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CD4+ CD8αα+ T Cells in the Gastric epithelium Mediate Chronic Inflammation Induced by Helicobacter Felis

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Abstract

CD4+ CD8αα+ double-positive intraepithelial T lymphocytes (DP T cells), a newly characterized subset of intraepithelial T cell, has been reported to contribute to local immunosuppression. However, whether DP T cells are present in *Helicobacter. pylori*-induced gastritis, and their relationship with disease prognosis remain to be elucidated. In this study, We established chronic gastritis models through *Helicobacter felis* (*H. felis*) infection. Gastric infiltrating lymphocytes were isolated from *H. felis*-induced gastritis mice and analyzed by flow cytometry. Our results suggest that DP T cells frequency in *H. felis*-induced gastritis mice was higher than the uninfected mice. Gastric DP T cells derived from lamina propria cells, which distributed in the gastric epithelial layer. We found that DP T cells exhibited anti-inflammatory function. *In vitro*, DP T cells inhibited the maturation of dendritic cells and the proliferation of CD4+ T cell. The elimination of CD4+CD8αα+ T cells *in vivo* resulted in severe gastritis and a reduction of *H. felis* load. Additionally, vaccine with silk fibroin as delivery systems enhanced vaccine efficacy by reducing DP T cells. We demonstrated that DP T cells performed an immunosuppressive role in *Helicobacter felis*-induced gastritis. These findings revealed that DP T cells may affect the prognosis of the disease and the vaccine efficacy.

1. Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral- shaped and microaerophilic bacterium that colonizes in gastric mucosa of more than half of the world's population [9]. This bacterium is involved in several diseases such as chronic gastritis, gastric ulcer and gastric cancer [3, 17]. Current research finds that CD4 + T helper cells (Th cells) play an important role in *H. pylori* infection diseases. For example, studies have shown that both Th1 and Th17 cells may have pro-inflammatory function [26, 16], and Treg cells may play an anti-inflammatory role during *H. pylori* infection [19, 1]. Moreover, whether and how other CD4 + T cell subsets respond to *H. pylori* infection and affect the antibacterial immunity remains unclear.

CD4 + CD8αα + T cells (also known as double-positive T cells, DP T cells) proved to be a distinctive subset of CD4 + T cells, played an important role in inflammatory diseases. Some studies report that CD4 + CD8αα + intraepithelial lymphocytes (IEL) have cytotoxic potential[8, 20]. The antigen presentation by small IECs induced CD4 + CD8αα + intraepithelial lymphocytes expressed a large amount of Granzyme B (Granzyme B) and highly expresses CD107a, also known as Lysosomal associated membrane protein-1 (LAMP-1)[8]. In clinical studies [2], it was found that CD4 + CD8αα + T cells in patients with celiac disease are significantly reduced compared with the healthy one, suggesting that CD4 + CD8αα + T cells have potential regulatory functions. Studies show that CD4 + CD8αα + T cells can secrete cytokines similar to regulatory T cells (Regulatory cells, Treg). In addition, CD4 + CD8αα + T cells inhibit Th1-induced intestinal inflammation in an IL-10-dependent manner[4]. Studies also show that DP IELs and Treg cells synergistically regulate intestinal inflammation [25]. These studies show that CD4 + CD8αα + T cells are important subsets of CD4 + T lymphocytes in the intestinal epithelium and play an irreplaceable role in intestinal inflammation. However, the role of CD4 + CD8αα + T cells in gastritis is still not completely

understood. It is nothing is known about the effects of DP T cells in *H. pylori* persistent and vaccine efficacy.

There are two main animal models of gastric infection: *Helicobacter felis* (*H. felis*) infection model and human *H. pylori* strain infection model [22, 5]. *H. felis* has a strong ability to induce gastritis in mice. In addition, the gastritis caused by *H. felis* infection are more similar to that of Hp-infected patients' gastritis[22]. Therefore, *H. felis* infection model was used in our study. Studies show that regulatory T cells affect vaccine efficacy by inhibiting the protective immune response caused by vaccination [11]. In this study, we have identified a subset of mouse gastric T cells that co-express CD4 and CD8αα. We found that gastric CD4 + CD8αα + T cells increased significantly in *H. felis*-induced gastritis mice. We analyzed the location and phenotype of gastric CD4 + CD8αα + T cells. In addition, we found that gastric CD4 + CD8αα + T cells are able to inhibit the maturation of dendritic cells (DCs) and the proliferation of CD4 + T cells *in vitro*. We also showed that CD4 + CD8αα + T cells affected gastritis and *H. felis* load in vivo.

Liu. et al had demonstrated that the mice were vaccinated *H. pylori* vaccine in the gastric subserous layer (GSL) induced better protection against *H. pylori* infection.[12]However, we found that rather than other ways (such as p.o. and s.c.), vaccination in GSL induced a large number of CD4 + CD8αα + T cells. Studies showed that regulatory T cells affect vaccine efficacy by inhibiting the protective immune response caused by vaccination [11]. It suggests that the induction of CD4 + CD8αα + T cells may affect vaccine efficacy. Here, we compared two vaccine delivery systems used in the laboratory. We found that silk fibroin (SF)-loaded vaccine enhanced vaccine efficacy by inhibiting CD4 + CD8αα + T cells development. These studies suggest that the immunosuppression of *H. felis* induced-gastritis may be related to CD4 + CD8αα + T cells, which may affect the prognosis of the disease and vaccine efficacy.

2. Materials And Methods

2.1 Mice

Six-week-old female C57BL/6J mice were obtained from the Comparative Medicine Center of Yangzhou University and bred at the China Pharmaceutical University Animal Experimental Center. All animal experiments were approved by the Animal Ethical and Experimental Committee of China Pharmaceutical University. The animals allowed to acclimatize in the Animal Facility for one week before being randomly assigned to experimental groups.

