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Celiac Disease and Hypoparathyroidism: Cross-Reaction of Endomysial Antibodies with Parathyroid Tissue

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Celiac disease (CD) is a gluten-sensitive enteropathy characterized by the presence of serum antibodies to endomysial reticulin and gliadin antigens. CD has been associated with various autoimmune endocrine disorders, such as diabetes. We report a rare case of idiopathic hypoparathyroidism with coexistent CD characterized by the presence of serum autoantibodies. Studies were conducted to determine the specificities of these autoantibodies and to localize the antibody binding sites by indirect immunofluorescence and immunoelectron microscopy. Sera from a patient with idiopathic hypoparathyroidism and CD and from two patients with CD alone were tested by indirect immunofluorescence for autoantibodies to parathyroid and endomysial antigens. The specificities of the antibody reactions were determined by testing the sera before and after absorption with monkey stomach tissue. In addition, immunoelectron microscopic studies were performed to determine the localization of the endomysial antigen. Indirect-immunofluorescence studies on the patient's serum were positive with the parathyroid as well as the endomysial substrate. Similar reactions were also observed with the sera of endomysial antibody-positive patients with CD. Absorption of the sera with monkey stomach powder, which is known to have the endomysial antigen, abolished the antibody activities on both the endomysial substrate and the parathyroid tissue. Immunoelectron microscopic studies showed that endomysial antibody activity was associated with antigens localized on the myocyte plasma membrane and in the intercellular spaces. Thus, reactions of the patient's serum with the parathyroid tissue were due to endomysial antibodies and were not parathyroid specific as in patients with idiopathic hypoparathyroidism who did not have coexistent CD. In conclusion, indirect-immunofluorescence tests on parathyroid tissue detect not only tissue-specific antibodies but also cross-reactive antibodies, and this should be taken into consideration when these tests are performed.

Celiac disease (CD) is an autoimmune, gluten-sensitive enteropathy characterized by the presence of antigliadin antibodies, endomysial antibodies (EMA), and antireticulin antibodies (ARA) (1, 6–9, 13). CD is also associated with autoimmune diseases such as insulin-dependent diabetes and autoimmune thyroid disorders (2–4, 11). In a recent report we described a case of idiopathic hypoparathyroidism coexistent with CD; in this patient, besides the antibodies to intestinal smooth muscle endomysium and reticulin, there were also antibodies to parathyroid tissue (15). The question of whether there is a single antibody which reacts with both the parathyroid and endomysial substrates or whether there are two different antibodies with target tissue specificities was raised. Circumstantial evidence (temporal relationships) suggested that antibody reactions with parathyroid tissue in this case were not produced by parathyroid-specific antibodies but by EMA mimicking parathyroid-specific antibody reactions. Immunoabsorption and immunoelectron microscopic studies were therefore performed to characterize the tissue specificity of EMA and to determine the subcellular localization of the corresponding antigens. It appears that parathyroid antibody reactions can be of two types: one is specific for the parathyroid gland, and the other is not organ specific (e.g., the CD-associated EMA that mimic parathyroid antibodies). This distinction may be important in the evaluation of tests for the detection of parathyroid autoantibodies.

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MATERIALS AND METHODS

Clinical material. The serum from a patient with idiopathic hypoparathyroidism coexistent with CD has been described previously (15). In addition, sera from two patients with active CD were positive for EMA and ARA, as detected by indirect immunofluorescence (IF), on monkey esophagus tissue or other endomysial antigen substrates and on rat kidney tissue, respectively. Bovine parathyroid tissue and human parathyroid adenoma tissue were used as substrates for detecting parathyroid-associated antibodies.

Indirect IF. EMA and ARA reactivities were determined by indirect IF on primate intestinal smooth muscle and rat kidney tissues (IMMCO Diagnostics), respectively. Briefly, serial twofold dilutions of sera at 1:2.5 were placed on cryocut tissue sections, and the sections were incubated for 30 min at room temperature. The slides were washed in phosphate-buffered saline (PBS) for 15 min and treated with fluorescein-labeled goat anti-human immunoglobulin A (IgA) (IMMCO Diagnostics) for 30 min (molar fluorescein/protein ratio = 2.5). After another washing step with PBS, the slides were mounted with Gelvatol (IMMCO Diagnostics) containing antifading agents. The resulting indirect-IF reactions were read with a Nikon fluorescent microscope equipped with a narrow-band filter. The titers of EMA and ARA were determined by serial dilution. A titer of more than 1:2.5 was considered positive. An indirect-IF method also was used to determine antibody binding to bovine and parathyroid tissues.

