In situ characterization of the inflammatory cell infiltrates of hyperplastic denture stomatitis

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Cryostat sections from 14 surgical specimens were examined to determine whether selected factors of the immune response related to histopathological reactions are present in the palatal mucosa affected by hyperplastic denture stomatitis. By means of various immunological techniques the presence of IgG, IgA, IgM, complement factor C3c, receptors for the Fc region of IgG (FcR) and for complement factor C3b (C3bR), T lymphocytes, and macrophages were studied. The inflammatory infiltrate was mainly located in the papillary part of the lamina propria. IgG, IgA, and IgM appeared both in plasma cells and intercellularly. FcR, C3bR, and T lymphocytes were present in the areas with inflammatory cell infiltrate. Macrophages were found in the papillary part of the lamina propria and within the epithelium. The immunological response in the mucosa affected by denture stomatitis was in many respects similar to that of marginal and apical periodontitis. We conclude that hyperplastic denture stomatitis is a complex inflammatory lesion showing elements of both humoral and cellular immune responses. □ *Histology; immunology; oral pathology*

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Hyperplastic denture stomatitis has been regarded as a multifactorial disease (1–4). Antigens and toxins of microorganisms in denture plaque have been proposed to be significant factors in the inhibition and maintenance of hyperplastic denture stomatitis (4). Serological studies of patients with denture stomatitis have shown increased antibody titers to *Candida albicans* (5, 6). Bergendal & Holmberg (7) found an increased humoral antibody response against *C. albicans* in patients with hyperplastic denture stomatitis and concluded that these patients are more susceptible to local contamination and infection with *C. albicans*.

Eglitis et al. (8) have suggested that hyperplastic denture stomatitis is an endogenous bacterial disease arising from a locally imbalanced host–parasite relationship. The tissue destruction may therefore be a consequence of the immunopathologic response of the host. Cellular and humoral immune reactions occur in inflamed tissue, and deposits of IgG and complement factor C3 at certain sites in tissues from subjects with hyperplastic denture stomatitis have indicated that immune complex formation may occur (8).

This investigation was carried out to determine whether selected factors of the immune response are present in the oral mucosa affected by hyperplastic denture stomatitis.

Materials and methods

Tissues

Palatal tissue was obtained from 14 subjects with full maxillary dentures diagnosed as having hyperplastic denture stomatitis, which was surgically treated in accordance with the method described by Bergendal et al. (9) Immediately after the surgical removal, the tissue was mounted on a specimen holder with Tissue Tek (Miles Laboratories, Chicago, Ill., USA) and snap-frozen in carbon-dioxide-cooled hexane. The material was stored at −90°C until examined.

Consecutive cryostat sections of 6 μm were incubated for the various immunohistochemical reactions. For morphological studies sections were also stained with hematoxylin and eosin.

Sections from human spleen and placenta, treated in the same manner as the sections from the denture stomatitis tissue, served as control tissues.

Incubations with monospecific antibodies

The sections were air-dried for 1 h in room temperature, washed for 30 min in phosphate-buffered saline, pH 7.2 (PBS) (4°C), and incubated at room temperature for 30 min in a moist chamber with the following fluorescein isothiocyanate (FITC)-conjugated antisera: $F(ab')_2$ fragments of rabbit anti-human IgG, IgA, and IgM or rabbit anti-human complement factor C3c (Behringwerke AG, Marburg, FRG), using optimal dilutions determined in a chessboard titration. Rabbit anti-candida IgG (Dako Immunoglobulins, Copenhagen, Denmark) was used in an indirect immunofluorescence technique with FITC-conjugated swine anti-rabbit IgG (Dako Immunoglobulins) as the second step. The dilution of rabbit anti-candida IgG was determined in a chessboard titration of smears of a *C. albicans* culture (from The Laboratory of Oral Microbiology, University of Bergen, Bergen, Norway). After being washed in PBS, the sections were mounted in PBS–glycerol and examined under a Leitz Orthoplan microscope equipped with a Pleumopak, a Philips CS 200-W mercury lamp, and filter blocks K2 and L2. As controls, parallel sections were incubated with PBS instead of antiserum.

