**Name of Journal:** *World Journal of Methodology*

**Manuscript NO:** 62167

**Manuscript Type:** MINIREVIEWS

**Isolation of lymphocytes from the human gastric mucosa**

Iwamuro M *et al*. Lymphocyte isolation from the stomach

Masaya Iwamuro, Takahide Takahashi, Natsuki Watanabe, Hiroyuki Okada

**Masaya Iwamuro, Hiroyuki Okada,** Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

**Takahide Takahashi, Natsuki Watanabe,** Division of Medical Support, Okayama University Hospital, Okayama 700-8558, Japan

**Author contributions:** Iwamuro M organized the report and drafted the article; Takahashi T and Watanabe N critically revised the article for important intellectual content; Okada H approved the final article.

**Corresponding author: Masaya Iwamuro, MD, PhD, Assistant Professor,** Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-Ku, Okayama 700-8558, Japan. iwamuromasaya@yahoo.co.jp

**Received:** December 31, 2020

**Revised:** April 9, 2021

**Accepted:** July 9, 2021

**Published online:**

**Abstract**

Flow cytometry is widely used for lymphocyte immunophenotyping in clinical settings. However, few studies have applied it for analyzing lymphocytes of the gastric mucosa. This review offers an overview of methodologies for isolating lymphocytes from the human stomach. Previously reported articles were reviewed, focusing on procedures for isolating human gastric mucosal lymphocytes. *Helicobacter pylori*-associated peptic diseases and gastric cancer are two major subjects of research in this field. Enzymatic dissociation, mechanical dissociation, or a combination of the two have been used to isolate lymphocytes from the stomach. Intra-epithelial and lamina propria lymphocytes were separately isolated in several studies. We also summarize the history and present trends in analyzing lymphocytes in patients with gastric disease.

**Key Words:** Gastrointestinal biopsies; Gastrointestinal endoscopy; Gastric cancers; *Helicobacter pylori*; Gastric ulcer; Flow cytometry

Iwamuro M, Takahashi T, Watanabe N, Okada H. Isolation of lymphocytes from the human gastric mucosa. *World J Methodol* 2021; In press

**Core Tip:** This review provides an overview of methodologies used to analyze lymphocytes in the stomach. *Helicobacter pylori*-associated peptic diseases and gastric cancer are two major subjects of research in this field. Previously reported articles were reviewed, focusing on procedures for isolating human gastric mucosal lymphocytes. The history and present trends in analyzing lymphocytes in patients with gastric disease are also summarized.

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) infection is responsible for most peptic ulcers and chronic inflammation in the stomach. Such prolonged inflammation causes mucosal damage and regeneration, in turn leading to carcinogenesis of the gastric epithelium and lymphomagenesis. Additionally, recent advances in cancer immunology and immunology-based anticancer therapies highlight the contribution of tumor-infiltrating lymphocytes in gastric cancer treatment. Thus, chronic inflammation with lymphocyte infiltration in the stomach may be involved in various gastric diseases[1,2].

Flow cytometry is widely used for lymphocyte immunophenotyping. An advantage of flow cytometry over immunohistochemistry is its multicolor analysis, providing an accurate characterization of the surface antigen profile of specific cells. Despite its superiority, few studies have assessed gastric mucosal lymphocytes by flow cytometry. We believe that its widespread use will promote a better understanding of surface marker expression and function of lymphocytes in the stomach. Here, we review relevant basic studies and summarize the methods of isolating lymphocytes from the human stomach for flow cytometry. Additionally, the history and current trends in analyzing lymphocytes in patients with gastric disease are summarized.

**Literature search**

We performed a literature search on October 13, 2020, in the PubMed database. The search terms used were “lymphocyte isolation”, “stomach”, and “flow cytometry”. We retrieved 66 titles[1-66], and no previous systematic review of lymphocyte isolation from the gastric mucosa was identified (Figure 1). Articles not written in English (*n* = 4) were excluded from this review. A further 20 articles were excluded as the research subjects were cell lines (*n* = 4) or non-human mammals, including mice (*n* = 14), pigs (*n* = 1), or alpacas (*n* = 1). Among the remaining 42 articles, lymphocyte isolation from the stomach was not performed in 16 articles. Finally, we reviewed 26 articles and summarized the methodologies for lymphocyte isolation from the human gastric mucosa.

Lymphocyte isolation was performed using endoscopic biopsy specimens (*n* = 15), surgically resected specimens (*n* = 10), or both (*n* = 1). The number of endoscopically biopsied specimens was 1 (*n* = 2), 1–2 (*n* = 1), 2 (*n* = 3), 4 (*n* = 5), 5 (*n* = 1), or 6 (*n* = 1). The number used for lymphocyte isolation was not specified in the remaining three articles. Gastric mucosal lymphocyte analysis was performed in association with *H. pylori*-related peptic diseases (*n* = 12), gastric cancer (*n* = 9), or other diseases (*n* = 5).

During lymphocyte isolation, enzymatic degradation and/or mechanical shredding and grinding are performed to remove the epithelial cells and connective tissue of the stomach. Collagenase is commonly used for enzymatic processing, while mesh strainers or glass slides are used for mechanical processing. Hereafter, we review representative methods for lymphocyte isolation from the stomach.

