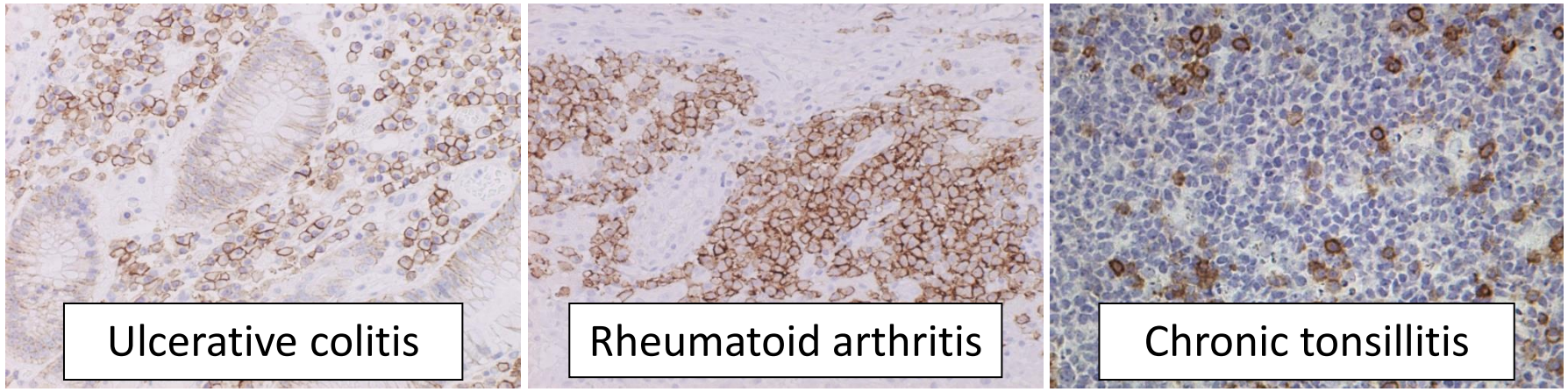
Periodontitis and enzyme-labeled antigen method

Dense plasma cell infiltration is commonly seen in infectious diseases, including *Porphyromonas gingivalis*-induced periodontitis and *Helicobacter pylori*-associated chronic gastritis. Antigens recognized by antibodies produced by locally infiltrating plasma cells remain unclear. We have developed an enzyme-labeled antigen method for microscopic identification of the antigen recognized by specific antibodies locally produced in plasma cells in inflammatory lesions. In fact, it was proven that plasma cells in periodontitis produce antibodies specific to *P. gingivalis*.

Ref.: Mizutani Y, et al. Enzyme-labeled antigen method: development and application of the novel approach for identifying plasma cells locally producing disease-specific antibodies in inflammatory lesions. Acta Histochem Cytochem 2016; 49(1): 7-19. doi: 10.1267/ahc.15030

Antigens recognized by antibodies secreted from plasma cells within pathology tissues are unclear.

Immunostaining for CD138 (plasma cell marker)

