Short Communication

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Simple Technique for Confirmation of Infection with *H. Pylori* in Mongolian Gerbils

Tesrou Seita, Shizuo Yamamoto and Takashi Kuribayashi*

Laboratory of Immunology, School of Life and Environmental Science, Azabu University, Japan

***Corresponding author:** Takashi Kuribayashi, Laboratory of Immunology, School of Life and Environmental Science, Azabu University, 1-17-71 Fuchinobe, Chou-ku, Sagamihara, Kanagawa 252-5201, Japan

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ABSTRACT

A simple method for measurement of serum antibodies against *Helicobacter pylori* (*H. pylori*) by indirect fluorescent antibody (IFA) technique using latex particles was developed. Sera were collected from Mongolian gerbils inoculated with *H. pylori*. Serum titers were measured using the IFA technique coupled with solubilized *H. pylori*. Fluorescence findings for latex particle-coupled soluble *H. pylori* were only confirmed in samples containing antibodies against *H. pylori*. Serum IgM and IgG titers increased after inoculation with *H. pylori*, and serum IgM titers increased before IgG titers. It is therefore possible to confirm infection at early stages using anti-IgM antibody as a secondary antibody. Moreover, the IFA technique needed only 10 µl of serum from Mongolian gerbils. This method will be useful for confirming *H. pylori* infection in individual animals.

Abbreviations: IFA: Indirect Fluorescent Antibody; FITC: Fluorescein Isothyocyanate; PBS: Phosphate-buffered Saline; VT: Verotoxin

Introduction

Helicobacter pylori (*H. pylori*), first identified by Marshall and Warren, is a gram-negative bacterium found on the luminal surface of the gastric epithelium [1]. Mongolian gerbils infected with *H. pylori* reflect human gastric disease caused by *H. pylori* [2-4]. Mongolian gerbils are considered to be a suitable experimental model for *H. pylori* infection [3,5,6]. Establishment of infection is confirmed by culture using the removed stomach from Mongolian gerbils in a satellite infection group before infection experiments. However, it is impossible to ensure that all Mongolian gerbils used in the study are infected, even if all Mongolian gerbils in the satellite group are confirmed to be infected with *H. pylori*. Measurement by indirect immunofluorescence assay (IFA) has high specificity and is a simple method for obtaining antibody titers. We have already reported that the antibody titer of bovine immune colostral antibody against verotoxin (VT) 2 can be measured using latex particles coupled with VT2 [7]. The aim of this study was to evaluate whether infection with *H. pylori* can be confirmed in individual Mongolian gerbils by IFA.

Materials and Methods

Animal Experiment

Five Mongolian gerbils were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). Mongolian gerbils under fasting for 18 hours were orally inoculated with *H. pylori* adjusted to 5 × 10^7 CFU/ml. Blood was collected from the jugular vein under anesthesia before inoculation and at 1-week intervals until 8 weeks after inoculation. Sera were obtained by centrifugation (9,200 × g, 15 minutes). The present animal experiments were approved by the Institutional Animal Care and Use Committee of Azabu University.

IFA Technique

Latex particles of 6.0 µm in diameter (Polyscience Inc., Warrington, PA) were used. Soluble antigens of *H. pylori* were bonded with the latex particles according to the method of Kuribayashi, et al. [8]. Latex particles sensitized with *H. pylori* soluble antigen were diluted fifty times. Diluted latex particles (10

μl) were smeared on one well of the slide glass (Matsunami Glass Ind., Ltd., Osaka, Japan), followed by drying at room temperature. Analyte sera were diluted between 5 and 80 times with phosphate buffered saline. Analyte sera (10 μl) was smeared on a slide glass (Matsunami Glass Ind., Ltd.) and reacted under a moist environment for 1 hour. Slide glass was washed with phosphate-buffered saline (PBS, pH7.2) for 5 minutes, and was then washed with PBS containing 5% glycerin. Finally, the slide glass was dried at room temperature after rinsing with distilled water for 3 minutes. Fluorescein isothyocyanate (FITC)-labeled goat anti-mouse IgG antibody (American Qualex International Inc., San Clemente, CA) or FITC-labeled goat anti-mouse IgM antibody (American Qualex International Inc.) was smeared onto a slide glass and reacted for 1 hour at room temperature. The slide glass was washed using the same method mentioned above and dried at room temperature. Antibody titers were determined at the maximum dilution before fluorescence was no longer observed.