Hemadsorption with tissue sections

Cryostat sections were used in a closed chamber technique (10) to demonstrate receptors for the Fc region of IgG (FcR), complement factor C3b receptors (C3bR), or T lymphocytes.

For detection of FcR activity, sheep erythrocytes (E) sensitized with rabbit IgG antibodies (A) to E to form particulate immune complexes (EA) (11, 12) were used as indicator cells. For detection of C3bR, E were sensitized with rabbit IgM antibody and coated with human C3b (13, 14). For detection of T lymphocytes, E were treated with 2-aminoethylisothiuronium bromide hydrobromide (AET) (15, 16). The closed chamber technique is briefly described as follows: The concavities of microculture slides are filled with indicator cells and sealed off by coverglasses. The slides are then inverted to enable the indicator cells to settle on the tissue sections, which have been placed centrally on the coverglasses. After incubation at room temperature for 30 min the slides are turned over and left for the indicator cells to detach from the glass and the non-reactive tissue.

Immediately after the sections had been read, those with positive reactions were fixed in Karnovsky's solution (17) and further processed for scanning electron microscopy (SEM) (12). The specimens were studied in a Philips SEM 500 microscope.

Enzyme histochemistry

Cryostat sections were incubated at 37°C for 2 h with α-naphthyl acetate esterase (ANAE) staining solution, in accordance with Müller et al. (18). After incubation the sections were washed in distilled water for 15 min, counterstained for 10–15 sec with 1% methyl green, and mounted in Depex mounting medium (Difco Laboratories, England). Monocytes and macrophages were identified by their diffuse cytoplasmic reddish-brown staining (19).

Results

Histological examination of hematoxylin- and eosin-stained sections showed a mucosa consisting of papillae and generally covered with a non-keratinized or occasionally parakeratinized epithelium with elongated rete pegs alternating with areas of epithelial atrophy. Most specimens showed a moderate to strong infiltration of mononuclear cells (Fig. 1). Morphologically, these cells were interpreted to be lymphocytes, plasma cells, and macrophages. The inflammatory cell infil-

Fig. 1. The tissue from hyperplastic denture stomatitis is characterized by elongated rete pegs alternating with areas of epithelial atrophy. An inflammatory cell infiltrate is located in the papillary part of the lamina propria. (Hematoxylin and eosin; bar = 400 μm.)

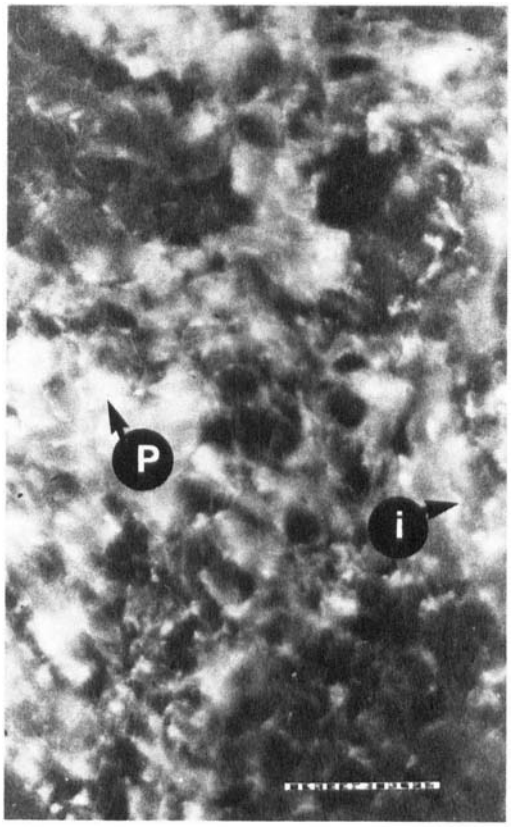

Fig. 2. With the immunofluorescence technique an abundance of IgG can be observed in plasma cells (P) and intercellularly (i) in the subepithelial area corresponding to the inflammatory cell infiltrate. (Bar = 50 μm.)

trate was located in the subepithelial loose connective tissue (papillary part of the lamina propria (20, 21)). Occasionally, the inflammatory cell infiltrate was intense, and the connective tissue was replaced by granulation tissue. Various numbers of inflammatory cells were present within the epithelium. Only a few scattered inflammatory cells were observed in the reticular part of the lamina propria (20).