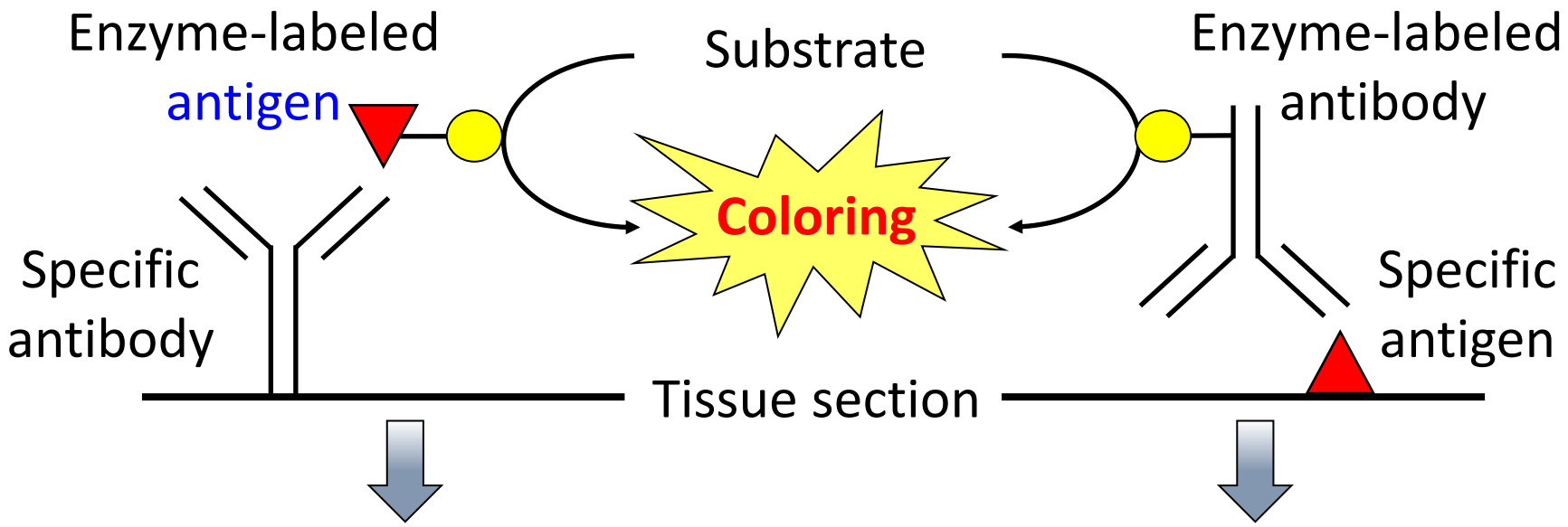


These immunocytes may locally produce disease-specific antibodies.

Detection of such antigens may give a breakthrough in analyzing the disease process or cause of the disease.

What is the “enzyme-labeled antigen method”?

Enzyme-labeled **antigen** method	Enzyme-labeled **antibody** method



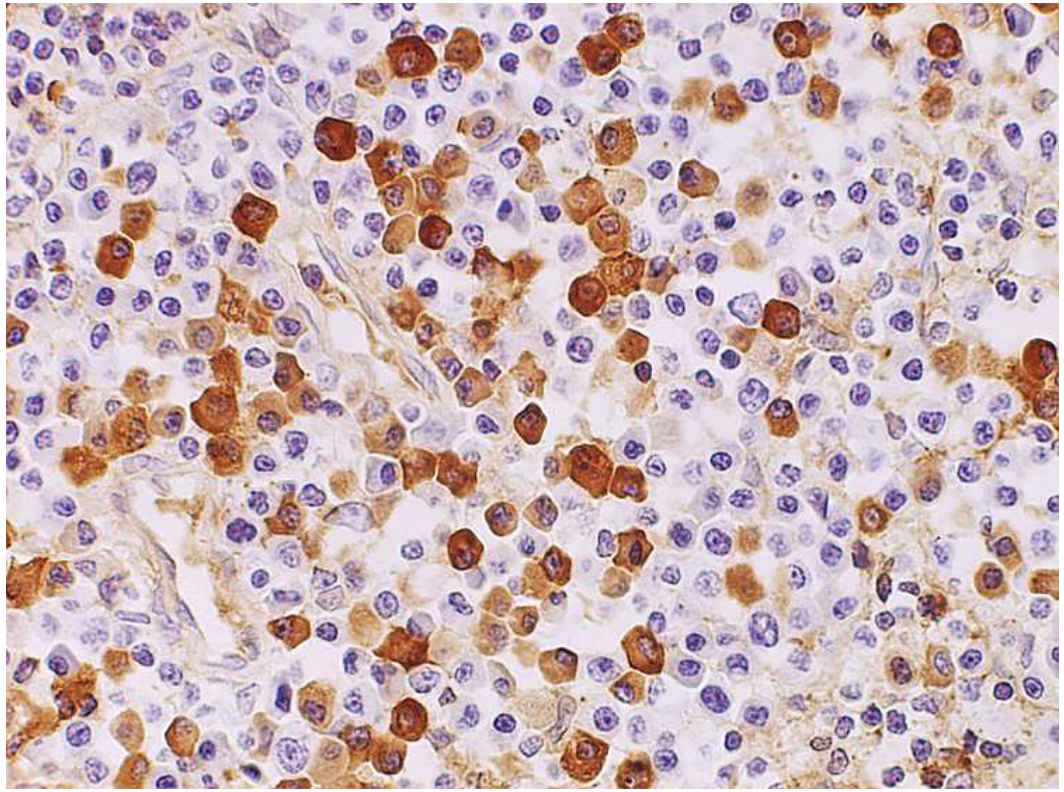
Specific antibodies are visualized.