Immunoabsorption. Monkey stomach tissue was lyophilized and pulverized in a coffee grinder. Lyophilized tissue (50 mg) was mixed with 1 ml of PBS containing protease inhibitors (25 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 3 mM *N*-ethylmaleimide, and 1 μg of pepstatin A per ml; all from Sigma Chemical Co., St. Louis, Mo.) for 30 min. The mixture was centrifuged at 16,000 × *g* for 10 min. The supernatant was discarded, and the pellet was mixed with 1 ml of serum diluted 1:5 with PBS containing the protease inhibitors. The absorption was done at 4°C for 24 h. The mixture was then centrifuged at 16,000 × *g* for 10 min, and the pellet was discarded. This procedure was repeated once more, and the absorbed sera were tested for EMA and ARA reactivities by indirect IF. A serum sample from a patient with pemphigus vulgaris which was positive for intercellular antibodies was similarly absorbed as a control for determining nonspecific absorption by the tissue.

Immunoelectron microscopy. Fresh frozen monkey stomach tissue was sectioned at a thickness of 10 μm on slides coated with 10% Elmer's glue (Borden,

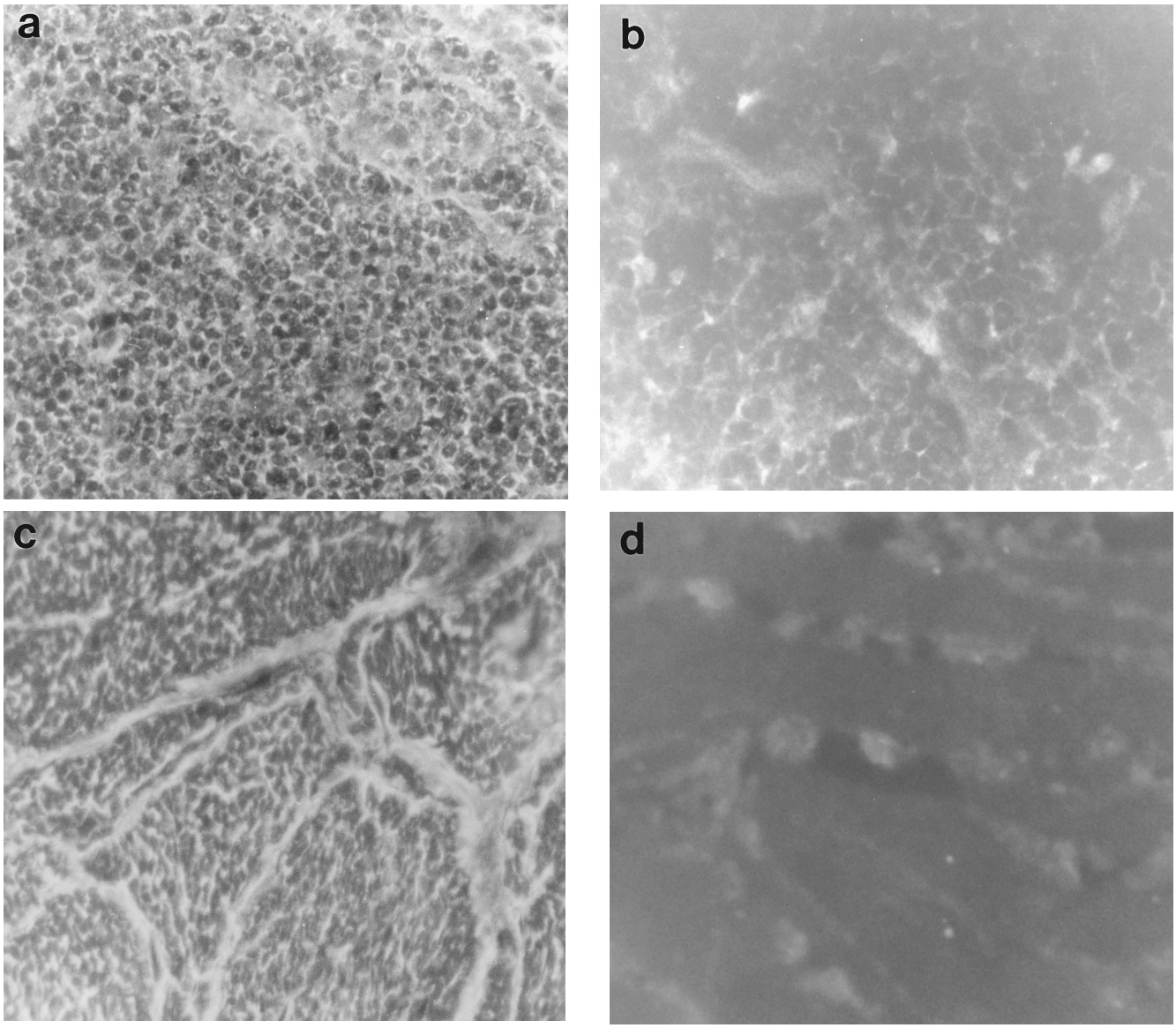

FIG. 1. Indirect immunofluorescence reactions on human parathyroid and monkey intestinal tissues before and after absorption. The photographs show similar exposures. Shown are parathyroid tissue before absorption (a) and after absorption (b) and monkey stomach tissue before absorption (c) and after absorption (d). Magnification, ×144.

