused to the Fc fragments of mouse IgG. This fusion targets FcRn by binding to the CH2, CH3, and hinge regions of the IgG Fc fragments, thereby mediating protein transport across mucosal barriers. As a teα helical bundle cytokine produced by activated T cells and NK cells, IL-2 controls the survival and proliferation of regulatory T cells and plays a role in enhancing the immune response in the fusion protein. Using SWISS-MODEL homology modeling, we predicted the three-dimensional structure of this fusion protein and revealed the structural characteristics of the HSV-1 gD protein, IgG Fc fragment, and IL-2 after fusion, as shown in Fig. 2.

The gD-IL-2 and gD-IL-2-Fc fused gene fragments were cloned into plasmid pUC57 and named pUC57-gD-IL-2 and pUC57-gD-IL-2-Fc, respectively (Synthesized by GenScript). Fusion PCR was performed to amplify gD-IL-2 and gD-IL-2-Fc using the synthesized plasmid as a template (Fig. 3a). The recombinant plasmids pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc were validated through NcoI/KpnI restriction enzyme digestion, which yielded a large fragment of the same size as pNZ8148 and a small fragment of the same size as the gD-IL-2(1419 bp) or gD-IL-2-Fc(2060 bp) genes, respectively (Fig. 3b). Recombinant *L. lactis* strains expressing the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc fusion genes were successfully constructed.

To confirm the expression of the fusion proteins of interest, cell lysates of the recombinant strains pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc were analyzed using SDS-PAGE and Western blotting. Figure 3 shows the expected immunoblot bands corresponding to the fusion protein gD-IL-2 (49 kDa) expressed by pNZ8148-gD-IL-2 (Fig. 3c and d) and the fusion protein gD-IL-2-Fc (72 kDa) expressed by pNZ8148-gD-IL-2-Fc (Fig. 3c and e). Importantly, these proteins were not detected in the control strain pNZ8148.

Evaluation of the immune effect of gD-IL-2-Fc fusion gene recombinant L. lactis

To evaluate the mucosal and systemic immune responses induced by pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc, we used a mouse model immunized with recombinant *L.lactis*. We measured the levels of HSV-1-specific IgA and IgG in serum samples collected on days 0, 14, 28, and 42 using ELISA. Both IgG and IgA levels progressively increased over time, with the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc groups showing significantly higher antibody levels compared to the PBS and pNZ8148 controls ($p < 0.001$), particularly at the 28- and 42-day post-immunization time points, as shown in Fig. 4a and b. During the study period, the pNZ8148-gD-IL-2-Fc and pNZ8148-gD-IL-2 groups demonstrated marked elevations in serum IgA and IgG levels from day 0 to day 42. However, the PBS and pNZ8148 groups did not show significant changes in antibody levels. Notably, the pNZ8148-gD-IL-2 group exhibited a significant increase in IgG and IgA levels by day 28, while the pNZ8148-gD-IL-2-Fc group showed earlier elevations by day 14. Throughout the study, the pNZ8148-gD-IL-2-Fc group consistently maintained the highest antibody levels, significantly surpassing those of the pNZ8148-gD-IL-2 group.

Lymphocyte proliferation in the blood, triggered by purified HSV-1, was analyzed using MTT assay on days 0, 14, 28, and 42. On day 0, there were no significant differences observed among the groups. From day 14 onward, the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc groups exhibited significantly higher proliferation compared to the PBS and pNZ8148 groups($p < 0.001$). Notably, the pNZ8148-gD-IL-2-Fc group showed a significantly stronger response than the pNZ8148-gD-IL-2 group ($p < 0.05$). These differences increased further by days 28 and 42, indicating effective stimulation of T lymphocyte proliferation by the gD-IL-2 and gD-IL-2-Fc constructs(Fig. 4c). The results in the spleen were consistent with those observed in peripheral blood(Fig. 4d). These results demonstrated that recombinant *L. lactis* targeting gD with IL-2 as an adjuvant performed better when fused with Fc fragments.

After immunizing mice with recombinant *L. lactis*, the serum and spleen were collected from the mice immunized for 42 days, and the contents of IFN-γ and IL-4 in the serum and spleen of mice were determined using