The antibody-producing plasma cells are stained

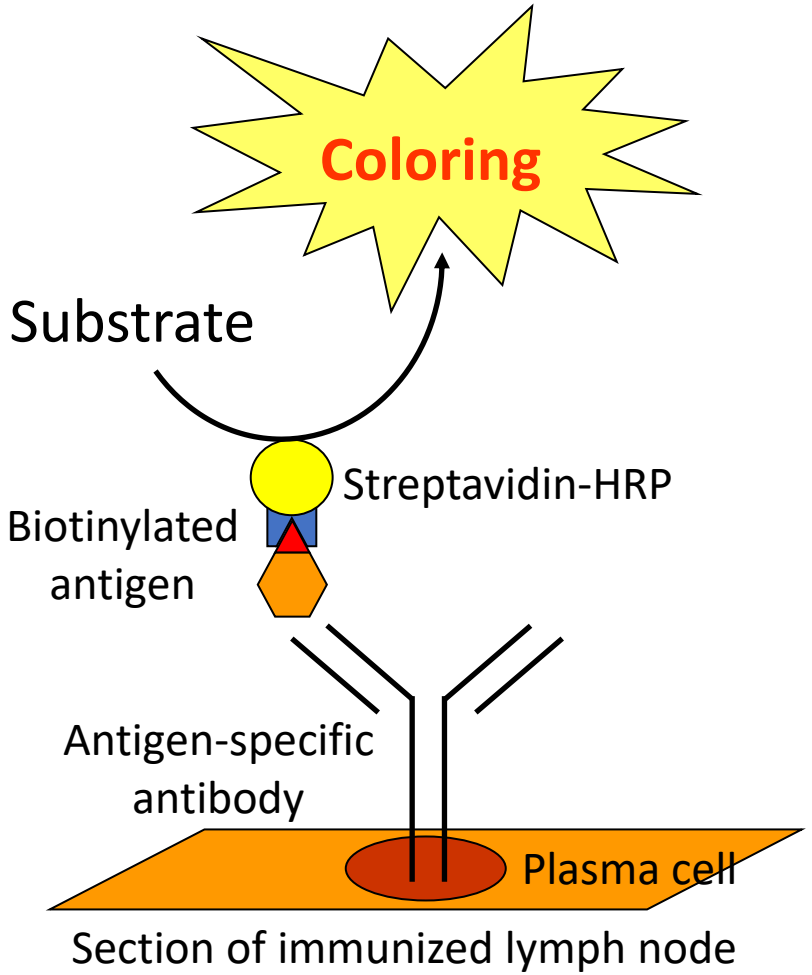
Specific antigens are visualized.

This method is just reverse of enzyme-labeled antibody method, a general immunohistochemistry. Enzyme-labeled antigen method is the immunohistochemistry that visualizes antigen-specific antibody producing cells in sections.

The enzyme-labeled antigen method to visualize specific antibody-producing plasma cells



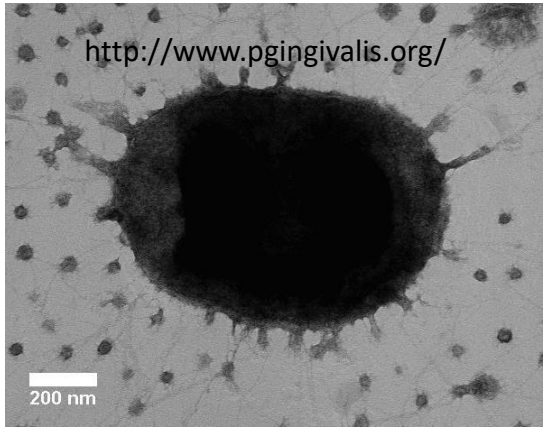
KLH-immunized lymph node stained with biotinylated KLH