***Combination of enzymatic and mechanical dissociation***

In previously reported studies, sequential processing with enzymatic and mechanical dissociation is most frequently used for lymphocyte isolation from the gastric mucosa. In one protocol[2,6,16], fresh tissues were obtained from the surgically resected stomach and washed three times with Hank’s solution containing 1% fetal calf serum. The specimen was cut into small pieces and collected in RPMI 1640 medium containing collagenase IV (1 mg/mL) and deoxyribonuclease I (10 mg/mL). Subsequently, mechanical separation was performed using gentleMACS Dissociator and the dissociated cell suspensions were incubated for 1 h under continuous rotation at 37 °C. After passing the cell suspension through a 70 μm cell strainer, cells were deemed ready for flow cytometry.

In another protocol[10], two specimens were obtained from the gastric mucosa by endoscopic biopsy. The specimens were placed on ice in RPMI 1640 medium containing 10% fetal calf serum, glutamine, and penicillin/streptomycin. The biopsy specimens were transferred to a dithiothreitol/EDTA solution and incubated for 15 min at 37 °C. The samples were finely sliced and digested in a collagenase A solution for 1 h at 37 °C. The digested cell suspensions were filtered through a 70 μm cell filter and collected in RPMI 1640 medium. After centrifugation, the pellet was resuspended in phosphate-buffered saline.

For enzymatic dissociation, other reagent combinations are also used, such as deoxyribonuclease 1 (0.02 mg/mL), collagenase (0.25 mg/mL), and hyaluronidase (0.1 mg/mL)[28]; collagenase (300 μg/mL) and Dispase II (500 U/mL)[33]; or collagenase (5 mg/mL), DNase (0.1 mg/mL), and protease (2 U/mL)[43,49,58,62].

***Enzymatic dissociation***

Isolation of lymphocytes can be performed by enzymatic dissociation alone. For instance[60], fresh gastric tissue can be digested in a rotating chamber (60 rpm, 37 °C, 12 h) with NaCl (8 mg/mL), KCl (0.4 mg/mL), CaCl2 (0.56 mg/mL), NaHPO4 (60 μg/mL), Na2HPO4-12H2O (151 μg/mL), HEPES (2.3 mg/mL), *Clostridium hystolyticum* collagenase (0.5 mg/mL), and soybean trypsin inhibitor (5 μg/mL). The lymphocytes are then dispersed and enriched on Ficoll-Paque density gradients.

***Mechanical dissociation***

Without enzymes, lymphocytes can be obtained by mechanical mincing of surgically resected or endoscopically biopsied specimens, followed by passing through a 40-100 μm filter to exclude tissue fragments[23,24,52] or by pressing and grinding in a coarse glass grinder[48].

***Isolation of intra-epithelial lymphocytes and lamina propria lymphocytes***

The predominance, heterogeneity, and distribution of lymphocytes are diverse at different locations within the gastric mucosa 22. Therefore, intra-epithelial lymphocytes and lamina propria lymphocytes have been investigated separately in several studies[22,47,54,55]. Briefly, biopsy samples were rotated at 37 °C for 1 h in calcium- and magnesium-free Hanks' balanced salt solution supplemented with 5% fetal calf serum, dithiothreitol (1 mmol/L), and EDTA (1 mmol/L). At this step, the epithelial layer is removed, leaving the lamina propria intact and attached to the basement membrane. Subsequently, the resulting single cell suspension was washed in RPMI 1640 medium supplemented with 10% fetal calf serum and penicillin/streptomycin. The remaining tissue was placed in 5 mL of RPMI 1640 medium containing collagenase Type 1A (130 U/mL) and rotated at 37 °C for 3 h to obtain lamina propria cells.

***Single-step lymphocyte isolation from an endoscopic biopsy specimen***

We recently introduced a simplified, one-step procedure for lymphocyte isolation from an endoscopic biopsy sample[67]. To isolate lymphocytes, we used a porcelain bowl with a spout and a wire mesh tea strainer. First, the porcelain bowl and wire mesh strainer were sterilized by autoclaving. During esophagogastroduodenoscopy, enteroscopy, or colonoscopy, a single tissue sample was collected from the gastrointestinal tract with a standard biopsy forceps. The collected gastrointestinal sample was put into a plastic centrifuge tube containing 5 mL of isotonic saline solution. Next, a wire mesh strainer was placed in a porcelain bowl and the specimen-containing saline solution was poured through the mesh. The solid specimens were crushed using the rubber portion of a 10 mL injection syringe plunger. The wire mesh tea strainer was then removed and thrown away. Subsequently, the saline solution containing lymphocytes was decanted into a plastic centrifuge tube and the cells were deemed ready for flow cytometry.

As far as we know, this is the easiest procedure reported to date; lymphocyte isolation by this method is achieved within 2 min. Besides, laboratory wares and apparatus are not necessary for this method. Therefore, our approach for lymphocyte separation can be completed in an endoscopy unit right after taking a biopsy sample. This method would allow widespread evaluation of lymphocytes in the field of gastroenterology.

**History and present trends of lymphocyte analysis in the stomach**

During the early development stages of flow cytometry for gastric disorders (1995-1999), the association between peptic ulcers and inflammation induced by mucosal lymphocytes was investigated in patients with *H. pylori* infection or duodenal ulcers[52,54,55,58,61,62]. Activation markers such as CD25 [interleukin (IL)-2 receptor alpha chain], CD69 (activation inducer molecule), CD71 (transferrin receptor protein 1), HLA-DR, and adhesion and emigration-related molecules, such as CD11a/CD18 (lymphocyte function-associated antigen 1: LFA-1), CD11b (integrin alpha-M), CD54 (intercellular adhesion molecule-1: ICAM-1), CD106 (vascular cell adhesion molecule-1: VCAM-1), and CD49d (very late antigen-4: VLA-4), were analyzed by flow cytometry in these patients. Also analyzed were the pan T cell markers CD2 and CD3, T-helper cell marker CD4, and T-cytotoxic cell marker CD8. Cytokines, such as interferon-gamma, tumor necrosis factor beta (TNF-β), IL-2, IL-4, and IL-5, were also examined by flow cytometry[52,55].