2.2 H. felis challenge

H. felis (ATCC 49179) was cultured as previously described [7, 28]. H. felis was routinely cultured at 37 °C with 120 rpm shaking in trypticase soy liquid medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) under microaerophilic conditions. After 96 h, H. felis were harvested by centrifugation and re-suspended in fresh medium. The absence of contaminants and bacterial motility in cultures was

checked by phase contrast microscopy. Mice were fasted 8 h and inoculated 3 times at 2-day intervals with 0.5×10^7 H. felis in 200μL medium.

2.3 Real-time qPCR to detect the number of H. felis in the gastric mucosa

To detect the colonization densities of H. felis, qRT-PCR was performed to detect flab expression in gastric tissue. The DNA was extracted with a Qiagen DNA Tissue Mini Kit according to the manufacturer's instructions. PCR amplification was performed with a conventional TaqMan method. TaqMan gene primers and probes were designed by Sangon Biotech Co. Led. (China, Shanghai) based on the sequence numbers listed in Table 1 [28, 7].

Table 1
Q-PCR primer sequences.

Gene	Sequences (5' to 3')
GAPDH forward	GGGGGTAGGAACACGGAA
GAPDH reverse	AAGGGTGGAGCCAAAAGG
FlaB forward	TTCGATTGGTCCTACAGGCTCAGA
FlaB reverse	TTCTTGTTGATGACATTGACCAACGCA

2.4 Preparation of Single-Cell Suspension from Gastric Tissue

Single-cell suspensions were prepared using a previously described technique[12]. Briefly, the whole stomach was isolated, cut through the lesser curvature, and the contents were removed before being placed into 15 ml RPMI 1640 containing 10 mM HEPES, 10% FBS, 4 mM EDTA, and 0.5 mM dithiothreitol. Gastric epithelial lymphocytes were isolated by shaking at 250 rpm and 37 ℃ for 30 min. Tissues were then incubated with another 15 ml RPMI 1640 containing 10 mM HEPES, 10% FBS, 4 mM EDTA, and 0.5 mM dithiothreitol for 30 min to isolate the remaining lymphocytes. Supernatants were passed through a 70 μm cell strainer. After washing and centrifugation, cell pellets were resuspended in an appropriate medium for further analysis or culture.

2.5 FTY720 Treatment

For FTY720 treatment, 1 mg/kg FTY720 was injected i.p. twice a week to block circulating T cell egress from the lymphoid nodes according to the design of the experiments [12, 27].

2.6 Neutralizing Antibody Experiments

Infected mice were i.p. injected with 100 μg anti-CD8α antibody (2.43, BioXcell), anti-α4β7 (DATK32, BioXcell) antibody or anti-Rat IgG2a every 2 days to deplete CD4 + CD8αα + T cells according to the design of experiments.

2.7 Flow cytometry

For cell frequency and phenotype detection, single-cell suspensions were stained with the following antibodies: anti-CD45 (30-F11), anti-CD4 (GK1.5 or RM4-4), anti-CD8α (GL-1 or 53 − 6.7), anti-CD8β (YTS156.7.7), anti-TCRγδ (UC7-13D5), anti-CD25 (3C7), anti-FOXP3 (MF-14), anti-CD103 (2E7), anti-LAMP-1 (DATK32), anti-CD80 (16-10A1), anti-CD86 (GL-1) anti-Ly6C (HK1.4), anti-Gr-1 (RB6-8C5) and anti-CD11c (N418) purchased from Biolegend or BD Pharmingen. Multiparameter analyses were performed on a BD FACS Aria II or a BD FACS Calibur flow cytometer.

2.8 Bone marrow-derived dendritic cell culture

The bone marrow cells were isolated from the femurs and tibias of male C57BL/6 mice. According to the method as previously mentioned [10]. The cells were seeded in 24-well plate containing complete medium (RPMI 1640 supplemented with glutamine, penicillin, streptomycin, 2-mercaptoethanol) at the concentration of 1×10^6 cells per well after removing red blood cells. Three-fourth of culture medium was replaced with fresh medium and supplemented with IL-4 and GM-CSF every 2 days. After 6 days, bone marrow cells were reseeded and continue to culture for 1 or 2 days. The purity of the BMDCs was detected by FASC.

2.9 Isolation of CD4 + CD8αα + T cells

The preparation of single-cell suspensions of stomach is the same way as before. Mouse single cell suspensions are either selected in a two-step separation process. Cell suspension was enriched for DP T cells by positive selection on magnetic separating columns using the MojoSort™ Mouse APC nanobeads (Biolegend). Purities were consistently greater than 90%.

2.10 Suppressive Assays: Inhibition of CD4 Proliferation and DC Maturation

In the presence or absence of CD4 + CD8αα + T cells, 5×10^4 CD4 + T cells per well were cultured in 96-well plates with plate-bound anti-CD3 (5 μg/mL, Biolegend)) in complete medium (RPMI-1640 containing 10% FBS, 2 mM L-glutamine, 30 μM β-mercaptoethanol, non-essential amino acids (NEAAs), 1 mM sodium pyruvate and streptomycin) together with IL-2 (5 ng/mL, PreproTech). The proliferation of target CD4 T cells was assessed by flow cytometry analysis of CFSE dilution among CD8-negative T cells, on day 4. In the presence or absence of CD4 + CD8αα + T cells, immature DC were cultured with LPS for 2 to 3 d (to induce DC maturation) in the presence or absence of CD4 + CD8αα + T cells. The cells were stained with APC-conjugated anti-CD8α (53 − 6.7) and FITC-conjugated anti-CD80 (16-10A1) and PE-conjugated anti-CD86 (GL-1), or isotype control antibodies. APC negative cells were analyzed by flow cytometry to determine the level of expression of CD80 and CD86.