Results

Typical negative and positive fluorescent observations of latex particles with adsorbed *H. pylori* soluble antigen after reaction with serum with or without antibodies against *H. pylori* are shown in (Figure 1). No fluorescence was observed with serum lacking antibodies against *H. pylori*. On the other hand, particles coupled with *H. pylori* soluble antigen reacted with serum antibodies against *H. pylori*, showing fluorescence. Serum IgG and IgM titers increased after inoculation with *H. Pylori* (Figure 2).

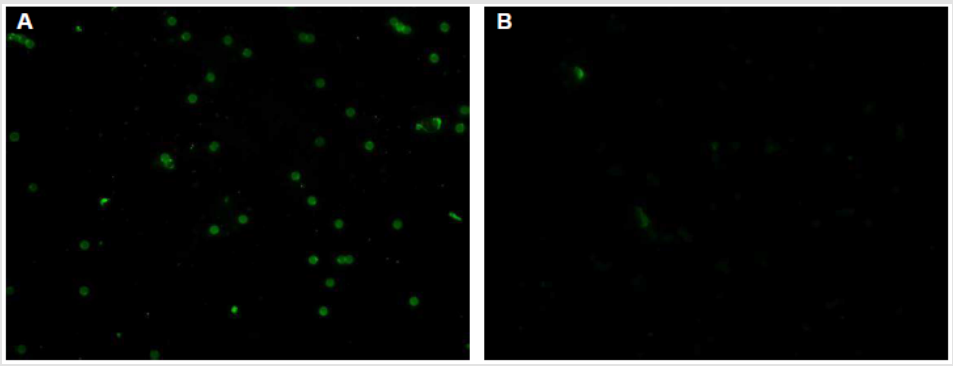

Figure 1: The typical positive and negative e immunofluorescent findings

A. positive,

B. negative.

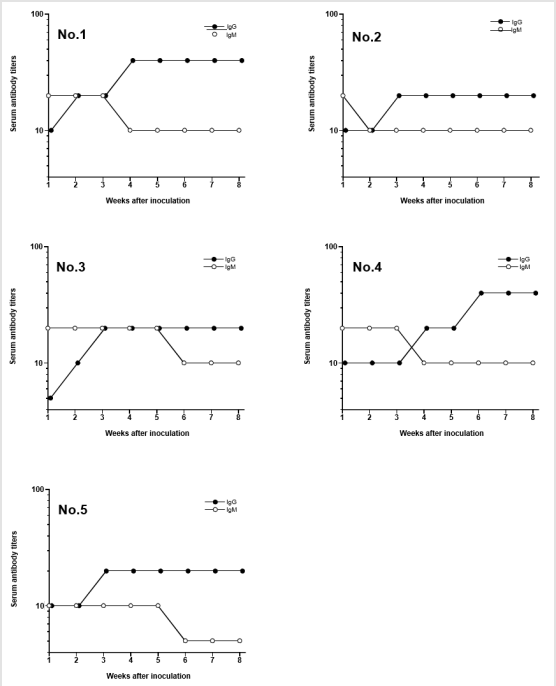

Figure 2: Changes of serum IgM and IgG titers in Mongolian gerbils after inoculation of *Helicobacter pylori* Each number was represented the animal number.

Discussion

Mongolian gerbils are essential experimental animals for evaluating *H. pylori* infection [2,4]. It is therefore important to confirm the establishment of infection in individual animals to ensure the accuracy of experimental infection studies. The IFA technique using latex particles with soluble antigens from *H. pylori* was therefore used to evaluate serum titers in Mongolian gerbils after inoculation. Elevation of serum antibodies was found to be correlated with infection by *H. pylori* in Mongolian gerbils [9]. Serum IgM titers increased before IgG titers at 1 week after inoculation with *H. pylori*. It is therefore possible to confirm infection at early stages using anti-IgM antibody as a secondary antibody. Only 10 µl of serum was needed to measure serum titers using the IFA technique. Therefore, all Mongolian gerbils were confirmed to be infected with *H. pylori* using the IFA technique in an *H. pylori* infection experiment, and only required the collection of a very small quantity of blood from each animal. Thus, only Mongolian gerbils confirmed to be infected with *H. pylori* would be used in the infection study, thus improving experimental precision. Moreover, it would be unnecessary to confirm *H. pylori* infection in a satellite group, which would reduce the number of experimental animals.

Conclusion

In summary, serum titers in individual Mongolian gerbils could be measured using the IFA technique. Establishment of infection with *H. pylori* in each Mongolian gerbil could therefore be confirmed, and this approach will improve the accuracy of experimental *H. pylori* infection studies.

Acknowledgment

None.

Conflicts of Interest

The author has no organizations that could inappropriately influence or bias the content of the article.

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