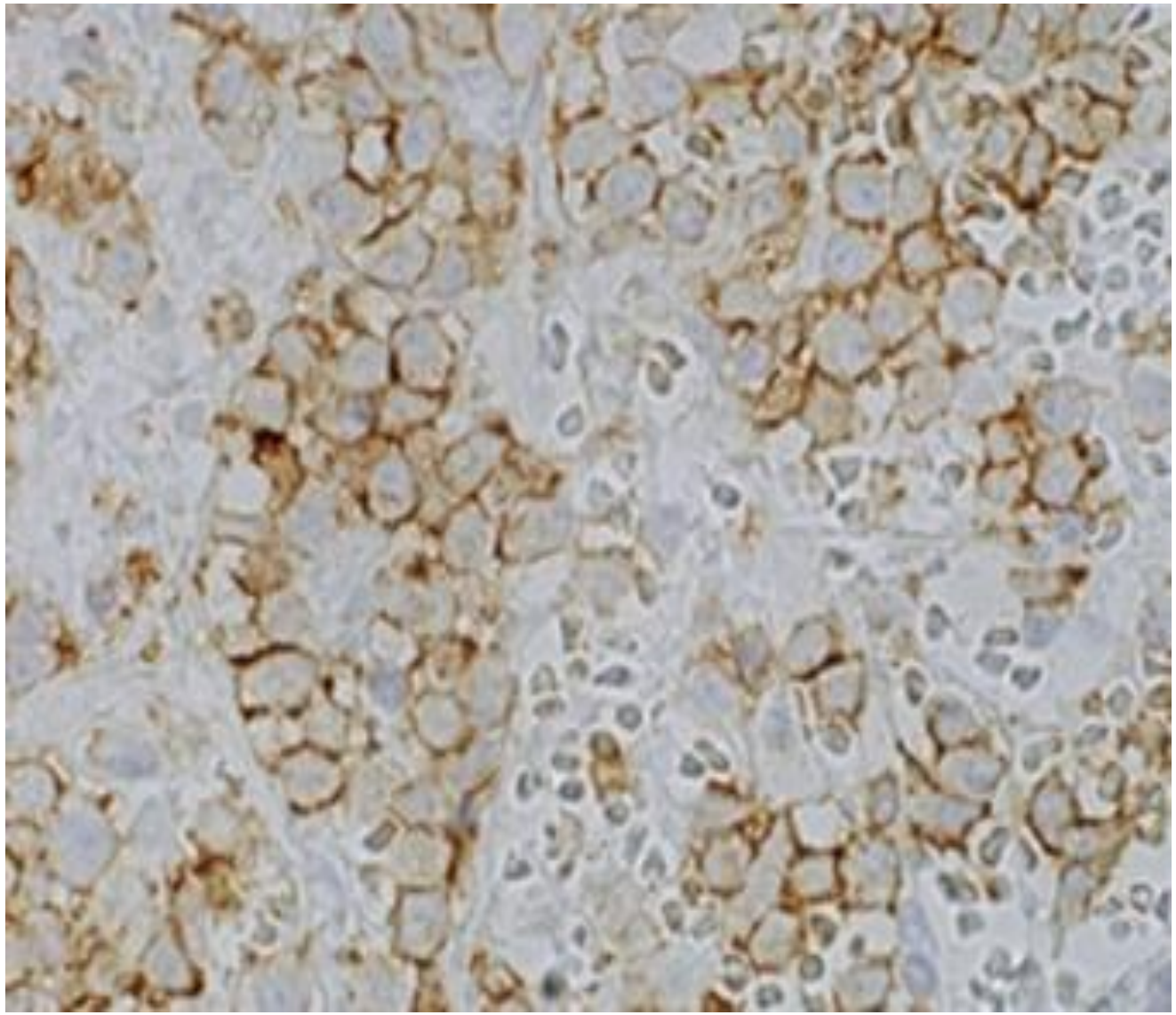
Biotinylated antigens bind to specific antibodies in plasma cells on the section. The biotin labels are detected with HRP-labeled streptavidin. This picture shows the KLH-immunized lymph node section stained with biotinylated KLH. A large number of anti-KLH antibody-producing plasma cells are observed.