Columbus, Ohio), fixed in Zamboni's paraformaldehyde for 10 min, and subjected to three 30-min washes in PBS. Rabbit anti-human IgA or IgG antisera (DAKO, Santa Barbara, Calif.) diluted 1:320 were applied, and the tissue sections were incubated for 2 h. The sections were washed with PBS, and then they were incubated with a 1:30 dilution of swine anti-rabbit IgG for 30 min. After being washed in PBS, the sections were incubated for 30 min with a 1:60 dilution of rabbit peroxidase-antiperoxidase complex (DAKO). After the sections were washed again with PBS and then treated for 10 min in 0.05 Tris-HCl, pH 7.6, containing diaminobenzidine (Polysciences, Warrington, Pa.) and 0.001% hydrogen peroxide, the reaction was terminated with a PBS wash. The sections were then fixed in 2% osmium tetroxide in distilled water for 1 h.

For embedding, the sections were dehydrated with two sequential 3-min changes each of 10, 50, 70, 95, and 100% ethanol. After dehydration, the sections were placed twice in propylene oxide and then in Epon 812. The slides were brought to near dryness by exposure to room air for 3 min. An inverted gelatin capsule containing Epon 812 was then placed over each of the sections, which were then incubated overnight at 37°C; this was followed by an additional 24-h incubation at 60°C. The solidified capsules were then cut out, and the tissues were examined with a Zeiss EM10 electron microscope.

RESULTS

Serum from the patient with idiopathic hypoparathyroidism coexistent with CD gave positive staining reactions on monkey intestinal smooth muscle and rat kidney substrates by indirect IF. Similar reactions were observed with sera from patients with CD alone. When sera from either source were tested on bovine or human parathyroid tissue, there was strong reactivity against the parenchymal cells and the endomysial components of the vascular smooth muscle of the parathyroid tissue. As the reactions on parathyroid tissue obtained with sera from patients with idiopathic hypoparathyroidism coexistent with CD mimicked the reactions obtained with sera from patients with CD alone, it was assumed that EMA could be responsible for the parathyroid parenchymal reactivity. Indeed, absorption of both sera from CD patients and hypoparathyroid-reactive sera

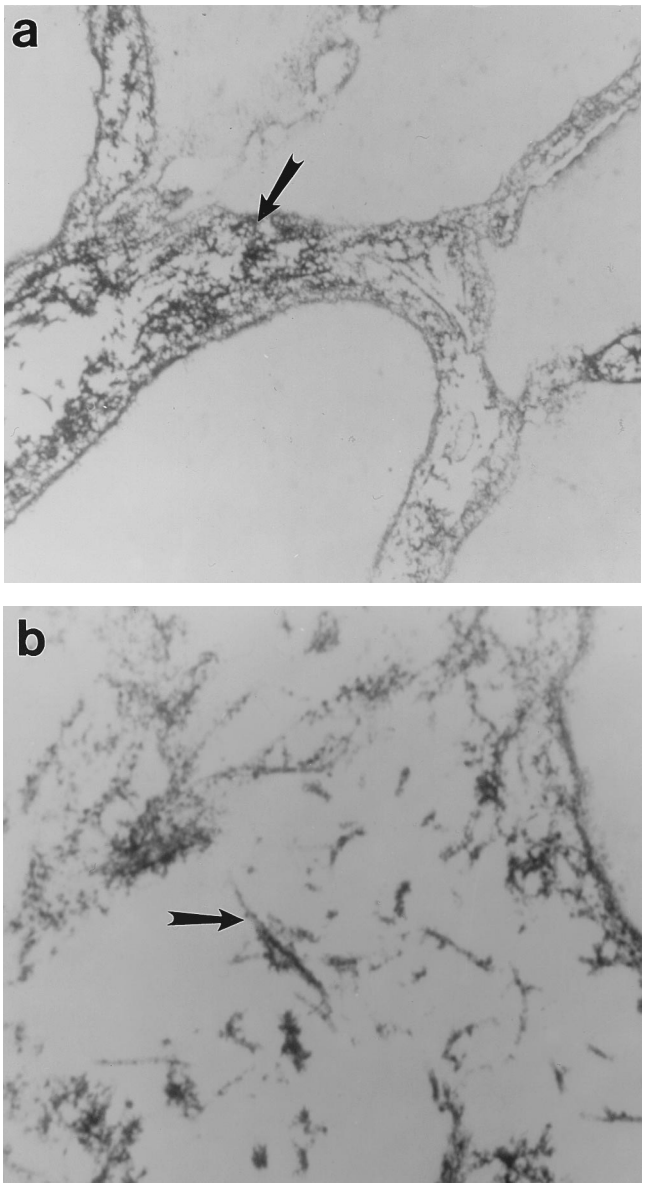

FIG. 2. Immunoelectron microscopy of monkey stomach smooth cells incubated with EMA-positive serum. (a) Electron-dense reaction products (arrow) between smooth muscle cells. Magnification, ×10,000. (b) Higher magnification showing reactive products (arrow) closely associated with cell surface and also in the intercellular space. Magnification, ×20,000.