In the year 2000, research was focused on CD95, also known as Fas or TNF receptor superfamily member 6[47,48]. CD95 is a death receptor localized on the surface of cells that triggers a signal transduction pathway upon binding its ligand, leading to programmed cell death (apoptosis). In *H. pylori*-associated gastritis, epithelial cell damage is mediated through Fas/Fas ligand interactions[47]. Simultaneously, apoptotic depletion of invading mucosal lymphocytes occurs with Fas ligand expression, providing a mechanism of immune privilege evading severe destruction of gastric epithelium in the *H. pylori*-infected stomach[48]. Meanwhile, flow cytometry was also performed for gastric mucosal lymphocytes in patients with duodenal ulcers[33,43] and in children[38].

To our knowledge, lymphocyte infiltration in gastric cancer was first assessed in 1996 in patients with lymphocyte-rich, Epstein-Barr virus-associated gastric carcinoma[60]. In 2006, tumor-infiltrating lymphocytes were investigated in two major types of gastric adenocarcinoma (Lauren classification): intestinal type or diffuse type[28]. The number of B cells was significantly higher, while the number of T cells was significantly lower in intestinal type compared with diffuse type tumors. Furthermore, cloning and characterization of tumor-infiltrating T cells isolated from gastric cancers revealed a specific type-1 T cell response to gastric cancer antigens[21]. Analysis of CD8+ cells that produce IL-17 (Tc17 cells) showed that the percentage of Tc17 cells increased with tumor progression and was associated with overall survival time[15]. Tc17 cells induce CXCR4-dependent chemotaxis of myeloid-derived suppressor cells and impair cytotoxic functions of anti-tumor CD8+ cells, promoting tumor progression.

Since 2008, regulatory T (Treg) cells have gained attention in association with gastric disorders owing to their involvement in immune regulation[9,23,24]. In these studies, Tregs were defined as CD4+CD25high[24], CD4+CD25+CD127low/-[23], or CD4+FOXP3+ cells[9]. Studies show that local Treg cells in gastric cancer express a suppressive cytokine profile characterized by high IL-10, low transforming growth factor-beta (TGF-β), and interferon-gamma production[9]. Thus, it is believed that Tregs suppress effector T cell proliferation and contribute to gastric cancer progression[23]. Furthermore, increase of IL-10 secretion by Tregs was confirmed in *H. pylori*-infected gastric mucosa[24]. IL-10 inhibits IL-8 expression, activates nuclear factor kappa B in the gastric epithelium, and enhances *H. pylori* growth *in vitro*, suggesting the participation of Tregs in gastric ulcer formation and persistent *H. pylori* infection.

In one study, T cells expressing natural killer cell receptors, defined as CD3+CD56+, CD3+CD161+, or CD3+CD94+ cells, were quantified in *H. pylori*-positive and -negative patients[22]. CD3+CD161+ cells were higher in the epithelium of *H. pylori*-infected gastric mucosa, whereas CD3+CD56+ cells were lower in the lamina propria, indicating a site-specific distribution of T cells bearing natural killer receptors. In another study, mast cells, defined as CD45+CD117+FcεRI+ cells, were investigated in patients with gastric cancer[2]. A significantly higher number of mast cells exist in gastric cancer tissues, and the mast cell levels increase with tumor progression and independently predict a reduced survival. Besides lymphocytes, tumor-infiltrating neutrophils were examined in gastric cancers by cell sorting against CD66b, which allows for the enrichment of mature neutrophils[6].

Using flow cytometry, specific lymphocyte subsets are defined based on their lineage-, developmental stage-, and function-specific cell surface markers. In addition, fluorescence-activated cell sorting technology enables diversion of individual cells from the fluid stream and collection into viable, homogeneous fractions. Efficient isolation of a lymphocyte population enables characterization of the specific fractions in *in vitro* and animal studies[6,9,15]. More recently, mass cytometry and single-cell RNA sequencing are available. These cutting-edge technologies may reveal distinct immune cell signatures of gastric disorders, such as gastric cancers and *H. pylori*-related peptic diseases.

**Diagnosis of gastric lymphoma**

In addition to the aforementioned technologies of evaluating gastric diseases, flow cytometry has been used for the diagnosis of lymphoma of the stomach. Flow cytometry is a rapid and practical diagnostic tool for B-cell lymphoma. Analysis of the distribution of surface immunoglobulin light chain kappa and lambda using flow cytometry offers evidence for the monoclonality of B-cell neoplasms because these lymphomas that typically arise from an expansion of a B-cell clone expressing only one class of immunoglobulin light chain, either a kappa or lambda chain. Thus, isolation of lymphocytes from the gastric mucosa and detection of monoclonality using flow cytometry lead to the prompt diagnosis of lymphoma of the stomach. This approach may be particularly useful for the detection and therapeutic monitoring of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), which is the most common non-Hodgkin lymphoma subtype arising in the stomach[68-70].