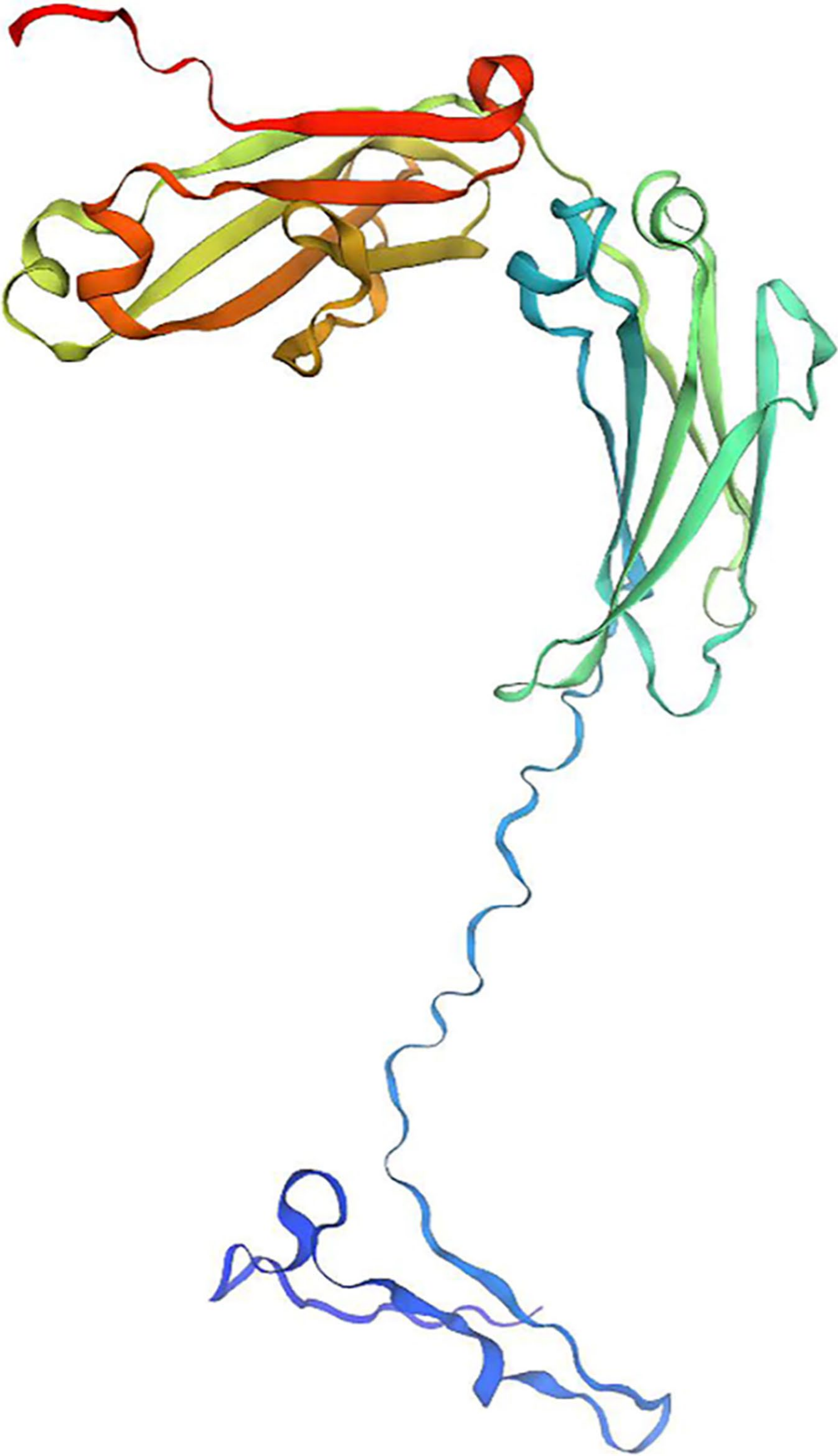

Fig. 2 Predicted structure of the fusion protein HSV-1 gD-IL-2-Fc. The predicted structure was generated using SWISS-MODEL. The fusion protein consists of three main domains: the extracellular domain of HSV-1 gD at the N-terminal, the IL-2 domain in the middle, and the Fc region of mouse IgG at the C-terminal

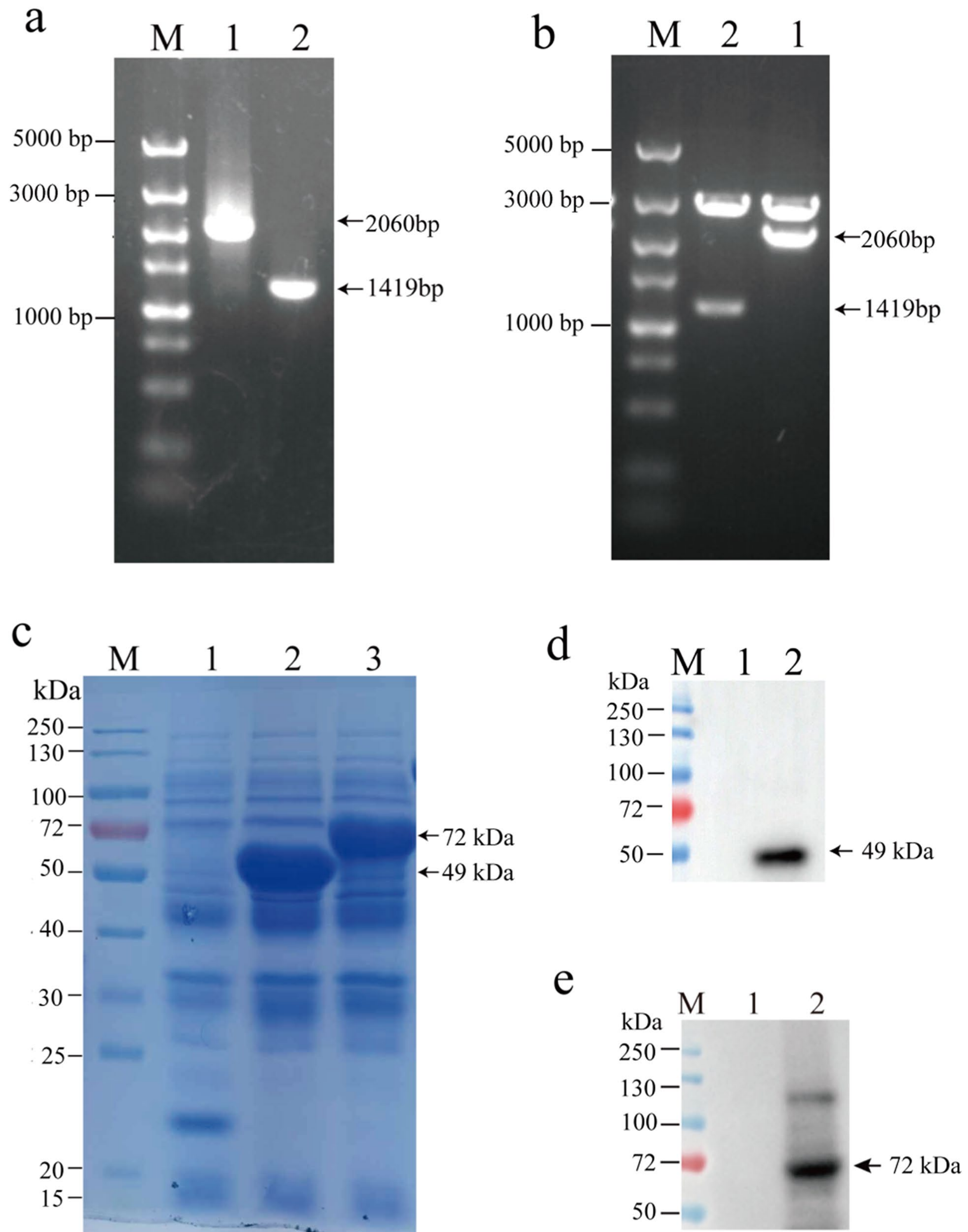

Fig. 3 Identification of the recombinant plasmids and Western blot analysis of the recombinant proteins. (**a**) Amplification of gD-IL-2 and Fc genes. M: Marker; Lane 1: gD-IL-2-Fc; Lane 2: gD-IL-2. (**b**) Identification of the recombinant plasmids by double enzyme digestion. M: Marker; Lane 1: pNZ8148-gD-IL-2-Fc; Lane 2: pNZ8148-gD-IL-2. Fusion proteins by recombinant *L. lactis* were detected by SDS-PAGE (**c**). (**d** and **e**). Fusion proteins by recombinant *L. lactis* were detected by anti-his mouse antibodies. M: Protein marker, Lane 1: Negative Control, Lane 2: Identification of fusion protein gD-IL-2, Lane 3: Identification of fusion protein gD-IL-2-Fc