2.11 Statistical analysis

All of the statistical analyses were performed with GraphPad Prism 5 software. The data were expressed as the mean ± standard deviation (SD). P-values of less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Result

3.1 CD4 + CD8αα + T lymphocytes are present in stomach after *H. felis* infection

To detect gastric CD4 + CD8αα + T cells upon *H. felis* infection, we analyzed the co-expression of CD4 and either CD8α or CD8β by T cells isolated from *H. felis* induced-gastritis mice and the uninfected one (Fig. S1). We noticed that the mice harbored substantial numbers of CD4 + CD8αα + T cells upon *H. felis* infection, whereas CD4 + CD8αα + T cells were negligible or absent in mice from the uninfected one (Fig. 1a). After *H. felis* infection, CD4 + CD8αα + T cells were observed on Day 20 (Fig. 1b, Fig. S2) and stabilized after Day 30 (data not shown), coincident with the progression of chronic gastritis (Fig. 1c, d).

Several reports suggest that inflammatory bowel disease is closely related to colonic CD4 + CD8αα + T cells [4, 2, 20]. We assessed the correlation between the formation of gastritis and the frequency of CD4 + CD8αα + T cells in *H. felis* infection mice. Notably, our data demonstrated that the induction of CD4 + CD8αα + T cells was accompanied by inflammation (Fig. 1e).

3.2 CD4 + CD8αα + T lymphocytes formed by lamina propria cells distributed in the gastric epithelial layer

We next examined the location of DP T cells induced by gastritis. Refer to the previous method[12], stomach was isolated and sniped longitudinally to use for immunohistological staining of CD4 + CD8αα + T cells. The results indicated that CD4 + CD8αα + T cells could be observed in the gastric epithelial layer while Treg cells were located close to the lamina propria (Fig. S3a, b). Parallelly, flow detection of CD4 + CD8αα + T cells in the epithelium or lamina propria also suggested that most of CD4 + CD8αα + T cells were located in epithelial regions (Fig. S3c). These data demonstrated that CD4 + CD8αα + T cells recruited by *H. felis* gastritis preferentially infiltrate the adjacent mucosa.

Previous studies suggest that CD4 + CD8αα + T cells are formed by migration and differentiation of CD4 + T cells in the lamina propria[15, 21, 25]. To investigate whether these CD4 + CD8αα + T cells experienced recirculation via the lymph vascular system, we treated *H. felis* infected mice with FTY720 twice a week to inhibit lymphocyte egress from lymph nodes[27, 12] for 14 or 30 days (Fig. 2a)[13]. The frequency and absolute number of CD4 + CD8αα + T cells were decreased in the group 3 but not group 2, the number of CD4 + CD8αα + T cells in group 2 was stable in stomach (Fig. 2b, c), suggesting the characteristic of migration and differentiation of CD4 + CD8αα + T cells.

3.3 Double Positive CD4 + CD8αα + T lymphocytes exhibit a regulatory phenotype and function

It had been hypothesized that CD4 + CD8αα + T lymphocytes might be regulatory cells [23, 6]. Therefore, we measured the regulatory phenotype of CD4 + CD8αα + T lymphocytes. As shown in Fig. 3a, CD4 + CD8αα + T lymphocytes expressed high intensity level of CD25 and CTLA4, activation and co-stimulatory markers (CD80 and CD86). We found that DP T cells do not express Foxp3. Moreover, CD4 + CD8αα + T lymphocytes expressed the homing/localization molecules (α4β7 and CD103).

Then, we addressed the regulatory potential of these cells in vitro. Similar to Foxp3 + Treg cells, DP T cells inhibited the maturation of dendritic cells (DCs), as revealed by inhibition of CD80/CD86 upregulation (Fig. 3b, Fig. S4). In addition, CD4 + CD8αα + T cells inhibited CD4 + T cell proliferation induced by anti-CD3 and anti-CD28 antibodies (Fig. 3c).

3.4 *In vivo* neutralization of anti-CD8α antibodies results in decreased colonization in *H. felis*-challenged mice

To directly address whether exaggerated CD4 + CD8αα + T cells differentiation could regulate chronic inflammation in *H. felis* infected mice, we depleted CD4 + CD8αα + T cells using neutralizing antibodies (Fig. 4a). We found that integrin α4β7 was up-regulated in CD4 + CD8αα + T cells (Fig. 3a). Furthermore, we found that the frequency of CD4 + CD8αα + T cells significantly decreased after the injection of both anti-α4β7 antibodies (DATK-32) and anti-CD8α antibodies (2.43), which could deplete CD4 + CD8αα + T cells (Fig. 4b). In addition,we also found that *H. felis* infected mice treated with anti-CD8α antibodies, but not control antibodies, showed severe intestinal inflammation and induced a reduction of *H. felis* load. (Fig. 4c).

However, the blockade of integrin α4β7 resulted in both a reduction of the frequency of CD4 + CD8αα + T cells and gastritis severity in stomach of *H. felis* infected mice (Fig. 4b, d). Interestingly, we found that integrin α4β7 was also up-regulated in Ly6c + T cells (Fig. S5). We asked whether the treatment of DATK-32 influences the number of neutrophils and monocytes in *H. felis* infected mice. We found that the frequency of Ly6c + CD11b + cells in the stomach decreased (Fig. 4e). These data indicated that the blockade of integrin α4β7 resulted in a decrease of the frequency of CD4 + CD8αα + T cells, neutrophils and monocytes, which leading to a reduction in inflammation. Taken together, these data indicated that the reduction of CD4 + CD8αα + T cells induced by neutralizing antibodies resulted in significantly increased gastritis and induced a drastic reduction of *H. felis* load. In addition, these data demonstrated that the immune homeostasis and the persistence of *H. felis* in the gastric mucosa is independent, at least in part, on the engagement of CD4 + CD8αα + T cells.

3.5 SF adjuvant enhances vaccine efficacy by reducing double positive T cells

Previous studies suggest that adjuvants can enhance the immunogenicity of T cell vaccines through different mechanisms, including restricting the development of T regulatory cells, and promote the T cell immunity. Our research showed CD4 + CD8αα + T cells are also one of regulatory cells involved in inflammatory response.