with monkey stomach powder abolished the previously detected antibody reactions against EMA and ARA substrates as well as those against human and bovine parathyroid tissue (Fig. 1), but similar treatment of serum from a patient with pemphigus vulgaris did not affect the intercellular antibody reactions. For the absorption to be considered significant, a decrease in titer of at least fourfold had to be observed for EMA and ARA. However, no such decrease in the titer for intercellular antibody was observed. This strongly suggests that the parathyroid reactivity was due to CD-specific antibody(ies) cross-reacting with smooth muscle endomysial antigens. Localizing immunoelectron microscopic studies on normal monkey stomach tissue with EMA-positive serum as the primary antibody revealed that electron-dense reactions were associated with the surfaces of smooth muscle cells. In addition, electron-dense products were also localized in the intercellular spaces between individual myocytes and could not be associated with any particular cell type (Fig. 2). When normal human serum was used as the primary antibody, there were no staining reactions on the monkey stomach tissue.

DISCUSSION

Serological methods for detecting antigliadin antibodies, ARA, and EMA are included in the European Society for Pediatric Gastroenterology and Nutrition criteria for the diagnosis of CD (14). According to these criteria, the serum of a patient must be positive for two of the three antibodies for a diagnosis of CD to be made. In a recent comparative study of the various serological methods, it was determined that these tests, if performed under standardized conditions, exhibit a high degree of reliability in their predictive value for the diagnosis of CD (9). The availability of noninvasive serological methods has enabled the recognition of an association of various autoimmune endocrine disorders with CD. We recently used these tests to diagnose a patient with late-onset idiopathic hypoparathyroidism coexistent with CD (15). The diagnosis of CD was confirmed by the findings of intestinal malabsorption, intestinal villus atrophy, and the presence of EMA, ARA, and antigliadin antibodies. These antibodies disappeared upon institution of a gluten-free diet. Indirect-IF reactions on various substrates showed that the serum from this patient reacted not only with primate intestinal smooth muscle, the antigenic substrate for EMA, but also with spleen, thyroid, adrenal, and parathyroid tissues. More specifically, the reactions on parathyroid tissue were directed against the endomysial components of the vascular smooth muscle and also against the parenchymal cells. It was therefore possible that the patient's serum contained two different antibodies, one specific for CD and the other specific for parathyroid cells. Alternatively, the serum could have contained only one antibody specific for CD, the staining on parathyroid parenchyma representing true EMA reactions mimicking parathyroid-specific antibody reactions. On the basis of our previous study, the latter appears to be the most probable scenario since the CD control sera exhibited similar staining reactions. This was confirmed by our absorption studies with monkey stomach powder, in which all EMA reactions on the primate intestinal tissue, the human and bovine parathyroid vascular tissues, and the parathyroid parenchymal cells were abolished. This absorption was specific for EMA, as absorption of intercellular-antibody-positive serum from a patient with pemphigus vulgaris had no effect on intercellular antibody titers.

The immunoelectron microscopic studies suggest that the endomysial antigen is localized both to the surfaces of myocytes and to the connective tissue between the myocytes. This finding differs from our previous observations in patients with idiopathic hypoparathyroidism not associated with CD, in whom parathyroid antibody reactivity was restricted to parenchymal cells (10). Therefore, it is apparent that the use of indirect-IF tests on parathyroid tissue in patients with idiopathic hypoparathyroidism allows the detection of two types of circulating autoantibodies. One, perhaps the most common, is selectively directed against parathyroid parenchymal cells, while the other (CD type) reacts with both parenchymal and interstitial parathyroid antigens.

Taken together, the present and previous studies suggest that idiopathic hypoparathyroidism associated with CD may be a result of in vivo EMA reactions on parathyroid tissue producing atrophy similar to the splenic atrophy reported for

patients with CD (10). Nevertheless, the rarity of hypoparathyroidism among CD patients suggests that an additional factor (perhaps length of exposure to antibodies) may also be important for the production of parathyroid disease in persons with CD. The irreversibility of the parathyroid damage and the reversibility of the intestinal malabsorption may be explained by the strong regenerative capacity of the intestinal mucosa compared with the parathyroid. Nonetheless, determination of the pathogenic role of EMA in CD, hypoparathyroidism, and other autoimmune endocrine disorders awaits investigation.

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