Porphyromonas gingivalis（*Pg*）
～a representative periodontitis pathogen～

- Gram negative obligate anaerobic rods
- Major antigen:
  - Outer membrane protein **Ag53** (53 kDa)
- Pathogenic protein（protease **gingipains**）
  - **Arg-pro**
  - **Arg-hgp**
  - **Lys-pro**
  - **Lys-hgp**

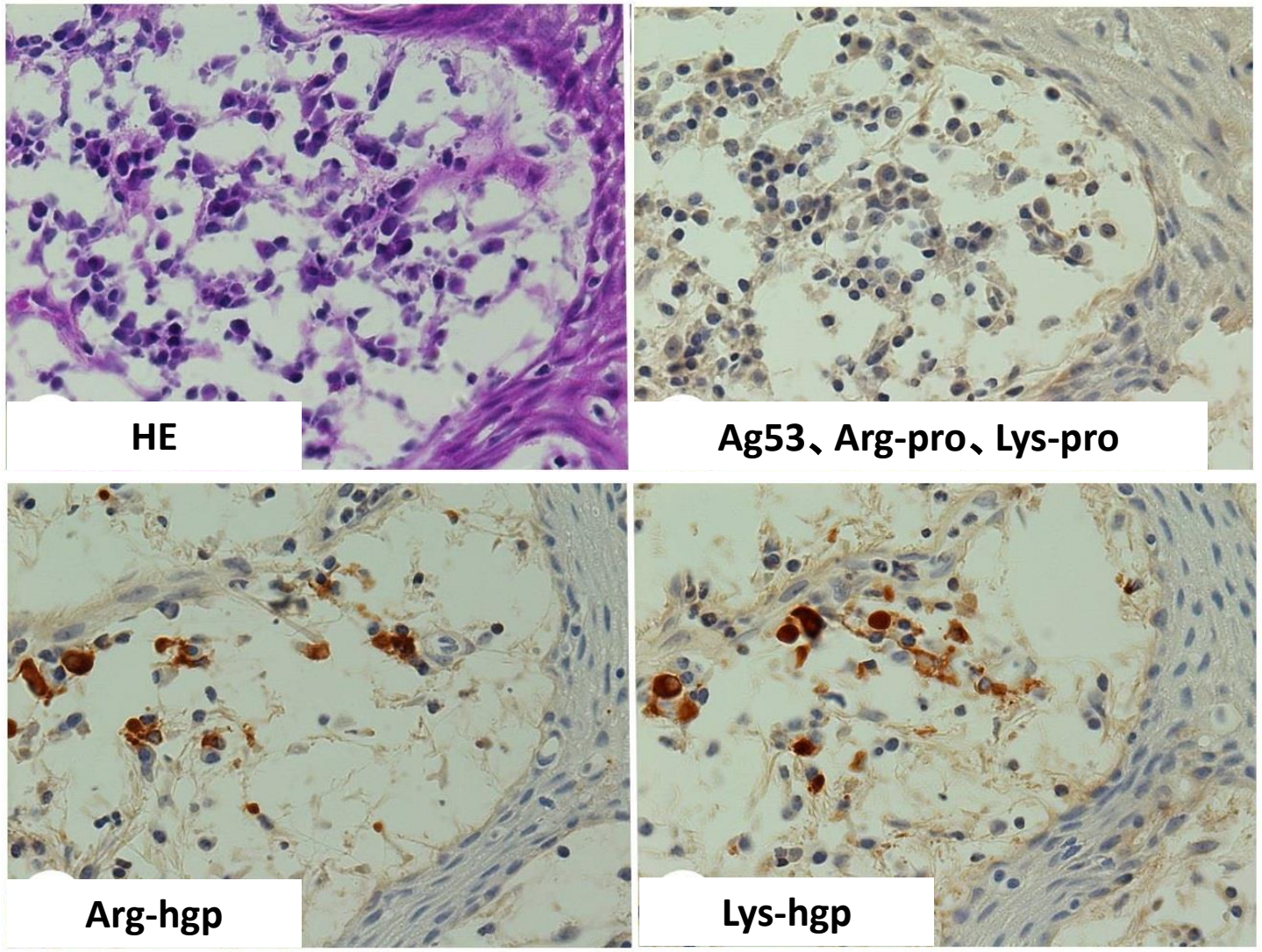


Antibodies against these five antigens were screened.