We have reported the utility of a single-step lymphocyte isolation procedure from an endoscopic biopsy specimen, which is described above, for the diagnosis of gastrointestinal lymphoma[67,71]. Our previous study included patients with gastric extranodal marginal zone MALT lymphoma (*n* = 8), duodenal follicular lymphoma (grade 1; *n* = 5), and benign lymphoid hyperplasia (ileum, *n* = 1, and rectum, *n* = 1). Lymphocytes were successfully isolated from 14 (93.3%) patients. The sensitivity and specificity of flow cytometric analysis of immunoglobulin light chain expression for the diagnosis of B-cell lymphoma were 83.3% and 100%, respectively. These results suggest that a single endoscopic biopsy specimen contains enough lymphocytes for flow cytometric analysis and can be used for the diagnosis of gastrointestinal lymphoma.

**CONCLUSION**

In this review, we provided methodologies for lymphocyte isolation from the gastric mucosa that are reported in the literature. We also described the history and current trends of lymphocyte analysis in the stomach. Owing to the multicolor analysis that accurately defines the surface antigen profile of specific lymphocyte populations, flow cytometry will continue to be a powerful tool for revealing the pathogenesis of gastric disorders. We believe that the methodologies described herein will provide a better understanding of the application of flow cytometry.

**REFERENCES**

1 **Fenton TM**, Jørgensen PB, Niss K, Rubin SJS, Mörbe UM, Riis LB, Da Silva C, Plumb A, Vandamme J, Jakobsen HL, Brunak S, Habtezion A, Nielsen OH, Johansson-Lindbom B, Agace WW. Immune Profiling of Human Gut-Associated Lymphoid Tissue Identifies a Role for Isolated Lymphoid Follicles in Priming of Region-Specific Immunity. *Immunity* 2020; **52**: 557-570.e6 [PMID: 32160523 DOI: 10.1016/j.immuni.2020.02.001]

2 **Lv Y**, Zhao Y, Wang X, Chen N, Mao F, Teng Y, Wang T, Peng L, Zhang J, Cheng P, Liu Y, Kong H, Chen W, Hao C, Han B, Ma Q, Zou Q, Chen J, Zhuang Y. Increased intratumoral mast cells foster immune suppression and gastric cancer progression through TNF-α-PD-L1 pathway. *J Immunother Cancer* 2019; **7**: 54 [PMID: 30808413 DOI: 10.1186/s40425-019-0530-3]

3 **Bagheri V**, Abbaszadegan MR, Memar B, Motie MR, Asadi M, Mahmoudian RA, Gholamin M. Induction of T cell-mediated immune response by dendritic cells pulsed with mRNA of sphere-forming cells isolated from patients with gastric cancer. *Life Sci* 2019; **219**: 136-143 [PMID: 30641083 DOI: 10.1016/j.lfs.2019.01.016]

4 **Martins MR**, Santos RLD, Jatahy KDN, Matta MCD, Batista TP, Júnior JIC, Begnami MDFS, Torres LC. Could OX40 agonist antibody promote activation of the anti-tumor immune response in gastric cancer? *J Surg Oncol* 2018; **117**: 840-844 [PMID: 29529339 DOI: 10.1002/jso.25001]

5 **Zhou Y**, Guo F. A selective sphingosine-1-phosphate receptor 1 agonist SEW-2871 aggravates gastric cancer by recruiting myeloid-derived suppressor cells. *J Biochem* 2018; **163**: 77-83 [PMID: 29036438 DOI: 10.1093/jb/mvx064]

6 **Wang TT**, Zhao YL, Peng LS, Chen N, Chen W, Lv YP, Mao FY, Zhang JY, Cheng P, Teng YS, Fu XL, Yu PW, Guo G, Luo P, Zhuang Y, Zou QM. Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. *Gut* 2017; **66**: 1900-1911 [PMID: 28274999 DOI: 10.1136/gutjnl-2016-313075]

7 **Topliff CL**, Alkheraif AA, Kuszynski CA, Davis WC, Steffen DJ, Schmitz JA, Eskridge KM, Charleston B, Henningson JN, Kelling CL. Experimental acute infection of alpacas with Bovine viral diarrhea virus 1 subgenotype b alters peripheral blood and GALT leukocyte subsets. *J Vet Diagn Invest* 2017; **29**: 186-192 [PMID: 28166712 DOI: 10.1177/1040638717690015]

8 **Hou M**, Zhou NB, Li H, Wang BS, Wang XQ, Wang XW, Wang KG, Xue FS. Morphine and ketamine inhibit immune function of gastric cancer patients by increasing percentage of CD4(+)CD25(+)Foxp3(+) regulatory T cells in vitro. *J Surg Res* 2016; **203**: 306-312 [PMID: 27363637 DOI: 10.1016/j.jss.2016.02.031]

9 **Kindlund B**, Sjöling Å, Yakkala C, Adamsson J, Janzon A, Hansson LE, Hermansson M, Janson P, Winqvist O, Lundin SB. CD4+ regulatory T cells in gastric cancer mucosa are proliferating and express high levels of IL-10 but little TGF-β. *Gastric Cancer* 2017; **20**: 116-125 [PMID: 26782287 DOI: 10.1007/s10120-015-0591-z]

10 **Bashir M**, Prietl B, Tauschmann M, Mautner SI, Kump PK, Treiber G, Wurm P, Gorkiewicz G, Högenauer C, Pieber TR. Effects of high doses of vitamin D3 on mucosa-associated gut microbiome vary between regions of the human gastrointestinal tract. *Eur J Nutr* 2016; **55**: 1479-1489 [PMID: 26130323 DOI: 10.1007/s00394-015-0966-2]

11 **Chen J**, Yang J, Jiang J, Zhuang Y, He W. Function and subsets of dendritic cells and natural killer cells were decreased in gastric cancer. *Int J Clin Exp Pathol* 2014; **7**: 8304-8311 [PMID: 25550889]