Immunofluorescence

In all specimens an abundance of IgG was found in the subepithelial area corresponding to the inflammatory cell infiltrate. IgG appeared in plasma cells and intercellularly (Fig. 2); the latter was interpreted as IgG circulating in the tissue.

The amount and distribution of IgA varied considerably between the specimens. Ten specimens showed numerous IgA-containing plasma cells (Fig. 3), and five of these also showed extracellular IgA, located in the papillary part of lamina propria. Four specimens were negative for IgA.

Sections from eight subjects showed a moderate amount of extracellular IgM, most often located in close connection with vessel walls. Only a few IgM-containing plasma cells were observed.

Granular deposits of C3c were found in all specimens in the areas corresponding to the inflammatory cell infiltrate. The epithelium appeared negative in all but two cases, in

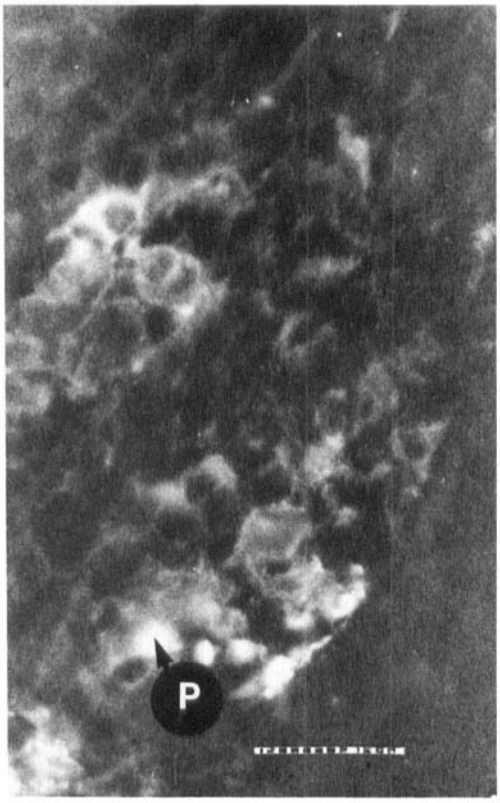

Fig. 3. In the papillary part of lamina propria IgA is seen mainly in plasma cells (P). (Bar = 50 μm.)

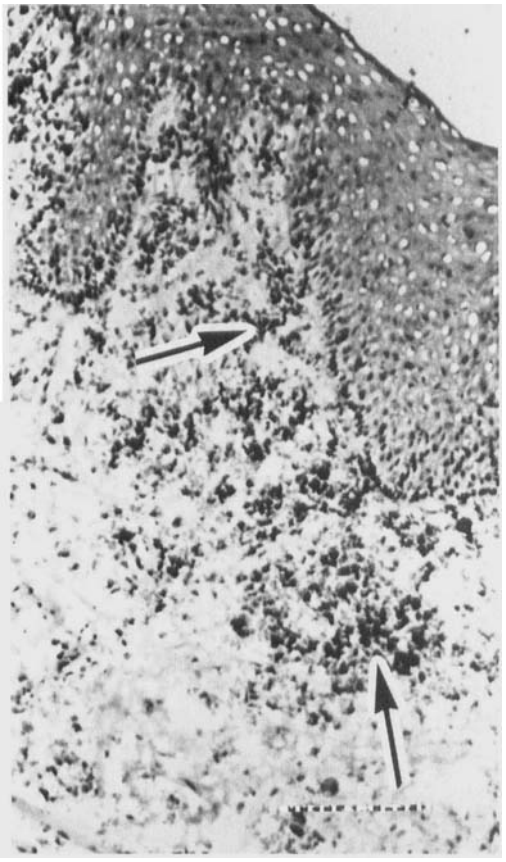

Fig. 4. Hemadsorption in a tissue section showing indicator cells binding to receptors for the Fc region of IgG (FcR) in the underlying tissue. FcR is confined to the papillary part of the lamina propria (arrows), but scattered clusters of indicator cells are also observed within the epithelium. (Bar = 400 μm.)

which the C3c showed granular deposits in the superficial part of the stratum spinosum.