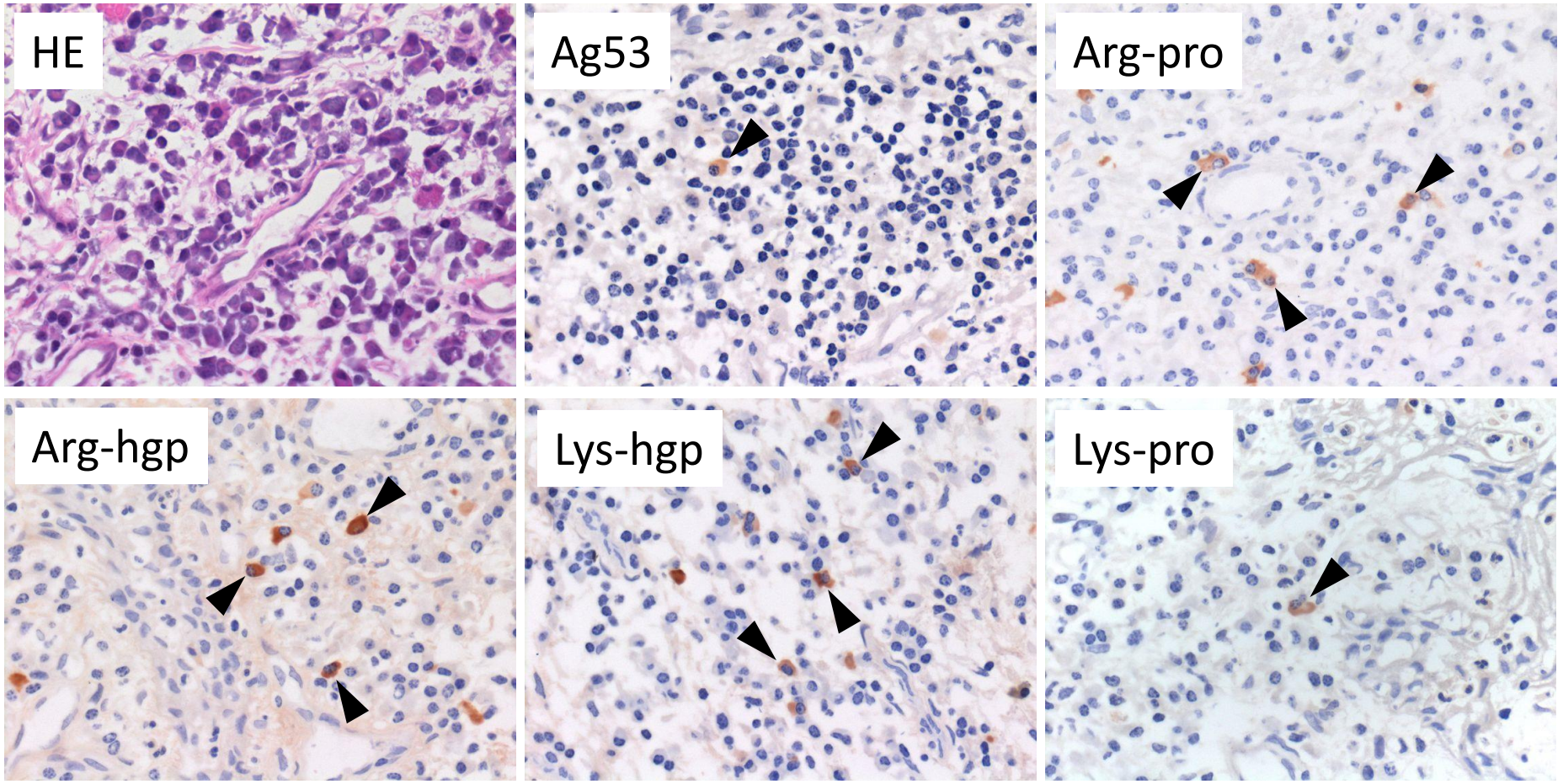
Immunostaining for CD138 (plasma cell marker) demonstrates numerous plasma cells in periodontitis lesion.

