IMMUNOCYTOCHEMICAL EVIDENCE FOR DIFFERENTIAL DISTRIBUTION OF GASTRIN FORMS USING REGION-SPECIFIC MONOClonAL ANTIBODIES

Takeshi AZUMA, M.D.*, Don ABRAHM, M.D.** and John H. WALSH, M.D.**
*Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kyoto 602, Japan and **Center for Ulcer Research and Education, VA Wadsworth Medical Center, University of California, Los Angeles, CA 90073, U.S.A.

Summary

Immunocytochemical identification of cellular origins of different forms of gastrin in canine and human antral mucosa has been carried out using region specific monoclonal antibodies. Three types of gastrin cells were identified. The first type of cell was stained with both the C-terminal specific antibody of G17 and the N-terminal specific antibody of G17. The second type of cell was stained only with the C-terminal specific antibody of G17 but not with the N-terminal specific antibody of G17. The third type of cell was stained only with the N-terminal specific antibody of G17.

From these findings we propose that the first type of cell contains gastrins with the amidated C-terminus of G17 such as component 1, G34, G17, or G14 as well as the free N-terminus of G17 such as G17, or C-terminal extended gastrins, the second type of cell contains gastrins only with the C-terminus of G17 but not with the N-terminus of G17 such as G34, or component 1, and the third type of cell contains C-terminal extended gastrins with intact N-terminus G17.

Key Words: Gastrin, Gastrin forms, Immunocytochemistry, Monoclonal antibody.

Introduction

It is well known that gastrin occurs in four main components with different lengths of the N-terminal extension of the bioactive C-terminal tetrapeptide amide, component 1, G34, G17 and G14. It has been suggested that different active forms of peptide hormones are synthesized not only by proteolytic cleavage in the N-terminal part of the translated polypeptide chain, but also by transamidation to form a C-terminal amide.

There have been numerous reports of the cellular localization of gastrins by the immunocytochemical method, however, there has not yet been any clear demonstration of the cellular origins of the different molecular forms of gastrin.

The purpose of the present study is to identify the cellular origins of different forms of gastrin using monoclonal antibodies specific for
the C- and N-termini of G17.

Materials and Methods

1) Antibodies

Polyclonal rabbit antisera 1802, specific for the C-terminus of G17, were described previously. Monoclonal C-terminal specific antibody of G17, OCTG XX136, and monoclonal N-terminal specific antibody of G17, NTG 1F9D6, originated from the fusion of spleen cells from a mouse immunized with human synthesized G17 and the mouse myeloma cell line FOX-NY, which were donated by Dr. T. Taggart, were described in detail elsewhere. Ascitic fluid was the source of antibody.

2) Immunocytochemistry

Fresh samples of antral mucosa were taken from 5 dogs and histologically normal antral biopsies were obtained from 5 patients during diagnostic endoscopy. Samples were fixed in Bouin's solution, routinely processed, embedded in paraffin and 4 µm serial section were made. For immunostaining, the unlabelled peroxidase-antiperoxidase complex (PAP) technique, as described by Sternberger, was applied.

3) Double staining

Sections were first stained with 1802 by the PAP procedure and subsequently stained for NTG 1F9D6 with the indirect immunofluorescence procedure, which was carried out according to the following process.

1. Incubation with the first antibody (NTG 1F9D6; 1:10,000 dilution in PBS) for 24 hrs at room temperature.
2. Incubation with FITC-conjugated goat anti-mouse IgG (1:10 dilution in PBS) for 1 hr.

The optimal dilution of antibody was established from serial dilutions of 1:100 to 1:100,000 and was defined as the highest dilution giving reproducible staining (1:1000 for 1802, 1:50,000 for OCTG XX136 and NTG 1F9D6 by PAP method, 1:10,000 for NTG 1F9D6 by indirect immunofluorescence method).

4) Relative proportions of three types of gastrin cells

Three types of gastrin cells were counted in transverse serial sections stained with OCTG XX136 and NTG 1F9D6. The results were evaluated as a percentage of the total gastrin cells which were estimated as the number of the second type plus the third type of cell. The total surface area of the sections averaged 0.2 cm².

5) Immunocytochemical specificity

The specificity of the immunocytochemical reaction was established first by showing that non-immune rabbit and mouse serum did not reveal gastrin cells. In addition, the specificity of particular antibodies was evaluated by absorption controls. The diluted antibodies were incubated with G17, the C-terminal fragment of G17 (3-17 G17) and the N-terminal fragment of G17 (1-13 G17) in concentrations of up to 10 nmol/ml for 48 hours at 4°C and then applied to tissue sections.

Results

In serial sections there were three types of gastrin cells. The first type of cell was stained with 1802, OCTG XX136, and NTG 1F9D6. The second type of cell was stained with 1802, and OCTG XX136, but not with NTG 1F9D6. The third type of cell was stained only with NTG 1F9D6, but not with 1802, and OCTG XX136 (Table 1). These three types of gastrin cells staining with OCTG XX136 and NTG 1F9D6 are shown in Fig. 1.

Table 1. Staining patterns of antral gastrin cells

<table>
<thead>
<tr>
<th>Antibody/Cell type</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1802</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>OCTG XX136</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NTG 1F9D6</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>