12 **Sparks D**, Bhalla A, Dodge J, Saldinger P. Isolated gastric amyloidoma in the setting of marginal zone MALT lymphoma: case report and review of the literature. *Conn Med* 2014; **78**: 277-280 [PMID: 24974561]

13 **Odiere MR**, Scott ME, Leroux LP, Dzierszinski FS, Koski KG. Maternal protein deficiency during a gastrointestinal nematode infection alters developmental profile of lymphocyte populations and selected cytokines in neonatal mice. *J Nutr* 2013; **143**: 100-107 [PMID: 23190758 DOI: 10.3945/jn.112.160457]

14 **Ruiz VE**, Sachdev M, Zhang S, Wen S, Moss SF. Isolating, immunophenotyping and *ex vivo* stimulation of CD4+ and CD8+ gastric lymphocytes during murine Helicobacter pylori infection. *J Immunol Methods* 2012; **384**: 157-163 [PMID: 22814402 DOI: 10.1016/j.jim.2012.07.002]

15 **Zhuang Y**, Peng LS, Zhao YL, Shi Y, Mao XH, Chen W, Pang KC, Liu XF, Liu T, Zhang JY, Zeng H, Liu KY, Guo G, Tong WD, Shi Y, Tang B, Li N, Yu S, Luo P, Zhang WJ, Lu DS, Yu PW, Zou QM. CD8(+) T cells that produce interleukin-17 regulate myeloid-derived suppressor cells and are associated with survival time of patients with gastric cancer. *Gastroenterology* 2012; **143**: 951-62.e8 [PMID: 22710190 DOI: 10.1053/j.gastro.2012.06.010]

16 **Yoneda A**, Ito S, Susumu S, Matsuo M, Taniguchi K, Tajima Y, Eguchi S, Kanematsu T, Nagata Y. Immunological milieu in the peritoneal cavity at laparotomy for gastric cancer. *World J Gastroenterol* 2012; **18**: 1470-1478 [PMID: 22509078 DOI: 10.3748/wjg.v18.i13.1470]

17 **Nishimoto T**, Satoh T, Takeuchi T, Ikeda Y, Kuwana M. Critical role of CD4(+)CD25(+) regulatory T cells in preventing murine autoantibody-mediated thrombocytopenia. *Exp Hematol* 2012; **40**: 279-289 [PMID: 22240606 DOI: 10.1016/j.exphem.2012.01.001]

18 **Lulla P**, Bandeali S, Baker K. Fatal paraneoplastic systemic leukocytoclastic vasculitis as a presenting feature of chronic lymphocytic leukemia. *Clin Lymphoma Myeloma Leuk* 2011; **11 Suppl 1**: S14-S16 [PMID: 22035741 DOI: 10.1016/j.clml.2011.03.030]

19 **Modi N**, Gulati N, Solomon K, Monaghan T, Robins A, Sewell HF, Mahida YR. Differential binding and internalization of Clostridium difficile toxin A by human peripheral blood monocytes, neutrophils and lymphocytes. *Scand J Immunol* 2011; **74**: 264-271 [PMID: 21595735 DOI: 10.1111/j.1365-3083.2011.02578.x]

20 **Becher D**, Deutscher ME, Simpfendorfer KR, Wijburg OL, Pederson JS, Lew AM, Strugnell RA, Walduck AK. Local recall responses in the stomach involving reduced regulation and expanded help mediate vaccine-induced protection against Helicobacter pylori in mice. *Eur J Immunol* 2010; **40**: 2778-2790 [PMID: 21038469 DOI: 10.1002/eji.200940219]

21 **Amedei A**, Niccolai E, Della Bella C, Cianchi F, Trallori G, Benagiano M, Bencini L, Bernini M, Farsi M, Moretti R, Del Prete G, D'Elios MM. Characterization of tumor antigen peptide-specific T cells isolated from the neoplastic tissue of patients with gastric adenocarcinoma. *Cancer Immunol Immunother* 2009; **58**: 1819-1830 [PMID: 19319530 DOI: 10.1007/s00262-009-0693-8]

22 **O'Keeffe J**, Gately CM, O'Donoghue Y, Zulquernain SA, Stevens FM, Moran AP. Natural killer cell receptor T-lymphocytes in normal and Helicobacter pylori-infected human gastric mucosa. *Helicobacter* 2008; **13**: 500-505 [PMID: 19166415 DOI: 10.1111/j.1523-5378.2008.00641.x]

23 **Shen LS**, Wang J, Shen DF, Yuan XL, Dong P, Li MX, Xue J, Zhang FM, Ge HL, Xu D. CD4(+)CD25(+)CD127(low/-) regulatory T cells express Foxp3 and suppress effector T cell proliferation and contribute to gastric cancers progression. *Clin Immunol* 2009; **131**: 109-118 [PMID: 19153062 DOI: 10.1016/j.clim.2008.11.010]

24 **Robinson K**, Kenefeck R, Pidgeon EL, Shakib S, Patel S, Polson RJ, Zaitoun AM, Atherton JC. Helicobacter pylori-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. *Gut* 2008; **57**: 1375-1385 [PMID: 18467372 DOI: 10.1136/gut.2007.137539]

25 **Xu HY**, Xu L, Gao JH, Li KZ, Dou KF. [T lymphocytes with chimeric receptor induce carcinoembryonic antigen-positive specific gastric carcinoma cells apoptosis]. *Zhonghua Yi Xue Za Zhi* 2007; **87**: 1053-1057 [PMID: 17672971]