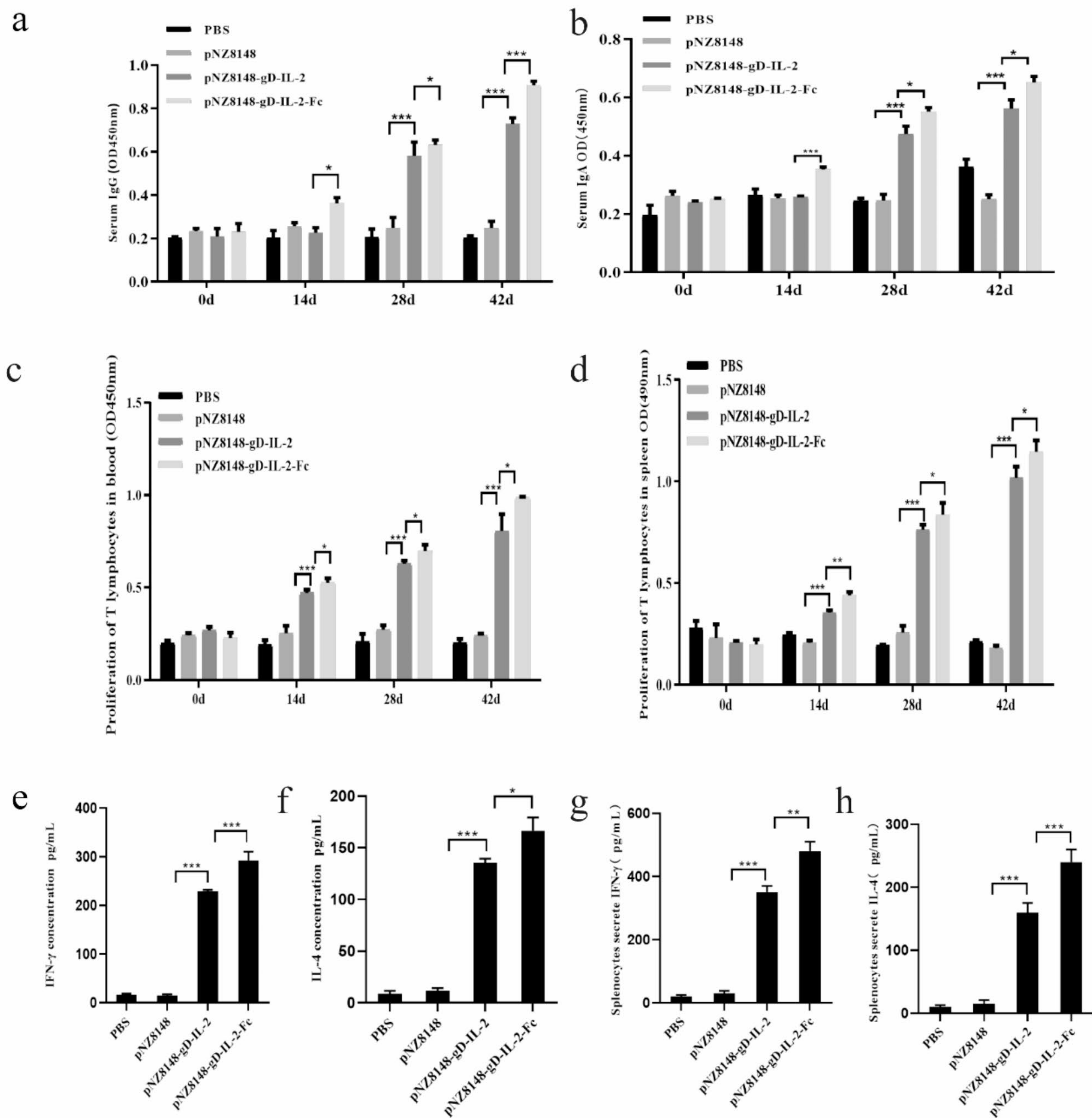

Fig. 4 ELISA was used to detect specific antibody, T lymphocyte proliferation, and serum cytokine levels. Detection of IgG antibodies in serum (**a**), IgA antibodies in serum (**b**), and T lymphocyte proliferation levels in the blood (**c**) and spleen (**d**) on days 0, 14, 28, and 42. Detection of IFN-γ (**e**) and IL-4 (**f**) in serum on day 42. Detection of IFN-γ (**g**) and IL-4 (**h**) in spleen on day 42. All values shown in the figures are the means ± SD of 3 independent experiments. *, $p < 0.05$;**, $p < 0.01$, ***, $p < 0.001$

double antibody sandwich ELISA. As shown in Fig. 4e and f, relative to the PBS and pNZ8148 control groups, the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc groups exhibited significantly elevated levels of IFN-γ and IL-4 ($p<0.001$). Moreover, the levels of IL-4 in the pNZ8148-gD-IL-2-Fc group were notably higher than those in the pNZ8148-gD-IL-2 group ($p<0.05$), and the difference in IFN-γ levels was even more significant($p<0.001$). We also detected that IL-4 and IFN-γ levels in the spleen were highest in pNZ8148-gD-IL-2-Fc treated mice, followed by immunization with pNZ8148-gD-IL-2. IL-4 and IFN-γ levels in the pNZ8148-gD-IL-2 group were significantly higher than those in the PBS and pNZ8148 group ($p<0.001$) (Fig. 4g and h).

These findings suggested that the recombinant L. lactis vaccine had good immunogenicity and could significantly improve the cellular and humoral immune response levels of the body.

Immune-related genes expression and antibody levels in intestinal tissue following immunization

Immune-related gene expression in immunized and control mice was analyzed through RT-qPCR on cDNA from intestinal tissues. The relative expression levels of TLR2, MyD88, TRAF6, p65, and IRF7, excluding GAPDH, used as a control, exhibited significant or greatly significant upregulation in the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc immunization groups on days 14, 28. There was a notable rise in the intestinal expression levels of TLR2, MyD88, p65, and IL-1β on days 14 and 28, which returned to initial levels by day 42(Fig. 5a-f). At 42 days post-oral immunization, intestinal tissue was collected to assess antibody levels. As shown in Fig. 5g and h, the results observed in the intestines were consistent with those seen in the serum, showing significantly increased levels of IgA and IgG antibodies in the vaccine groups compared to the PBS and pNZ8148 groups ($p<0.001$). Notably, the pNZ8148-gD-IL-2-Fc group exhibited a significantly greater increase in antibody levels than the pNZ8148-gD-IL-2 group ($p<0.01$). This indicated a comparable immune response in both the systemic and mucosal compartments following vaccination.

These results suggested that the vaccine strain could induce an inflammatory reaction in mice following oral immunization.

Protection effect in mice after oral immunization

The mice were challenged with HSV-1 and viral titers in the lungs of the infected mice were determined 7 days post-challenge. The viral copy number was normalized to the amount of DNA used in the RT-qPCR. Compared to the control groups (PBS group, pNZ8148 group), the HSV-1 gD copy numbers of the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc groups were significantly reduced, and the pNZ8148-gD-IL-2-Fc group had the lowest copy number ($p<0.01$)(Fig. 6a), indicating that the recombinant *L. lactis* group could resist HSV-1 infection.