The enzyme-labeled antigen method using biotinylated *Pg* antigens

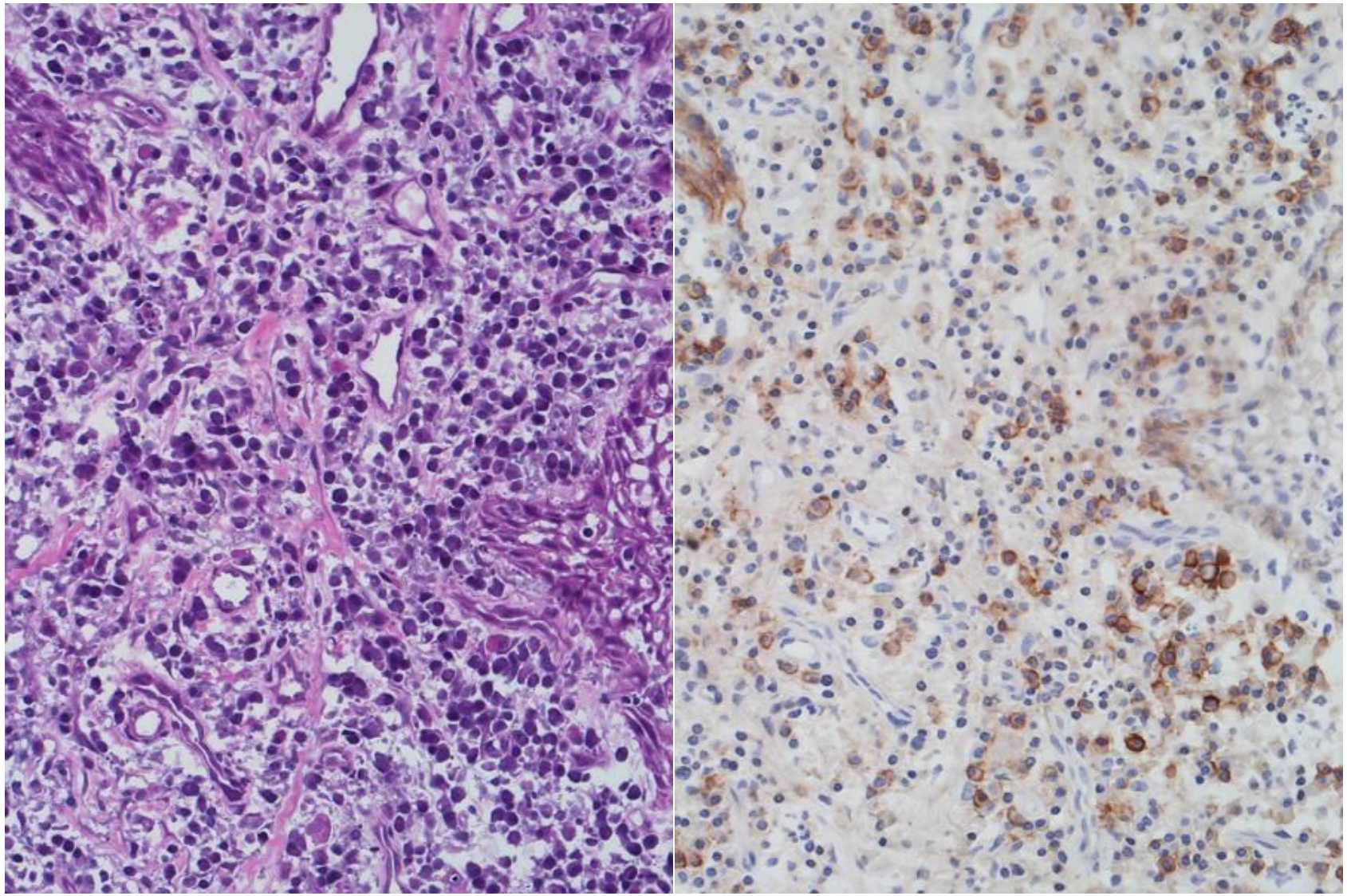


Specific antibody producing cells are detectable in tissue sections with enzyme-labeled antigen method.