26 **Wang LY**, Zeng Y, Pan ZZ, Zhu ZH. [Detection of intracellular and extracellular cytokines of CD4+CD25+ regulatory T cells in gastric cancer patients]. *Ai Zheng* 2007; **26**: 270-273 [PMID: 17355789]

27 **Rad R**, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, Reindl W, Dossumbekova A, Friedrich M, Saur D, Wagner H, Schmid RM, Prinz C. CD25+/Foxp3+ T cells regulate gastric inflammation and Helicobacter pylori colonization in vivo. *Gastroenterology* 2006; **131**: 525-537 [PMID: 16890606 DOI: 10.1053/j.gastro.2006.05.001]

28 **van den Engel NK**, Winter H, Rüttinger D, Shau I, Schiller M, Mayer B, Moudgil T, Meimarakis G, Stolte M, Jauch KW, Fox BA, Hatz RA. Characterization of immune responses in gastric cancer patients: a possible impact of H. pylori to polarize a tumor-specific type 1 response? *Clin Immunol* 2006; **120**: 285-296 [PMID: 16765089 DOI: 10.1016/j.clim.2006.04.566]

29 **Hatzifoti C**, Roussel Y, Harris AG, Wren BW, Morrow JW, Bajaj-Elliott M. Mucosal immunization with a urease B DNA vaccine induces innate and cellular immune responses against Helicobacter pylori. *Helicobacter* 2006; **11**: 113-122 [PMID: 16579841 DOI: 10.1111/j.1523-5378.2006.00385.x]

30 **Dutta N**, Gupta A, Mazumder DN, Banerjee S. Down-regulation of locus-specific human lymphocyte antigen class I expression in Epstein-Barr virus-associated gastric cancer: implication for viral-induced immune evasion. *Cancer* 2006; **106**: 1685-1693 [PMID: 16541432 DOI: 10.1002/cncr.21784]

31 **Hase K**, Murakami T, Takatsu H, Shimaoka T, Iimura M, Hamura K, Kawano K, Ohshima S, Chihara R, Itoh K, Yonehara S, Ohno H. The membrane-bound chemokine CXCL16 expressed on follicle-associated epithelium and M cells mediates lympho-epithelial interaction in GALT. *J Immunol* 2006; **176**: 43-51 [PMID: 16365394 DOI: 10.4049/jimmunol.176.1.43]

32 **Li ZY**, Chen FB, Chen J. [CD4+ and CD8+ T cells in gastric mucosa in children infected with Helicobacter pylori]. *Zhonghua Er Ke Za Zhi* 2005; **43**: 453-456 [PMID: 16053734]

33 **Itoh T**, Seno H, Kita T, Chiba T, Wakatsuki Y. Th response to Helicobacter pylori differs between patients with gastric ulcer and duodenal ulcer. *Scand J Gastroenterol* 2005; **40**: 641-647 [PMID: 16036523 DOI: 10.1080/00365520510015520]

34 **Velin D**, Bachmann D, Bouzourene H, Michetti P. Mast cells are critical mediators of vaccine-induced Helicobacter clearance in the mouse model. *Gastroenterology* 2005; **129**: 142-155 [PMID: 16012944 DOI: 10.1053/j.gastro.2005.04.010]

35 **Johansson C**, Ahlstedt I, Furubacka S, Johnsson E, Agace WW, Quiding-Järbrink M. Differential expression of chemokine receptors on human IgA+ and IgG+ B cells. *Clin Exp Immunol* 2005; **141**: 279-287 [PMID: 15996192 DOI: 10.1111/j.1365-2249.2005.02843.x]

36 **Wu YY**, Tsai HF, Lin WC, Chou AH, Chen HT, Yang JC, Hsu PI, Hsu PN. Helicobacter pylori enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in human gastric epithelial cells. *World J Gastroenterol* 2004; **10**: 2334-2339 [PMID: 15285015 DOI: 10.3748/wjg.v10.i16.2334]

37 **Valeri AP**, Pérez-Blas M, Gutiérrez A, López-Santalla M, Aguilera N, Rodríguez-Juan C, Sala-Silveira L, Martín J, Lasa I, Mugüerza JM, López A, García-Sancho L, Granell J, Martín-Villa JM. Intrinsic defects explain altered proliferative responses of T lymphocytes and HVS-derived T-cell lines in gastric adenocarcinoma. *Cancer Immunol Immunother* 2003; **52**: 708-714 [PMID: 12830324 DOI: 10.1007/s00262-003-0413-8]

38 **Bontems P**, Robert F, Van Gossum A, Cadranel S, Mascart F. Helicobacter pylori modulation of gastric and duodenal mucosal T cell cytokine secretions in children compared with adults. *Helicobacter* 2003; **8**: 216-226 [PMID: 12752734 DOI: 10.1046/j.1523-5378.2003.00147.x]

39 **Wolf AM**, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* 2003; **9**: 606-612 [PMID: 12576425]

40 **Kudo T**, Iwai T, Kubota T, Iwasaki H, Takayma Y, Hiruma T, Inaba N, Zhang Y, Gotoh M, Togayachi A, Narimatsu H. Molecular cloning and characterization of a novel UDP-Gal:GalNAc(alpha) peptide beta 1,3-galactosyltransferase (C1Gal-T2), an enzyme synthesizing a core 1 structure of O-glycan. *J Biol Chem* 2002; **277**: 47724-47731 [PMID: 12361956 DOI: 10.1074/jbc.M205839200]