Lung tissue sections from each group were prepared and pathological changes were examined to further validate the protective effect of the oral vaccine (Fig. 6b). The control group of mice infected with HSV-1 showed more severe structural tissue damage, thickening of the lung interstitium, infiltration of a significant number of inflammatory cells, reduction of alveolar septa, and tissue fluid leakage. Mice immunized with recombinant *L. lactis* were infected with HSV-1, and the lung tissue structure was intact without obvious inflammatory cell infiltration or tissue fluid leakage. On the seventh-day post-virus challenge, TLR2, MyD88, and TRAF6 levels were significantly elevated in the control group compared to pre-challenge levels ($p<0.001$), whereas no significant changes were observed in the lungs of the recombinant vaccine group (Fig. 6c-e). IL-1β levels in the control group showed changes consistent with those described above. In the recombinant Lactococcus lactis pNZ8148-gD-IL-2-Fc group, no significant changes of IL-1β were observed, while the pNZ8148-gD-IL-2 group showed a slight increase ($p<0.05$) Fig. 6f.

Following the viral challenge, the pulmonary lavage fluid IgG and IgA levels were assessed in each group on days 0 and 7 to further evaluate the immune response induced by the oral vaccine (Fig. 6g and h). Compared to the control groups (PBS and pNZ8148), both IgG and IgA levels were significantly elevated in the pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc groups by day 7 post-challenge, with the highest levels observed in the pNZ8148-gD-IL-2-Fc group ($p<0.001$). This increase in pulmonary antibodies, particularly in the pNZ8148-gD-IL-2-Fc group, suggests a robust mucosal immune response, which correlates with the observed reduction in viral titers and the preservation of lung tissue structure.

These results indicate that recombinant *L. lactis* inhibited viral replication in vivo and alleviated the damage caused by HSV-1 infection in the body.

Discussion

The most cost-effective method for preventing and treating HSV-1 infections currently remains the development and application of vaccines. In recent years, research on live vector vaccines has become the main focus of research and development of herpes virus vaccines. LAB samples exhibited a significant inhibitory effect on HSV-1 replication, successfully shortening and limiting existing herpes recurrence, disrupting the two stages of viral replication, and limiting viral movement and transmission between cells [21]. *L. lactis* is not pathogenic and can present foreign proteins to the host immune system and stimulate an immune response after modification. In recent years, it has been widely studied as a safe, effective, and quality-controllable bacterial vaccine and genetic engineering vector [22–25]. Notably, the oral administration of engineered *L. lactis* allows for noninvasive delivery and direct in situ expression of viral antigens in the gastrointestinal tract, enhancing immune responses and avoiding the discomfort and risks associated with injections. Furthermore, it reduces protease activity and avoids adverse reactions caused by endotoxins [26–28]. Based on the above experimental findings, we selected *L. lactis* as the expression vector for exogenous genes to prepare a recombinant *L. lactis* oral vaccine expressing the HSV-1 glycoprotein gD.

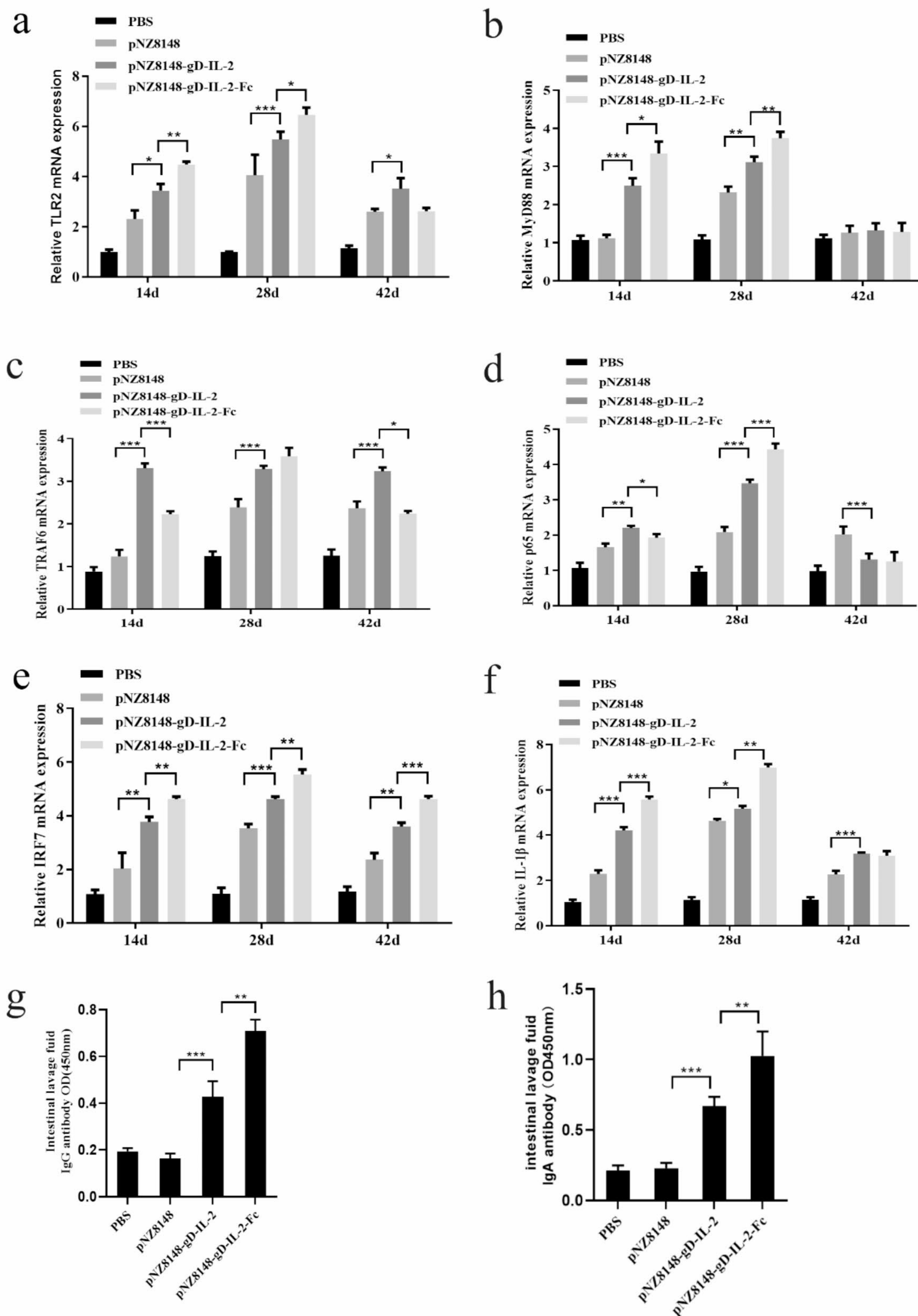

Fig. 5 RT-qPCR analysis of the expression of immune-related genes in intestinal tissues of the mice, along with ELISA detection of antibody levels in the intestine. (**a**) TLR2, (**b**) MyD88, (**c**) TRAF6, (**d**) p65, (**e**) IRF7, and (**f**) IL-1β. The mRNA levels of each gene were standardized relative to the expression of the GAPDH gene. Detection of (**g**) IgG and (**h**) IgA antibodies in the intestine. *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$