The positive reactions for the antisera used were in all cases confined to the papillary part of lamina propria. Candida antigens could not be demonstrated with certainty in any sections.

Hemadsorption with tissue sections

The subepithelial area showed in all cases a strong hemadsorption reaction of EA (FcR-positive cells) appearing in clusters or scattered in the tissue (Fig. 4). The deeper part of the connective tissue (reticular part of the lamina propria) was most often negative. Small clusters of indicator cells were observed scattered in the epithelium, predominantly in the basal part of the stratum spinosum.

Moderate C3bR activity was found in the subepithelial area in six specimens, corresponding to the papillary part of the lamina propria (Fig. 5). The other subjects had weak or absent C3bR activity.

In all cases a moderate to strong reaction of AET-E (T lymphocytes) appeared in foci in the papillary part of the lamina propria corresponding to the inflammatory cell infiltrate. Clusters of AET-E were seen scattered throughout the epithelium. Binding of indicator cells was verified by means of the SEM (Figs. 6 and 7), showing pseudopodium-like projections between the indicator cells and the underlying tissue.

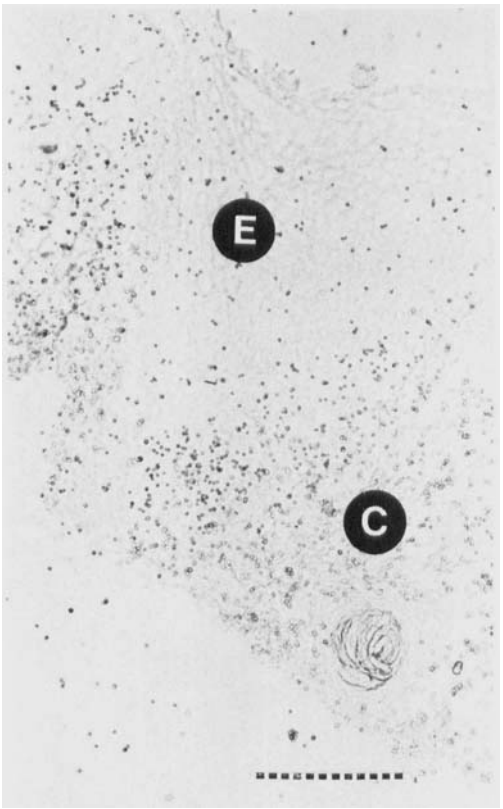

Fig. 5. Hemadsorption in a tissue section indicating C3bR activity. The indicator cells appear as small black dots in the epithelium (E) and papillary part of the lamina propria (C). (Bar = 400 μm.)

Control sections from spleen and placenta showed the distribution of FcR, C3bR, and T lymphocytes to be as previously described (11, 13, 15).

Enzyme histochemistry

A diffuse reddish-brown staining was observed in large irregular cells, especially in the upper part of the connective tissue papillae, close to the epithelium. These cells were interpreted to be macrophages (Figs. 8 and 9). T lymphocytes, which after optimal fixation show brown cytoplasmic dots (22), could not be identified with certainty, owing to the morphological limitations of the cryostat sections.

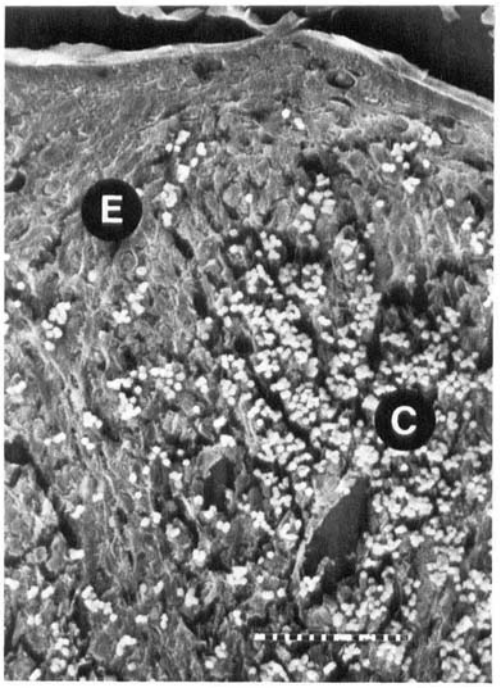

Fig. 6. Scanning electron micrograph showing adsorption of AET-E indicator cells to the papillary part of lamina propria (C). Clusters of indicator cells can be observed within the epithelium (E). (Bar = 200 μm.)