41 **Zavros Y**, Rieder G, Ferguson A, Merchant JL. Gastritis and hypergastrinemia due to Acinetobacter lwoffii in mice. *Infect Immun* 2002; **70**: 2630-2639 [PMID: 11953405 DOI: 10.1128/iai.70.5.2630-2639.2002]

42 **Li X**, Guo M, Mori E, Mori T. Active roles of caspase-3 in human gastric carcinoma cell death by apoptosis inducing nucleosides from CD57+HLA-DRbright natural suppressor cell line. *Int J Oncol* 2001; **18**: 837-842 [PMID: 11251182 DOI: 10.3892/ijo.18.4.837]

43 **Ihan A**, Tepes B, Gubina M. Diminished Th1-type cytokine production in gastric mucosa T-lymphocytes after H. pylori eradication in duodenal ulcer patients. *Pflugers Arch* 2000; **440**: R89-R90 [PMID: 11005624]

44 **Fan X**, Gunasena H, Cheng Z, Espejo R, Crowe SE, Ernst PB, Reyes VE. Helicobacter pylori urease binds to class II MHC on gastric epithelial cells and induces their apoptosis. *J Immunol* 2000; **165**: 1918-1924 [PMID: 10925273 DOI: 10.4049/jimmunol.165.4.1918]

45 **Michetti M**, Kelly CP, Kraehenbuhl JP, Bouzourene H, Michetti P. Gastric mucosal alpha(4)beta(7)-integrin-positive CD4 T lymphocytes and immune protection against helicobacter infection in mice. *Gastroenterology* 2000; **119**: 109-118 [PMID: 10889160 DOI: 10.1053/gast.2000.8548]

46 **Zavros Y**, Van Antwerp M, Merchant JL. Use of flow cytometry to quantify mouse gastric epithelial cell populations. *Dig Dis Sci* 2000; **45**: 1192-1199 [PMID: 10877237 DOI: 10.1023/a:1005514422187]

47 **Wang J**, Fan X, Lindholm C, Bennett M, O'Connoll J, Shanahan F, Brooks EG, Reyes VE, Ernst PB. Helicobacter pylori modulates lymphoepithelial cell interactions leading to epithelial cell damage through Fas/Fas ligand interactions. *Infect Immun* 2000; **68**: 4303-4311 [PMID: 10858249 DOI: 10.1128/iai.68.7.4303-4311.2000]

48 **Koyama S**. Apoptotic depletion of infiltrating mucosal lymphocytes associated with Fas ligand expression by Helicobacter pylori-infected gastric mucosal epithelium: human glandular stomach as a site of immune privilege. *Dig Dis Sci* 2000; **45**: 773-780 [PMID: 10759249 DOI: 10.1023/a:1005408113467]

49 **Ihan A**, Tepez B, Gubina M, Malovrh T, Kopitar A. Diminished interferon-gamma production in gastric mucosa T lymphocytes after H. pylori eradication in duodenal ulcer patients. *Hepatogastroenterology* 1999; **46**: 1740-1745 [PMID: 10430335]

50 **Wieckiewicz J**, Krzeszowiak A, Ruggiero I, Pituch-Noworolska A, Zembala M. Detection of cytokine gene expression in human monocytes and lymphocytes by fluorescent in situ hybridization in cell suspension and flow cytometry. *Int J Mol Med* 1998; **1**: 995-999 [PMID: 9852637 DOI: 10.3892/ijmm.1.6.995]

51 **Stallmach A**, Schäfer F, Hoffmann S, Weber S, Müller-Molaian I, Schneider T, Köhne G, Ecker KW, Feifel G, Zeitz M. Increased state of activation of CD4 positive T cells and elevated interferon gamma production in pouchitis. *Gut* 1998; **43**: 499-505 [PMID: 9824577 DOI: 10.1136/gut.43.4.499]

52 **Sommer F**, Faller G, Konturek P, Kirchner T, Hahn EG, Zeus J, Röllinghoff M, Lohoff M. Antrum- and corpus mucosa-infiltrating CD4(+) lymphocytes in Helicobacter pylori gastritis display a Th1 phenotype. *Infect Immun* 1998; **66**: 5543-5546 [PMID: 9784570 DOI: 10.1128/IAI.66.11.5543-5546.1998]

53 **Sikora J**, Dworacki G, Trybus M, Batura-Gabryel H, Zeromski J. Correlation between DNA content, expression of Ki-67 antigen of tumor cells and immunophenotype of lymphocytes from malignant pleural effusions. *Tumour Biol* 1998; **19**: 196-204 [PMID: 9591046 DOI: 10.1159/000030007]

54 **Terrés AM**, Pajares JM. An increased number of follicles containing activated CD69+ helper T cells and proliferating CD71+ B cells are found in H. pylori-infected gastric mucosa. *Am J Gastroenterol* 1998; **93**: 579-583 [PMID: 9576451 DOI: 10.1111/j.1572-0241.1998.168\_b.x]

55 **Bamford KB**, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, Brooks EG, Graham DY, Reyes VE, Ernst PB. Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype. *Gastroenterology* 1998; **114**: 482-492 [PMID: 9496938 DOI: 10.1016/s0016-5085(98)70531-1]

56 **Ermak TH**, Ding R, Ekstein B, Hill J, Myers GA, Lee CK, Pappo J, Kleanthous HK, Monath TP. Gastritis in urease-immunized mice after Helicobacter felis challenge may be due to residual bacteria. *Gastroenterology* 1997; **113**: 1118-1128 [PMID: 9322506 DOI: 10.1053/gast.1997.v113.pm9322506]