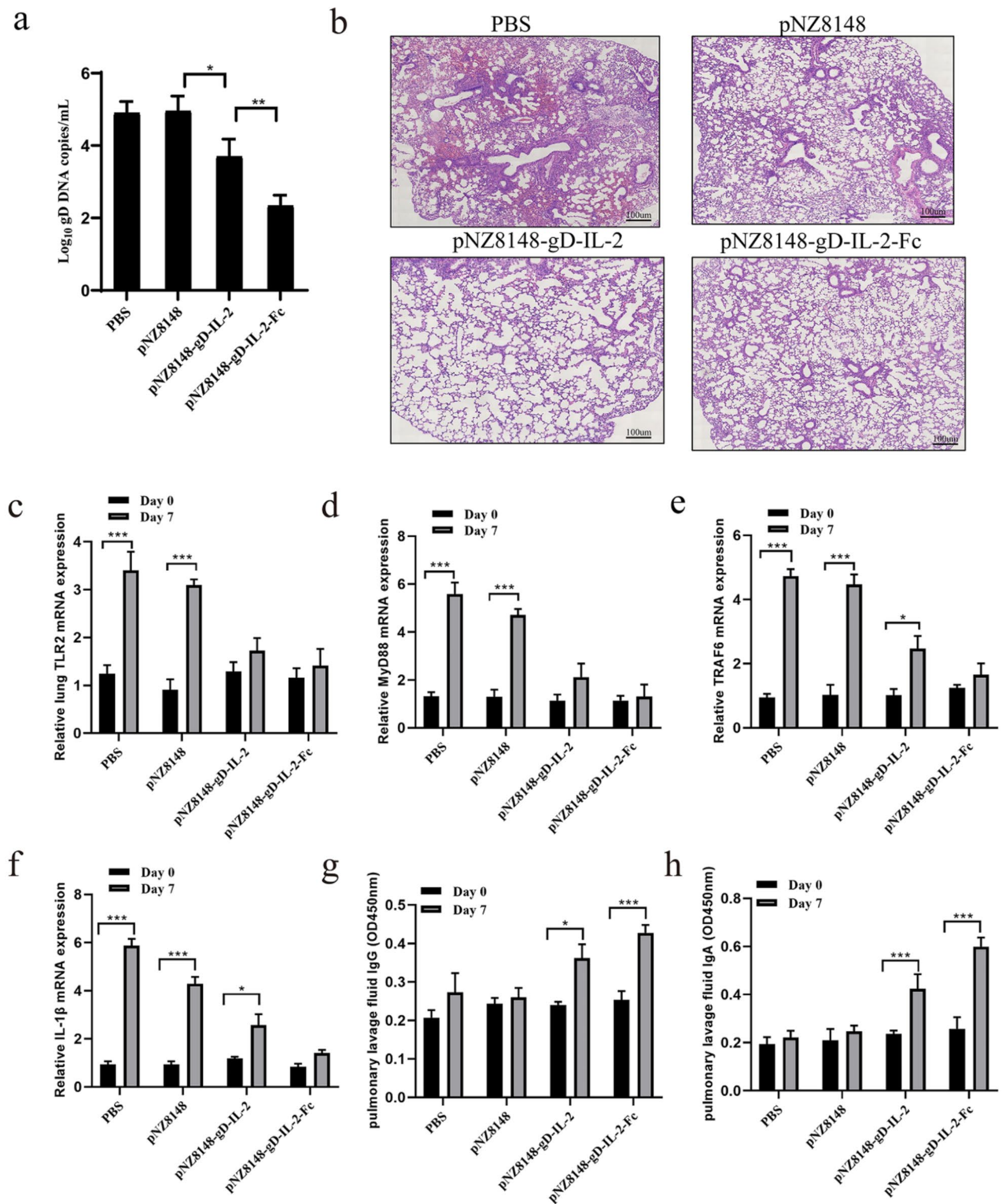

Fig. 6 Enhanced immunoprotection against HSV-1 infection induced by oral immunization vaccine. (**a**) For real-time quantitative PCR (RT-PCR), viral DNA was extracted from the lung tissues of four groups of mice infected with HSV-1 for 7 days after the end of immunization, and the copy number of the virus gene was determined by RT-qPCR. (**b**) Varying degrees of inflammation in the lungs of immunized mice, observed 7 days post HSV-1 challenge. (**c**-**e**) RT-qPCR analysis of the expression levels of TLR2, MyD88, and TRAF6 in lung tissues before (Day 0) and after the challenge (Day 7). (**f**) Relative mRNA expression levels of IL-1β in lung tissues as determined by RT-qPCR. (**g**-**h**) ELISA detection of IgG and IgA levels in pulmonary lavage fluid, comparing values before and after the HSV-1 challenge. *, $p < 0.05$;**, $p < 0.01$, ***, $p < 0.001$

The glycoprotein gD of HSV-1 is one of the most abundant viral antigens found on the surface of infected cells and in the viral envelope. Its main function is to bind to intracellular receptors and activate viral fusion with target cells [29]. In human clinical trials of Genocea vaccines, both HSV-1 gD and ICP4 induced equivalent CD8T cell responses, confirming that gD contains CD8T cell epitopes [30]. Hence, in this study, we used HSV-1 gD as the primary antigen for virus neutralization and IL-2 as an immune adjuvant to enhance the immunogenicity of gD. IL-2 exhibits a broad spectrum of biological functions, including the facilitation of T lymphocyte, B lymphocyte, and NK cell proliferation and differentiation, stimulation of cytokine secretion such as IFN and tumor necrosis factor, reinforcement of vaccine immune effects, and mitigation of vaccine-associated adverse effects [31]. It was reported that IL-2 and IL-6, when used as adjuvants and co-expressed with tetanus toxin fragment C (TTFC) in *L. lactis*, significantly enhanced the immune response. The intensity of the immune response produced by intranasal immunization in mice was 10–15 times higher than that of *L. lactis* expressing TTFC alone [32]. This suggests that the co-expression of vaccine antigens and adjuvants with *L. lactis* can generate stronger immune responses, indicating that constructing herpes virus vaccines using *L. lactis* as a vector is a feasible strategy.