To detect the gastric CD4 + CD8αα + T cells upon vaccinations, we performed different vaccine administrations[12] on the mice and compared the gastric CD4 + CD8αα + T cells at Day 30 (Fig. S6a). Results revealed that few CD4 + CD8αα + T cells were detected in these groups except for GSL (Fig. S6b, c). The regulatory T cells affect vaccine efficacy by inhibiting the protective immune response caused by vaccination [11]. To verify this, we compared two adjuvant systems,SF/CCF and Al/CCF.We found that GSL injection of SF/CCF did not recruit CD4 + CD8αα + T cells into stomach (Fig. 5a, b), and show a stronger protective inflammatory response in gastric tissues and reduced *H. felis* colonization (Fig. 5c). These findings suggested that GSL administration provided better immune protection, but produced a large number of CD4 + CD8αα + T cells. Moreover, the use of SF adjuvant can reduce the proportion of CD4 + CD8αα + T cells and achieve better vaccine efficacy.

4. Discussion

The reduction of CD4 + CD8αα + T cells have been shown in patients with inflammatory bowel disease (IBD) [24, 2]. CD4 + CD8αα + T cells can cooperate with Treg cells to regulate intestinal inflammation in mice [25]. However, there is currently little evidence describing how CD4 + CD8αα + T cells regulate gastritis. Therefore, in this study, we demonstrated that CD4 + CD8αα + T cells in the stomach of the gastritis mice increased significantly, and the induction of CD4 + CD8αα + T cells was coincident with the progression of chronic gastritis.

CD4 + CD8αα + T cells displayed regulatory and cytolytic activity in intestinal immunity. For example, CD4 + CD8αα + T cells have been shown to express some T regulatory cells-type cytokines [4]. CD4 + CD8αα + T cells can suppress T helper 1-induced intestinal inflammation in an IL-10-dependent fashion. In addition, CD4 + CD8αα + T cells displayed functional features that were similar to cytotoxic T lymphocytes (CTL). The activated CD4 + CD8αα + T cells could effectively kill target cells *in vitro* [15]. In this study, CD4 + CD8αα + T cells induced by gastritis can inhibit the maturation of DC and the proliferation of CD4 + T cells. These findings indicated that CD4 + CD8αα + T cells played a protective role in the development of chronic gastritis through suppressing DC and CD4 + T cells.

Research proves that anti-CD8α antibody can inhibit CD4 + CD8αα + T cells in the intestine [25]. In the present study, our findings confirmed that anti-CD8α antibodies can effectively reduce the proportion of CD4 + CD8αα + T cells in the stomach. Inhibiting CD4 + CD8αα + T cells in stomach significantly promote gastric inflammation and reduce colonization of *H. felis*, which is consistent with our observation that CD4 + CD8αα + T cells are related to inflammation. Furthermore, we have demonstrated that CD4 + CD8αα + T cells highly express integrin α4β7. Studies show that integrin α4β7 plays an important role in the homing of CD4 + T cells to gastric tissues [14].Interestingly, our findings demonstrated that α4β7 antibodies can reduce the proportion of CD4 + CD8αα + T cells and Ly6c + CD11b + T cells, which reduced the severity of gastritis and had no effect on colonization of *H. felis*, suggesting that α4β7 antibodies targeted other inflammatory cells in addition to CD4 + CD8αα + T cells in regulating gastritis.

Previous studies indicate that GSL vaccination method is more effective in enhancing the efficacy of the vaccine [12]. As a new vaccination method, GSL is to select the sub serosal layer of the avascular region of the great gastric curvature as the inoculation point, which can induce TRM cells in the stomach to enhance the vaccine efficacy [12, 27]. However, we found that GSL administration could induce more CD4 + CD8αα + T cells than other traditional immunization routes. Regulatory T cells affect vaccine efficacy by inhibiting the protective immune response caused by vaccination [11]. Therefore, we believe that *in situ* inoculation of aluminum adjuvant vaccine GSL can induce the production of CD4 + CD8αα + T cells in the stomach and affects the efficacy of the vaccine. Silk fibroin (SF) is a biological slow-release drug delivery material with good biocompatibility, degradability, stability and controllability [18]. Previous studies have found that SF as an adjuvant instead of aluminum adjuvant can obtain a wider range of immune, which effectively reduces colonization of Hp [27]. Therefore, we investigated whether SF enhanced the vaccine efficacy by inhibiting CD4 + CD8αα + T cells. In this study, we found that the use of SF adjuvant can effectively reduce the frequency of CD4 + CD8αα + T cells, and effectively reducing the colonization of *H. felis* compared to Alum adjuvant. These results implied that SF adjuvant may enhance the efficacy of the vaccine by reducing the frequency of CD4 + CD8αα + T cells caused by vaccination in the stomach *in situ*.

5. Conclusion

In summary, our study showed that gastric CD4 + CD8αα + T cells were significantly increase in gastritis mice induced by *H. felis*. CD4 + CD8αα + T cells were located in the gastric epithelium. Moreover, CD4 + CD8αα + T lymphocytes exhibited a Treg phenotype and anti-inflammatory function. In addition, SF-loaded vaccines enhanced efficacy by inhibiting the production of CD4 + CD8αα + T cells. These findings suggested an immunosuppressive role for CD4 + CD8αα + T cells in the pathogenesis of *H. felis*-induced gastritis and vaccine efficacy. In the future, therapeutic strategies and vaccine adjuvant design aimed at interfering with CD4 + CD8αα + T cells may benefit to *Helicobacter pylori* infected patients

Declarations

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Conflicts of interest/Competing interests :

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval (include appropriate approvals or waivers):

The approval of all animal's treatments was obtained from the Animal Ethics Committee of China Pharmaceutical University

Consent to participate (include appropriate statements):

Consent to participate

Consent for publication (include appropriate statements):

Consent to publish

Availability of data and material (data transparency):

The data is true and reliable

Code availability (software application or custom code):

Not applicable

Authors' contributions:

Yingying Xing conceived and designed the project. Guojing Ruan and An Huang and performed most experiments and data analysis. Chupeng Hu, Ningyin Xu, Zhenxing Zhang, Menghui Fan and Yue Wang participated in some experiments. Guojing Ruan and An Huang wrote the paper. All authors contributed to discussion of the results, reviewed the manuscript, and approved the final article.