57 **Shiyan SD**, Bovin NV. Carbohydrate composition and immunomodulatory activity of different glycoforms of alpha1-acid glycoprotein. *Glycoconj J* 1997; **14**: 631-638 [PMID: 9298696 DOI: 10.1023/a:1018544711767]

58 **Ihan A**, Tepez B, Kavcic I, Gubina M. Il-2 receptor expression on gastric mucosa T lymphocytes is enhanced in duodenal ulcer patients compared with non-ulcer dyspeptic patients. *Hepatogastroenterology* 1996; **43**: 1665-1670 [PMID: 8975986]

59 **Krakowka S**, Ringler SS, Eaton KA, Green WB, Leunk R. Manifestations of the local gastric immune response in gnotobiotic piglets infected with Helicobacter pylori. *Vet Immunol Immunopathol* 1996; **52**: 159-173 [PMID: 8809998 DOI: 10.1016/0165-2427(95)05547-9]

60 **Saiki Y**, Ohtani H, Naito Y, Miyazawa M, Nagura H. Immunophenotypic characterization of Epstein-Barr virus-associated gastric carcinoma: massive infiltration by proliferating CD8+ T-lymphocytes. *Lab Invest* 1996; **75**: 67-76 [PMID: 8683941]

61 **Fan X**, Long A, Fan X, Keeling PW, Kelleher D. Adhesion molecule expression on gastric intra-epithelial lymphocytes of patients with Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 1995; **7**: 541-546 [PMID: 7552637]

62 **Ihan A**, Krizman I, Ferlan-Marolt V, Tepez B, Gubina M. HLA-DR expression on CD8 Lymphocytes from gastric mucosa in urease-positive and urease-negative gastritis. *FEMS Immunol Med Microbiol* 1995; **10**: 295-299 [PMID: 7773247 DOI: 10.1111/j.1574-695X.1995.tb00047.x]

63 **Alderuccio F**, Toh BH, Gleeson PA, van Driel IR. A novel method for isolating mononuclear cells from the stomachs of mice with experimental autoimmune gastritis. *Autoimmunity* 1995; **21**: 215-221 [PMID: 8822279 DOI: 10.3109/08916939509008018]

64 **Yamaguchi Y**, Takashima I, Funakoshi M, Kawami H, Toge T. Defective natural killer activity in gastric cancer patients: possible involvement of suppressor factor receptor. *In Vivo* 1994; **8**: 279-283 [PMID: 7803704]

65 **Ebihara T**, Sakai N, Koyama S. Suppression by sorted CD8+CD11b- cells from T-cell growth factor-activated peripheral blood lymphocytes on cytolytic activity against tumour in patients with gastric carcinoma. *Eur J Cancer* 1991; **27**: 1654-1657 [PMID: 1838262 DOI: 10.1016/0277-5379(91)90439-k]

66 **Yamaue H**, Tanimura H, Tsunoda T, Iwahashi M, Tani M, Inoue M, Tamai M. [Clinical application of adoptive immunotherapy by cytotoxic T lymphocytes induced from tumor-infiltrating lymphocytes]. *Nihon Gan Chiryo Gakkai Shi* 1990; **25**: 978-989 [PMID: 2391445]

67 **Iwamuro M**, Takahashi T, Watanabe N, Omote S, Matsueda K, Tanaka T, Ennishi D, Otsuka F, Yoshino T, Okada H. Technique for single-step lymphocyte isolation from an endoscopic biopsy specimen for the diagnosis of gastrointestinal lymphoma. *MethodsX* 2020; **7**: 101095 [PMID: 33102158 DOI: 10.1016/j.mex.2020.101095]

68 **Oka S**, Muroi K, Sato K, Uskudar Teke H, Sahin Mutlu F, Gulbas Z. Flow cytometric evaluation of endoscopic biopsy specimens from patients with gastrointestinal tract B-cell lymphoma: a preliminary report. *Jichi Med Univ J* 2007; **30**: 129-135

69 **Oka S**, Muroi K, Sato K, Fujiwara S, Oh I, Matsuyama T, Ohmine K, Suzuki T, Ozaki K, Mori M, Nagai T, Fukushima N, Fukushima N, Tanaka A, Ozawa K. Flow cytometric analysis of kappa and lambda light chain expression in endoscopic biopsy specimens before the diagnosis of B-cell lymphoma. *J Clin Exp Hematop* 2012; **52**: 127-131 [PMID: 23037629 DOI: 10.3960/jslrt.52.127]

70 **Almasri NM**, Zaer FS, Iturraspe JA, Braylan RC. Contribution of flow cytometry to the diagnosis of gastric lymphomas in endoscopic biopsy specimens. *Mod Pathol* 1997; **10**: 650-656 [PMID: 9237173]

71 **Iwamuro M**, Matsueda K, Takahashi T, Omote S, Tanaka T, Ennishi D, Otsuka F, Yoshino T, Okada H. An Endoscopic Biopsy Specimen Contains Adequate Lymphocytes for Flow Cytometric Analysis of Light Chain Expression in the Gastrointestinal Mucosa. *Ann Clin Lab Sci* 2020; **50**: 348-353 [PMID: 32581024]

**Footnotes**

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest associated with this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Peer-review started:** December 31, 2020

**First decision:** April 6, 2021

**Article in press:**

**Specialty type:** Medical laboratory technology

**Country/Territory of origin:** Japan

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Di Mario F **S-Editor:** Gao CC **L-Editor: P-Editor:**

**Figure Legends**



**Figure 1 Selection of articles for the review.**