The Th1 response is crucial in combating HSV-1 infection by producing IFN-γ, enhancing the cytotoxic activity of T lymphocytes, and the immune response of lymphocytes to target cells [33, 34]. The level of IL-4(Th2-type) is closely related to the production of antibodies and plays a crucial role in regulating humoral immunity [35]. Our results demonstrated that the level of IFN-γ and IL-4 in pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc was significantly higher compared with the control group PBS and pNZ8148, indicating that recombinant *L. lactis* with IL-2 as an adjuvant played a key biological role in enhancing cellular immunity and inhibiting HSV-1 infection. Such results were also expressed in the T lymphocyte proliferation index in peripheral blood and spleen. Our study showed that Th1-type (IFN-γ) and Th2-type (IL-4) cytokine responses specific to gD protein were significantly induced, which may have contributed to broadening the protection efficiency of our recombinant *L. lactis* vaccine.

In addition to enhancing cellular immunity through oral immunization with recombinant L. lactis pNZ8148-gD-IL-2 and pNZ8148-gD-IL-2-Fc, the production of specific IgG and IgA in the serum and intestines was also induced. Antibody levels in both the intestine and serum were significantly elevated after oral immunization, suggesting that the oral vaccine successfully stimulated both systemic and local mucosal immune responses. The above results demonstrated that the antibody titers of the animal IL-2 fusion vaccine were higher and the expansion of T follicular helper cells (Tfh) was higher than that of the vaccine alone, demonstrating that recombinant *L. lactis* with IL-2 as an adjuvant played a key biological role in inhibiting HSV-1 infection.

Notably, the pNZ8148-gD-IL-2-Fc group exhibited the highest levels of these immune markers, significantly surpassing those in the pNZ8148-gD-IL-2 group. The results showed that recombinant *L. lactis* with gD as the target and IL-2 as the adjuvant showed better results when fused with Fc fragments. FcRn is a specific receptor for immunoglobulin IgG, and fusion proteins can effectively cross mucosal barriers under the mediation of FcRn [18]. In mice inoculated with HSV-1 0ΔNLS, it was found that the absence of FcRn significantly reduced the neutralizing titer of antibodies against HSV-1 and the level of reactivity to HSV-1 [36]. Preliminary studies have shown that the recombinant *L. lactis* pNZ8148-gD-IL-2-Fc vaccine constructed at our institute has good immunogenicity and can significantly improve cellular and humoral immune responses in the body.

Pattern recognition receptors (PRRs) are responsible for detecting pathogen-associated molecular patterns and stimulating innate immunity in mice [37]. As one of the canonical PRRs, TLR2 is crucial for detecting the presence of invasive microbes and is involved in antiviral immunity [38, 39]. These effects are mediated by signaling pathways that are both MyD88-dependent and independent [40]. We found that intestinal tissues had much higher expression levels of TLR2, MyD88, and TRAF6. These findings suggest that the innate immune signaling pathways of intestinal immunological tissues can be directly activated by antigen processing cells (APCs). It was demonstrated that MyD88, a key adaptor protein in TLR signaling, was essential for the activation of downstream pathways involved in cytokine production and inflammation, such as the nuclear factor-kappa B (NF-κB) pathway [41]. Additionally, the recombinant vaccination strain stimulated the production of genes linked to cytokines and inflammation, including IL-1β, IRF7, and p65. In the gut, IL-1β was released after an increase in p65 [42].

Final immune protection was assessed using HSV-1 challenge monitoring post-challenge, which indicated that the HSV-1 gD copy number was significantly lower in the pNZ8148-gD-IL-2 group than in the PBS and pNZ8148 groups, and significantly reduced in the pNZ8148-gD-IL-2-Fc group. To examine the protective effect of recombinant *L. lactis* in mice, we conducted HE staining of mouse lung tissue. The results indicated that the recombinant *L. lactis* pNZ8148-gD-IL-2-Fc group did not show significant lung tissue damage. In contrast, the PBS and pNZ8148 groups showed severe lung tissue

damage, thickening of the lung interstitium, and infiltration by a significant number of inflammatory cells.

There weren't significant changes in the expression of TLR2, MyD88, and TRAF6 observed in the lungs of recombinant vaccine-immunized mice before and after viral challenge. This indicated that the vaccine had an immunomodulatory effect, capable of reducing excessive innate immune activation during viral infection, thus preventing tissue damage caused by an overreactive immune system. Furthermore, our study indicated that the vaccination can significantly elevate IgG and IgA levels in the lungs, enhancing defense against HSV-1. According to the experimental data, mice vaccinated with the prototype not only exhibited strong cellular immune responses at the immunization site (intestine) but also showed significant enhancement of cellular immune responses in mucosal sites distant from the immunization site (the lungs). This indicated that the oral vaccine in this study could effectively induce and enhance immune responses throughout the body, providing broad protective effects.

A previous study showed that mice intranasally vaccinated with pSIP401-HA1-ZN-3 exhibited strong mucosal immune responses [43]. During our preliminary intranasal experiments, we observed significant side effects that worsened infection in mice. Consequently, we focused exclusively on oral immunization for the remainder of our study and demonstrated that the recombinant *L .lactis* vaccine could generate systemic immune effects and resist HSV-1 viral infection.

Conclusions

In conclusion, recombinant *L. lactis* used for the preparation of the herpes simplex virus vaccine has potential developmental value. This study demonstrates that the oral *L. lactis* vaccine can effectively induce mucosal and systemic immune responses and provide immune protection against HSV-1 infection in a mouse model via the oral route, providing a new strategy and theoretical foundation for the prevention and control of herpes virus infection in clinical practice.

Abbreviations

L. lactis	Lactococcus lactis
HSV-1	Herpes simplex virus type 1
gD	glycoprotein D
IL	interleukin
IFN	interferon
HRP	horseradish peroxidase
ELISA	enzyme-linked immunosorbent assay
RT-qPCR	Real-time quantitative PCR
HE	hematoxylin and eosin
Ct	cycle threshold
TTFC	tetanus toxin fragment C
PRR	pattern recognition receptor
APCs	antigen processing cells

Acknowledgements
Not applicable.