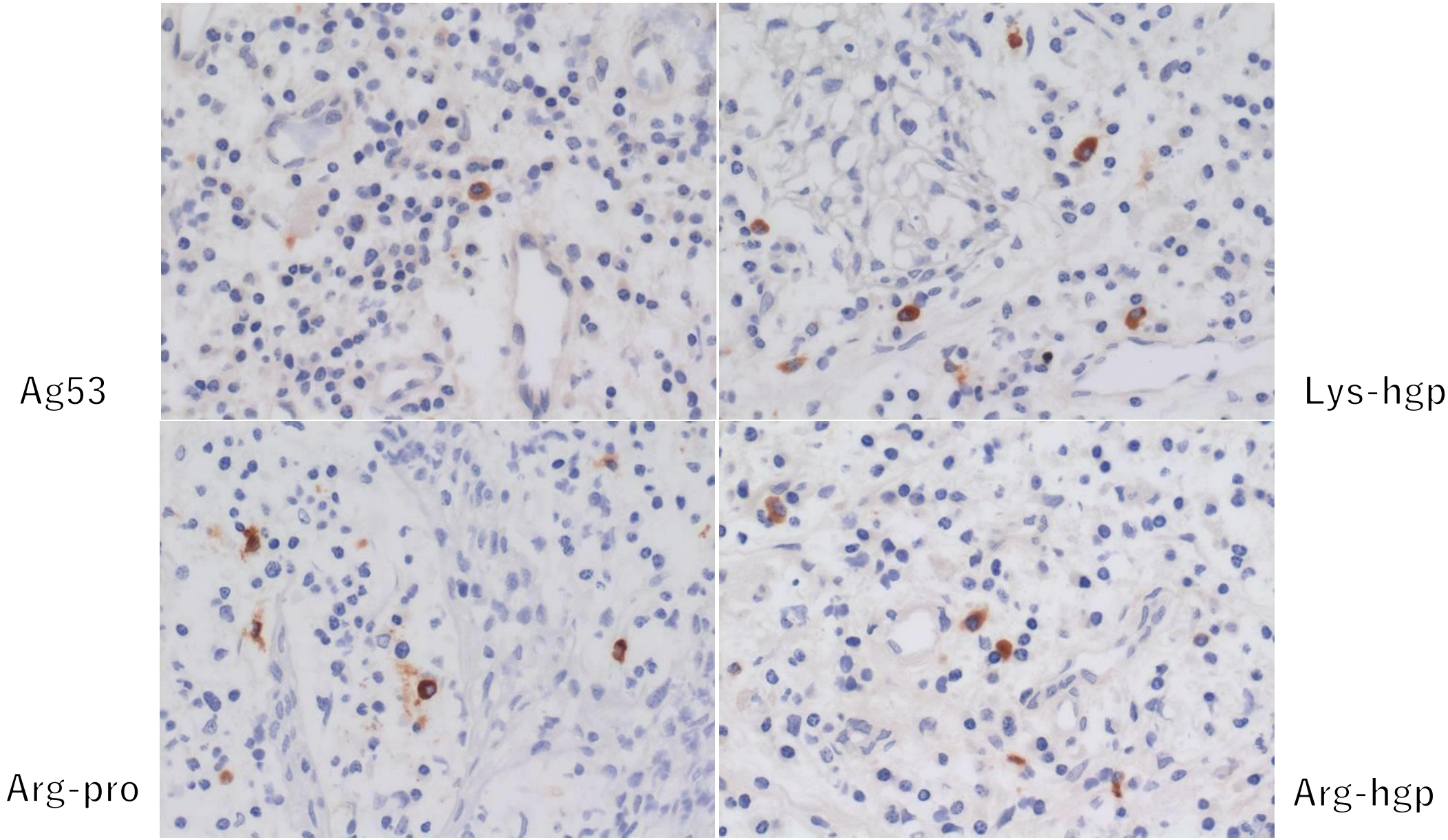
The enzyme-labeled antigen method for Pg antigens in fixed frozen sections of periodontitis



In this periodontitis lesion, specific antibodies against all five Pg antigens are visualized in plasma cells.



Another case of periodontitis. Biopsy reveals infiltration of numerous CD138-positive plasma cells (left: H&E, right: immunostaining for CD138)



The enzyme-labeled antigen method identifies local production of antibodies specific to *P. gingivalis* antigens (Ag53, Lys-hgp, Arg-pro and Arg-hgp).