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Figures

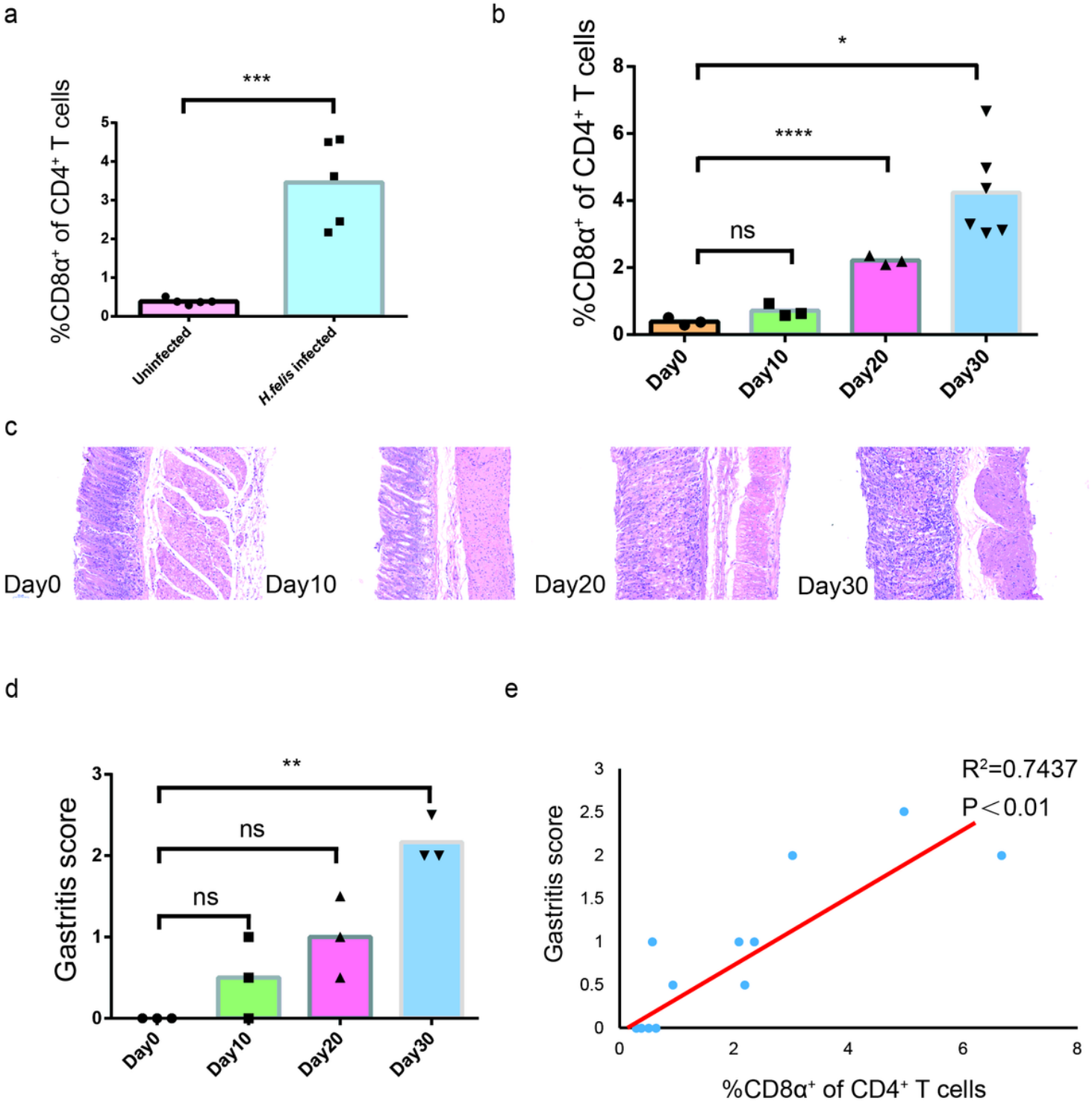

Figure 1

CD4+ CD8αα+ T cells are increase in gastritis induced by H. felis. a Frequencies of CD4+ CD8αα+ T cells (gated on CD45+ CD8β- CD4+ CD8α+) from H. felis infected mice and uninfected mice (n = 5). b and c Representative H&E staining and histological scores of stomachs from mice in each group (n = 3). d Frequencies of gastric CD4+ CD8αα+ T cells among total CD4+ T cells at Day 0、10、20 and 30 were quantified (n = 3-6). e Correlation between histological scores of stomachs with frequencies of CD4+CD8αα+ T cells among CD4+ T cells in H. felis infected mice. Each point is an individual mouse. Significance was calculated by unpaired t test: *, P <0.05; **, P<0.01; ***, P <0.001; ****, P <0.0001; ns, not significant.