Author contributions
S.Q. and R.L. conceived and designed research; S.Q., Y.H., H.W., and D.Z. conducted experiments; D.Z., A.S. analyzed data; S.Q., R.L., and Y.H. wrote the manuscript; L.Y., X.S., T.Z., Z.C., and Z.Y. revised this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding
This study was funded by the Startup Foundation for Doctor of Xinxiang Medical University (XYBSKYZZ202159 and XYBSKYZZ201824), the Key Scientific Research Foundation of the Higher Education Institutions of Henan Province (20B310009), the Key specialized research and development breakthrough of Henan Province (212102310752) and the provincial training program of innovation and entrepreneurship for undergraduates(202310472040).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All animal experiments were carried out following the guidelines for the Use and Care of Experimental Animals and were approved by the Ethics Committee of Xinxiang Medical University, China(approval number XYLL-20230271).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 23 January 2024 / Accepted: 31 August 2024
Published online: 09 September 2024

References

1. Simoons-Smit AM, Kraan EM, Beishuizen A, van Strack RJ, Vandenbroucke-Grauls CM. Herpes simplex virus type 1 and respiratory disease in critically-ill patients: real pathogen or innocent bystander? Clin Microbiol Infect. 2006. https://doi.org/10.1111/j.1469-0691.2006.01475.x
2. Periaswamy B, Maier L, Vishwakarma V, Slack E, Kremer M, Andrews-Polymenis HL, McClelland M, Grant AJ, Suar M, Hardt WD. Live attenuated S. Typhimurium vaccine with improved safety in immuno-compromised mice. PLoS ONE. 2012. https://doi.org/10.1371/journal.pone.0045433
3. Kolomaznik M, Nova Z, Calkovska A. Pulmonary surfactant and bacterial lipopolysaccharide: the interaction and its functional consequences. Physiol Res. 2017. https://doi.org/10.33549/physiolres.933672
4. Radziwill-Bienkowska JM, Zochowska D, Bardowski J, Mercier-Bonin M, Kowalczyk M. Lactococcus lactis IBB477 presenting adhesive and muco-adhesive properties as a candidate carrier strain for oral vaccination against influenza virus. Acta Biochim Pol 2014.
5. Zhai K, Zhang Z, Liu X, Lv J, Zhang L, Li J, Ma Z, Wang Y, Guo H, Zhang Y, Pan L. Mucosal immune responses induced by oral administration of recombinant Lactococcus lactis expressing the S1 protein of PDCoV. Virology. 2023. https://doi.org/10.1016/j.virol.2022.12.010
6. Torkashvand A, Bahrami F, Adib M, Ajdary S. Mucosal and systemic immune responses elicited by recombinant Lactococcus lactis expressing a fusion protein composed of pertussis toxin and filamentous hemagglutinin from Bordetella pertussis. Microb Pathog. 2018. https://doi.org/10.1016/j.micpath.2018.05.008
7. Lee SH, Beck BR, Hwang SH, Song SK. Feeding olive flounder (Paralichthys olivaceus) with Lactococcus lactis BFE920 expressing the fusion antigen of Vibrio OmpK and FlaB provides protection against multiple Vibrio pathogens: a universal vaccine effect. Fish Shellfish Immunol. 2021. https://doi.org/10.1016/j.fsi.2021.05.007
8. Gouran ST, Doosti A, Jami MS. Brucella abortus antigen omp25 vaccines: Development and targeting based on Lactococcus lactis. Vet Med Sci. 2023. https://doi.org/10.1002/vms3.1173
9. Sáez D, Fernández P, Rivera A, Andrews E, Oñate A. Oral immunization of mice with recombinant Lactococcus lactis expressing Cu,Zn superoxide dismutase