Discussion

In hyperplastic denture stomatitis the inflammatory reactions are confined to a narrow zone close to the epithelium, the papillary part of the lamina propria (23, 24). It is obvious that the inflammatory cell infiltrate does not extend underneath the well-vascularized barrier zone of the papillary part of the lamina propria.

The abundance of T lymphocytes in all cases, as shown by the AET-E hemadsorption technique, indicated that cellular immune reactions played a role, both by direct cytotoxic effector mechanisms of the T cells and by immune regulatory mechanisms of B lymphocytes (25).

FcR are present on macrophages and monocytes (26), subpopulations of T lymphocytes (27), B lymphocytes (28), and neutrophils (29). C3bR is a marker for macrophages, B lymphocytes, and granulocytes (30, 31). The FcR and C3bR activity in the

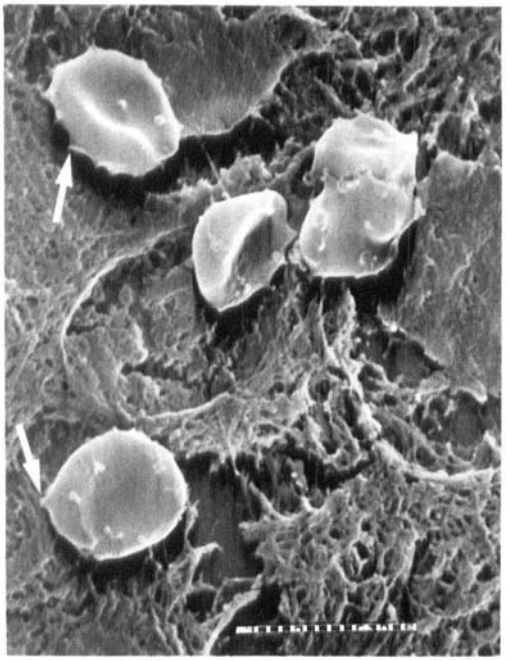

Fig. 7. Scanning electron micrograph showing adsorption of AET-E to cells within the epithelium. Note the pseudopodium-like projections (arrows) between the tissue and the indicator cells. (Bar = 10 μm.)

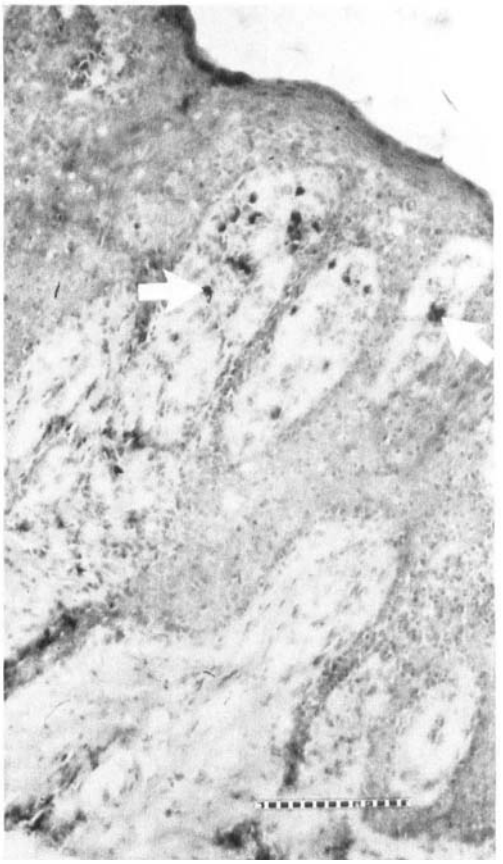

Fig. 8. α-Naphthyl acetate esterase (ANAE) staining reaction showing diffusely staining cells (arrows), interpreted as macrophages, in the superficial part of the connective tissue papillae. (Bar = 400 μm.)

papillary part of the lamina propria is most likely mainly due to macrophages or, to some extent, B lymphocytes or granulocytes. The presence of macrophages was also shown by the ANAE staining reaction. Macrophages are known to be essential in immune regulation and antigen presentation. Their ability to phagocytose may also explain their localization in the tissue of hyperplastic denture stomatitis.