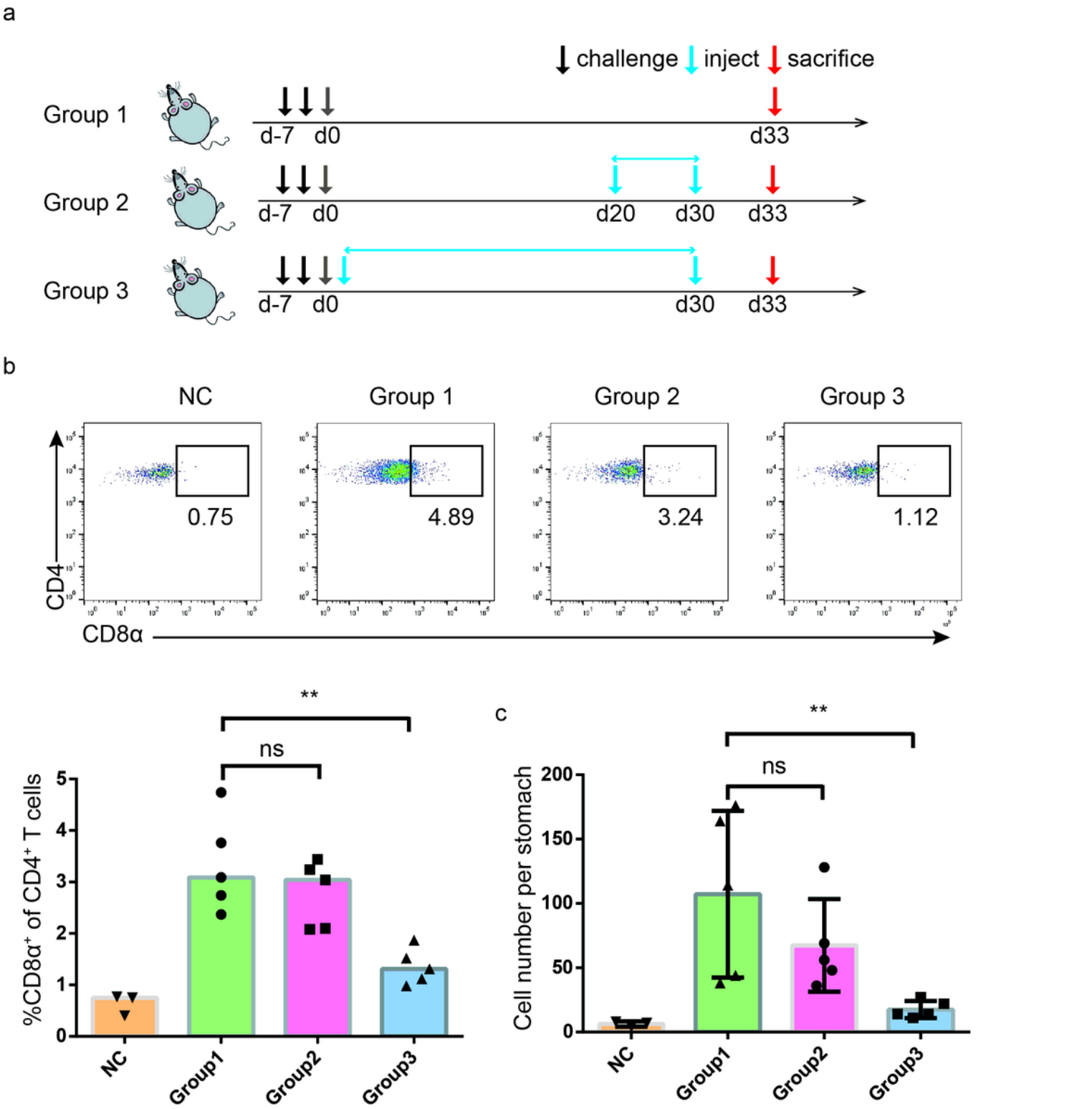

Figure 2

Localization and migration of CD4+ CD8αα+ T cells in the stomach. a Schematic representation of the experimental protocol. C57BL/6 mice were infected with H. felis (5 × 105 PFU/mouse). At day 0 or day 20, mice were injected with FTY720. At day 33 post infected mice were sacrificed for analysis of the CD4+ CD8αα+ T cells in the stomach. b Representative flow cytometry plots and frequencies of CD4+ CD8αα+ T cells among total CD4+ T cells in each group. c Absolute number of gastric CD4+ CD8αα+ T cells were

quantified. Data were shown as mean ± SD (n = 3-5), which pooled from two individual experiments. Significance was calculated by unpaired t test: **, P<0.01; ns, not significant.