of Brucella abortus triggers protective immunity. Vaccine. 2012. https://doi.org/10.1016/j.vaccine.2011.12.088
10. Burbelo PD, Hoshino Y, Leahy H, Krogmann T, Hornung RL, Iadarola MJ, Cohen JI. Serological diagnosis of human herpes simplex virus type 1 and 2 infections by luciferase immunoprecipitation system assay. Clin Vaccine Immunol. 2009. https://doi.org/10.1128/cvi.00350-08
11. Watson RJ. DNA sequence of the herpes simplex virus type 2 glycoprotein D gene. Gene. 1983. https://doi.org/10.1016/0378-1119(83)90203-2
12. Lasky LA, Dowbenko DJ. DNA sequence analysis of the type-common glycoprotein-D genes of herpes simplex virus types 1 and 2. *DNA*. 1984. https://doi.org/10.1089/dna.1.1984.3.23
13. Arii J, Shindo K, Koyanagi N, Kato A, Kawaguchi Y. Multiple roles of the Cytoplasmic Domain of Herpes Simplex Virus 1 envelope glycoprotein D in infected cells. J Virol. 2016. https://doi.org/10.1128/jvi.01396-16
14. Bernstein DI, Cardin RD, Smith GA, Pickard GE, Sollars PJ, Dixon DA, Pasula R, Bravo FJ. The R2 non-neuroinvasive HSV-1 vaccine affords protection from genital HSV-2 infections in a guinea pig model. NPJ Vaccines. 2020. https://doi.org/10.1038/s41541-020-00254-8
15. Lu Z, Brans R, Akhrameyeva NV, Murakami N, Xu X, Yao F. High-level expression of glycoprotein D by a dominant-negative HSV-1 virus augments its efficacy as a vaccine against HSV-1 infection. J Invest Dermatol. 2009. https://doi.org/10.1038/jid.2008.349
16. Egan KP, Hook LM, Naughton A, Pardi N, Awasthi S, Cohen GH, Weissman D, Friedman HM. An HSV-2 nucleoside-modified mRNA genital herpes vaccine containing glycoproteins gC, gD, and gE protects mice against HSV-1 genital lesions and latent infection. PLoS Pathog. 2020. https://doi.org/10.1371/journal.ppat.1008795
17. Lu L, Palaniyandi S, Zeng R, Bai Y, Liu X, Wang Y, Pauza CD, Roopenian DC, Zhu X. A neonatal fc receptor-targeted mucosal vaccine strategy effectively induces HIV-1 antigen-specific immunity to genital infection. J Virol. 2011. https://doi.org/10.1128/jvi.05441-11
18. Qian S, Zhang W, Jia X, Sun Z, Zhang Y, Xiao Y, Li Z. Isolation and identification of Porcine Epidemic Diarrhea Virus and its effect on host Natural Immune Response. Front Microbiol. 2019. https://doi.org/10.3389/fmicb.2019.02272
19. Ye L, Zeng R, Bai Y, Roopenian DC, Zhu X. Efficient mucosal vaccination mediated by the neonatal fc receptor. Nat Biotechnol. 2011. https://doi.org/10.1038/nbt.1742
20. Li H, Tao Y, Zhao P, Huai L, Zhi D, Liu J, Li G, Dang C, Xu Y. Effects of Pholiota Nameko polysaccharide on NF-κB pathway of murine bone marrow-derived dendritic cells. Int J Biol Macromol. 2015. https://doi.org/10.1016/j.ijbiomac.2015.03.011
21. Vilhelmova-Ilieva N, Atanasov G, Simeonova L, Dobreva L, Mancheva K, Trepechova M, Danova S. Anti-herpes virus activity of lactobacillus' postbiotics. Biomed (Taipei). 2022. https://doi.org/10.37796/2211-8039.1277
22. Bahey-El-Din M. Lactococcus lactis-based vaccines from laboratory bench to human use: an overview. Vaccine 2012https://doi.org/10.1016/j.vaccine.2011.11.098
23. Tian H, Li J, Chen X, Ren Z, Pan X, Huang W, Bhatia M, Pan LL, Sun J. Oral delivery of mouse β-Defensin 14 (mBD14)-Producing Lactococcus lactis NZ9000 attenuates experimental colitis in mice. J Agric Food Chem. 2023. https://doi.org/10.1021/acs.jafc.2c07098
24. Wu J, Tian X, Xu X, Gu X, Kong J, Guo T. Engineered Probiotic Lactococcus lactis for Lycopene production against ROS stress in intestinal epithelial cells. ACS Synth Biol. 2022. https://doi.org/10.1021/acssynbio.1c00639
25. Tavares LM, de Jesus LCL, da Silva TF, Barroso FAL, Batista VL, Coelho-Rocha ND, Azevedo V, Drumond MM, Mancha-Agresti P. Novel strategies for efficient production and delivery of live biotherapeutics and Biotechnological uses of Lactococcus lactis: the Lactic Acid Bacterium Model. Front Bioeng Biotechnol. 2020. https://doi.org/10.3389/fbioe.2020.517166
26. Bron PA, Kleerebezem M. Lactic acid Bacteria for delivery of Endogenous or Engineered Therapeutic molecules. Front Microbiol. 2018. https://doi.org/10.3389/fmicb.2018.01821
27. Barra M, Danino T, Garrido D. Engineered Probiotics for Detection and Treatment of Inflammatory Intestinal diseases. Front Bioeng Biotechnol. 2020. https://doi.org/10.3389/fbioe.2020.00265
28. Gorain C, Singh A, Bhattacharyya S, Kundu A, Lahiri A, Gupta S, Mallick AI. Mucosal delivery of live Lactococcus lactis expressing functionally active JlpA antigen induces potent local immune response and prevent enteric colonization of Campylobacter jejuni in chickens. Vaccine 2020https://doi.org/10.1016/j.vaccine.2019.12.064
29. Stanberry LR, Spruance SL, Cunningham AL, Bernstein DI, Mindel A, Sacks S, Tyring S, Aoki FY, Slaoui M, Denis M, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. N Engl J Med. 2002. https://doi.org/10.1056/NEJMoa011915
30. Flechtner JB, Long D, Larson S, Clemens V, Baccari A, Kien L, Chan J, Skoberne M, Brudner M, Hetherington S. Immune responses elicited by the GEN-003 candidate HSV-2 therapeutic vaccine in a randomized controlled dose-ranging phase 1/2a trial. Vaccine. 2016. https://doi.org/10.1016/j.vaccine.2016.09.001
31. Hernandez R, Põder J, LaPorte KM, Malek TR. Engineering IL-2 for immunotherapy of autoimmunity and cancer. Nat Rev Immunol. 2022. https://doi.org/10.1038/s41577-022-00680-w
32. Qian S, Jia X, Gao Z, Zhang W, Xu Q, Li Z. Isolation and identification of Porcine Deltacoronavirus and Alteration of Immunoglobulin Transport Receptors in the intestinal mucosa of PDCoV-Infected piglets. Viruses. 2020. https://doi.org/10.3390/v12010079
33. Di Carlo E, Comes A, Orengo AM, Rosso O, Meazza R, Musiani P, Colombo MP, Ferrini S. IL-21 induces tumor rejection by specific CTL and IFN-gamma-dependent CXC chemokines in syngeneic mice. J Immunol. 2004. https://doi.org/10.4049/jimmunol.172.3.1540
34. Sobol PT, Boudreau JE, Stephenson K, Wan Y, Lichty BD, Mossman KL. Adaptive antiviral immunity is a determinant of the therapeutic success of oncolytic virotherapy. Mol Ther. 2011. https://doi.org/10.1038/mt.2010.264
35. Miyauchi K, Adachi Y, Tonouchi K, Yajima T, Harada Y, Fukuyama H, Deno S, Iwakura Y, Yoshimura A, Hasegawa H, et al. Influenza virus infection expands the breadth of antibody responses through IL-4 signalling in B cells. Nat Commun. 2021. https://doi.org/10.1038/s41467-021-24090-z
36. Petkova SB, Akilesh S, Sproule TJ, Christianson GJ, Al Khabbaz H, Brown AC, Presta LG, Meng YG, Roopenian DC. Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease. Int Immunol. 2006. https://doi.org/10.1093/intimm/dxl110
37. Aoki T, Hikima J, Hwang SD, Jung TS. Innate immunity of finfish: primordial conservation and function of viral RNA sensors in teleosts. Fish Shellfish Immunol. 2013. https://doi.org/10.1016/j.fsi.2013.02.005
38. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010. https://doi.org/10.1016/j.cell.2010.01.022
39. Cao LP, Du JL, Jia R, Ding WD, Xu P, Yin GJ, Zhang T. Effects of cyclophosphamide on antioxidative and immune functions of Nile tilapia (Oreochromis Niloticus) via the TLR-NF-κB signaling pathway. Aquat Toxicol. 2021. https://doi.org/10.1016/j.aquatox.2021.105956
40. Liao Z, Su J. Progresses on three pattern recognition receptor families (TLRs, RLRs and NLRs) in teleost. Dev Comp Immunol. 2021. https://doi.org/10.1016/j.dci.2021.104131
41. Zhao M, Zhang Y, Yang X, Jin J, Shen Z, Feng X, Zou T, Deng L, Cheng D, Zhang X, et al. Myeloid neddylation targets IRF7 and promotes host innate immunity against RNA viruses. PLoS Pathog. 2021. https://doi.org/10.1371/journal.ppat.1009901
42. Chen S, Liu H, Li Z, Tang J, Huang B, Zhi F, Zhao X. Epithelial PBLD attenuates intestinal inflammatory response and improves intestinal barrier function by inhibiting NF-κB signaling. Cell Death Dis. 2021. https://doi.org/10.1038/s41419-021-03843-0
43. Zhang Y, Yang L, Zhang J, Huang K, Sun X, Yang Y, Wang T, Zhang Q, Zou Z, Jin M. Oral or intranasal immunization with recombinant Lactobacillus plantarum displaying head domain of Swine Influenza A virus hemagglutinin protects mice from H1N1 virus. Microb Cell Fact. 2022. https://doi.org/10.1186/s12934-022-01911-4

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.