With the various methods used in our study it was shown that both T lymphocytes and cells with FcR activity were present within the epithelium affected by denture stomatitis. Bergendal & Isacsson (24) have shown that there is a significant correlation between the amount of inflammatory cells within the epithelium and the intensity of the palatal erythema—that is, the clinical criteria of severity of the denture stomatitis. C3c and IgM observed in relation to vessel walls indicate that an immune-complex-mediated vasculitis may occur in the papillary part of the lamina propria.

We were not able to demonstrate candida antigens in the epithelium by the immunofluorescent technique. It is, however, likely that these antigens are present in the tissue in hyperplastic denture stomatitis and that they are an important factor in initiation and maintenance of the disease, as suggested by Budtz-Jörgensen et al. (4). It may be difficult to demonstrate a small amount of antigen by the methods used. In apical and marginal periodontitis many studies have been performed to characterize the effect of bacterial enzymes and toxins of the tissue (32–36). In these lesions it is generally accepted that bacterial invasion of the tissue may aggravate the existing disease. Thus,

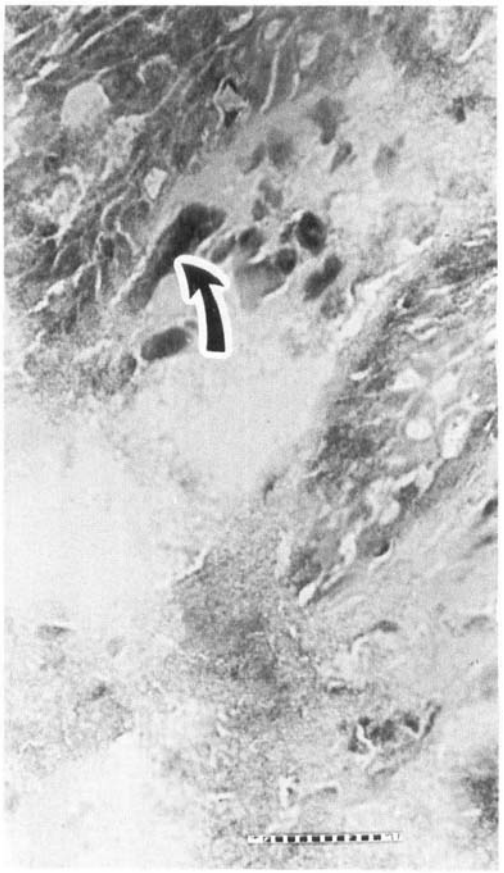

Fig. 9. Macrophages (arrow) in subepithelial connective tissue showing staining for ANAE. (Bar = 50 μm.)

microbial strategies for overcoming the phagocytic cell system and immune defences to enable invasion may be a critical aspect of the pathogenesis of apical and marginal periodontitis. A similar conclusion can be drawn about the pathogenesis of hyperplastic denture stomatitis.

In the present study we have shown that the inflammatory cell infiltrate of hyperplastic denture stomatitis contains various amounts of T lymphocytes, plasma cells, and macrophages. When the cellular content of denture-stomatitis-affected mucosa is compared with that characteristic for chronic marginal periodontitis (37) and chronic apical periodontitis (38, 39), it is obvious that there is a similarity between these lesions. This correspondence may reflect a comparable host response to noxious agents from the dental plaque in marginal periodontitis, from the necrotic pulp in apical periodontitis, or from the plaque on the denture base or interpapillary deposits in hyperplastic denture stomatitis. It is therefore reasonable to conclude that hyperplastic denture stomatitis is a complex inflammatory lesion showing elements of both humoral and cellular responses.

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