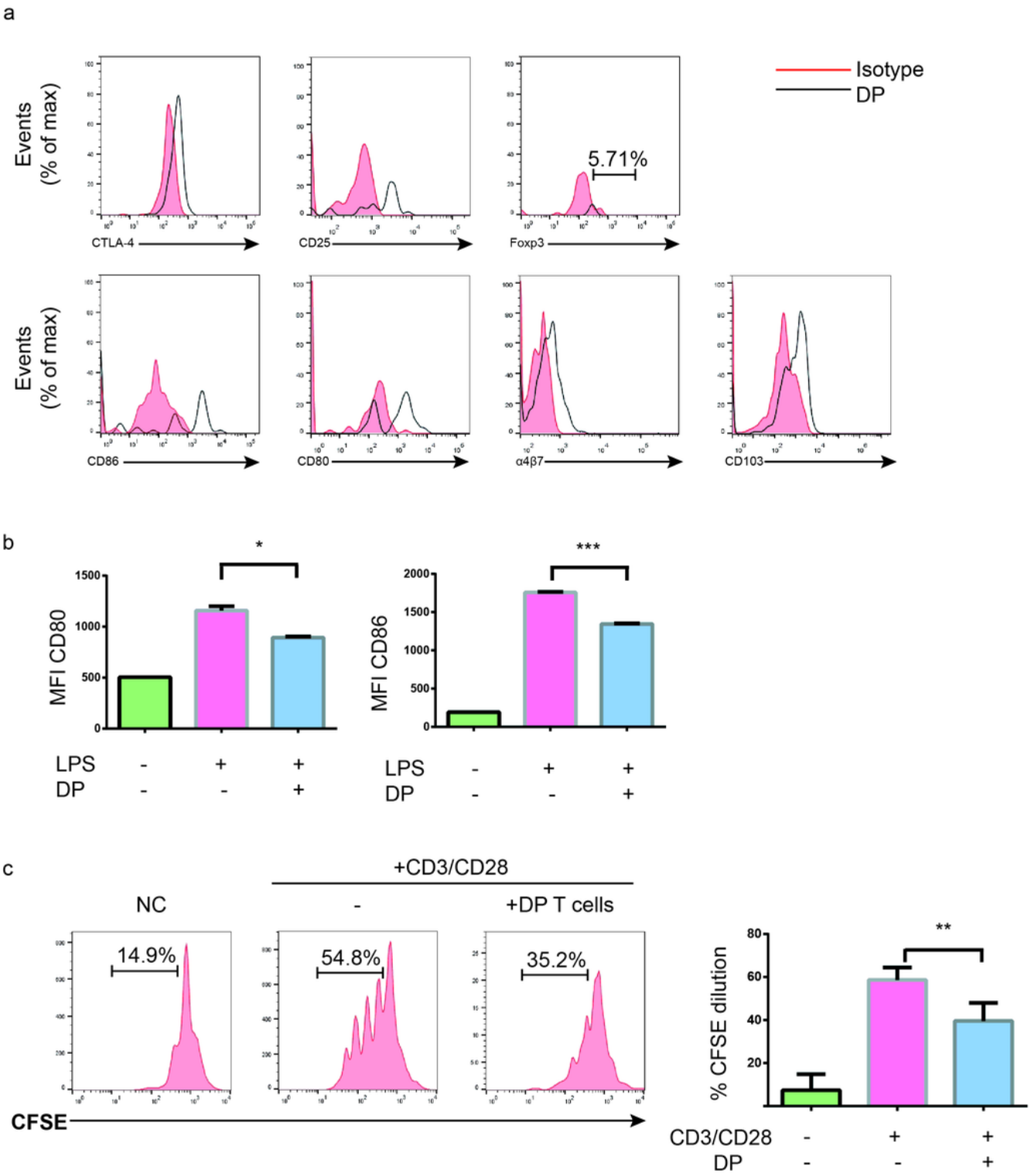

Figure 3

Regulatory phenotype and function of CD4+ CD8αα+ T cells. a Comparison of the phenotypes of CD4+ CD8αα+ and CD4+ lymphocytes among CD45+ T cells freshly dissociated from gastritis mice. b BMDCs from C57BL/6 mice were cultured 2 days with CD4+ CD8αα+ T cells, and then CD80 and CD86 expression

on BMDCs was determined. c Inhibition of the proliferative response of CD4+T cells by the CD4+ CD8αα+ T cells as measured by CFSE dilution. Splenic CD4+T cells were stimulated with anti-CD3 and anti-CD28 in the presence or absence of CD4+ CD8αα+ T cells for 3 d at a ratio of 1:1: representative cytometry data and histograms showing the CFSE dilution in APC negative lymphocytes: unstimulated (green histograms), stimulated (red histograms) and stimulated in the presence of CD4+ CD8αα+ T cells (blue histograms). Data were shown as mean ± SD (n = 4), which pooled from two individual experiments. Significance was calculated by unpaired t test: *, P <0.05; **, P<0.01; ***, P <0.001.

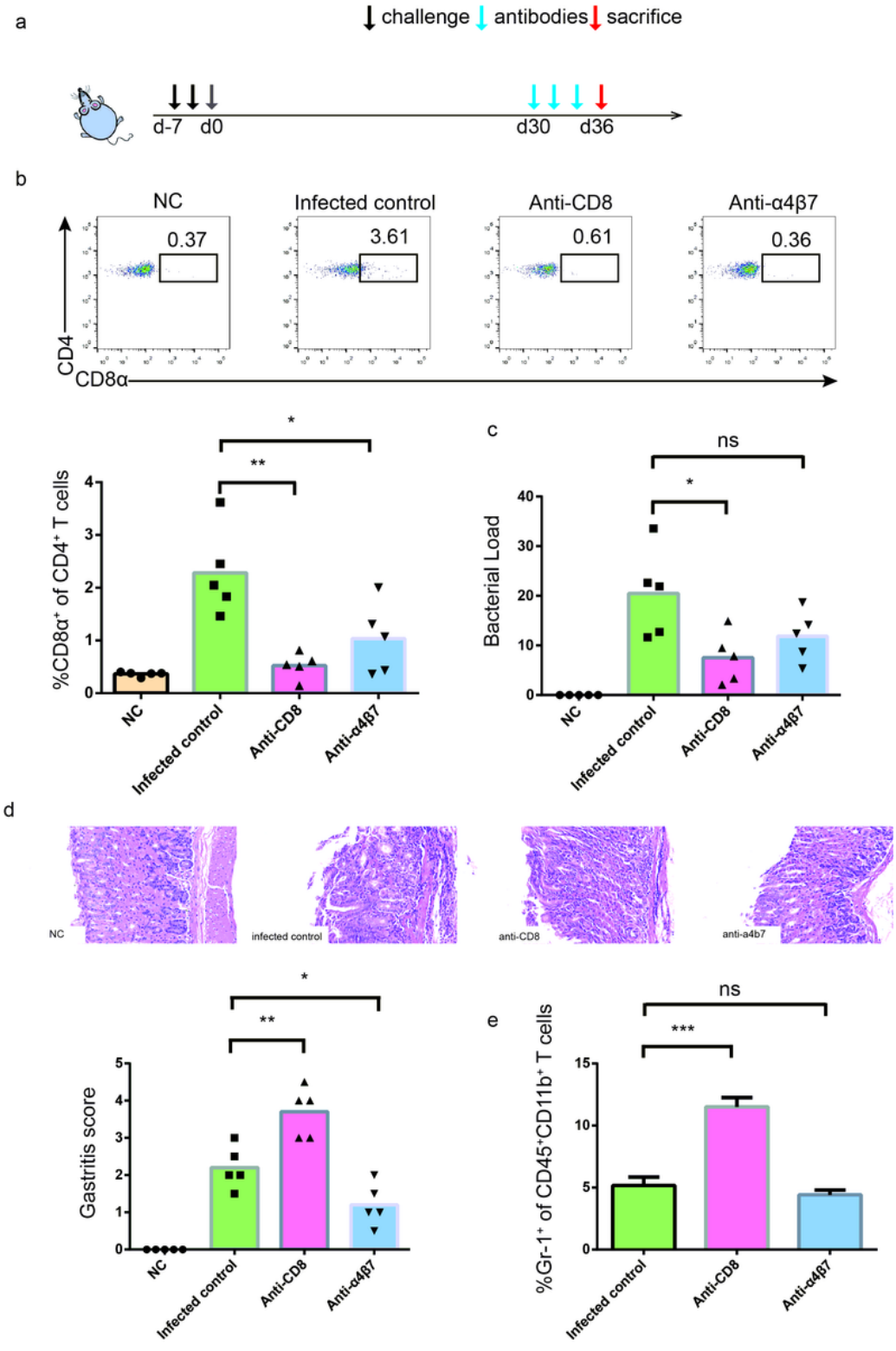

Figure 4

CD4+ CD8αα+ T cells control gastric inflammation. aSchematic representation of the experimental protocol. C57BL/6 mice were infected with H. felis (5 × 105 CFU/mouse) from day -7 to -1. Infected mice were injected with antibodies from day 30 to 34. b Representative flow cytometry plots and frequencies of CD4+ CD8αα+ T cells among total CD4+ T cells in each group. c Relative Hf colonization levels in mice determined by real-time qPCR. d Representative H&E staining and histological scores of stomachs from mice in each group. e The frequencies of Ly6C+ CD11b+ T cells among total CD11b+ T cells. Data were shown as mean ± SD (n = 3-5), which pooled from two individual experiments. Significance was calculated by unpaired t test: *, P <0.05; **, P<0.01; ***, P <0.001; ns, not significant.

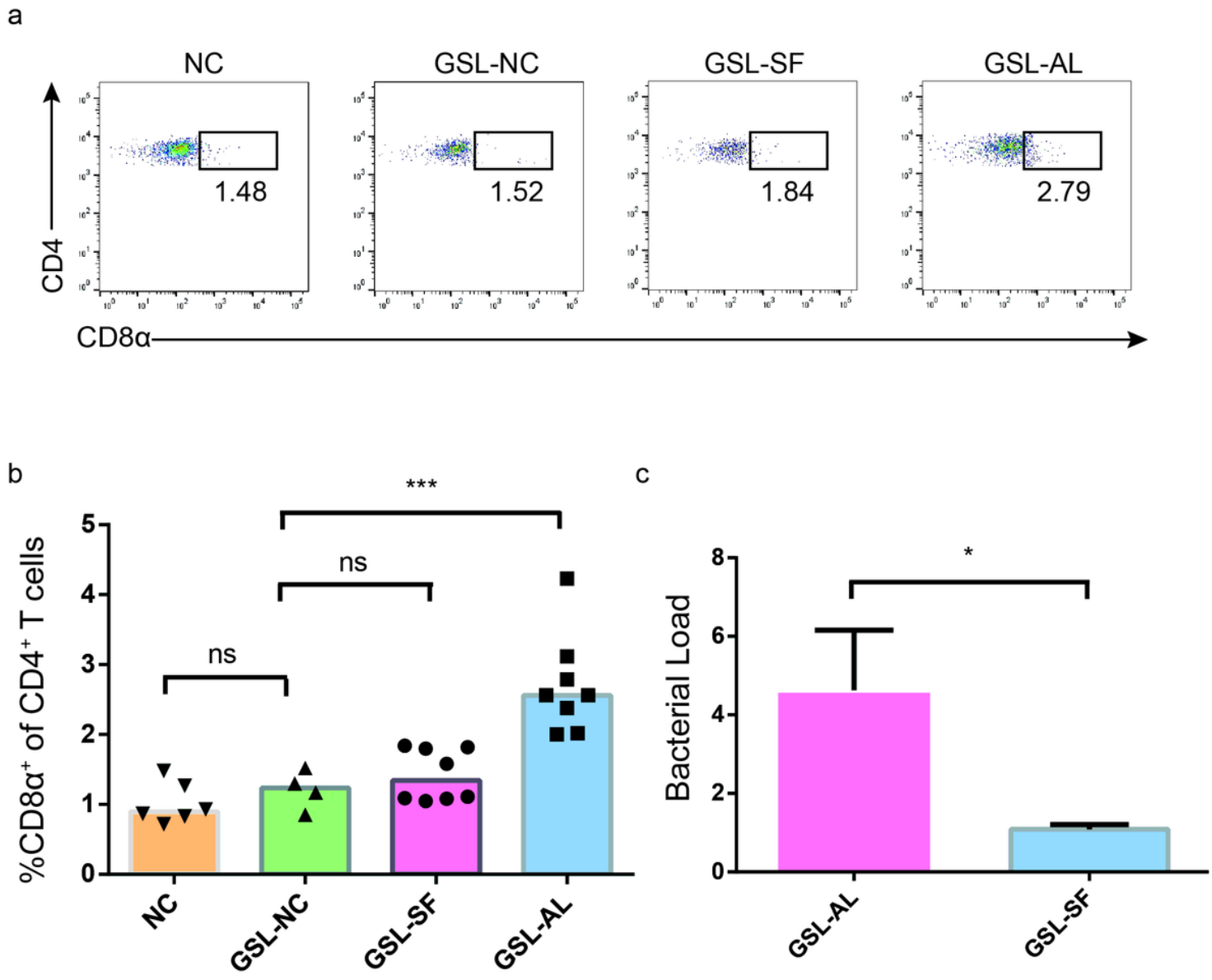

Figure 5

SF-loaded vaccines enhance efficacy by inhibiting the production of CD4+ CD8αα+ T cells. a Representative flow cytometry plots and frequencies of CD4+ CD8αα+ T cells among total CD4+ T cells in

each group. b Bacterial burden was evaluated in the stomach by real-time qPCR. Data were shown as mean ± SD (n = 4-8), which pooled from two individual experiments. Significance was calculated by unpaired t test: *, P <0.05; ***, P <0.001; ns, not significant.

Supplementary Files

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