

Immunological and histological studies on denture stomatitis in children: a pilot study

Fumio Shimizu, DMD, PhD Keiko Shimizu, DDS
Hiroshi Itoh, MD Kikuo Kamiyama, DDS, DDSc

Abstract

The observation that denture stomatitis could be closely associated with infections by oral microbes, especially *Candida*, suggested that interferon (IFN) might be produced in patients with atrophic denture stomatitis. We measured the saliva level of IFN in 24 juvenile patients affected or not with denture stomatitis (erythema score 0-3) and 26 healthy children. No salivary IFN activity was found except in one patient with an erythema score of 3. Our data suggest that the presence of IFN in saliva might depend on the disease activity including the period and stage of its development. Immunocytochemical studies by an avidin-biotin-peroxidase complex method and hematoxylin-eosin (H&E) staining on affected palatal mucosa from patients with erythema score 2 and 3 showed a typical atrophic lesion which is usually seen in adult patients with denture stomatitis. In contrast to adult patients, however, leukocyte penetration was not observed in the lesion. This observation suggests that an immunological response to denture stomatitis in children might be somewhat different from that in adults.

Denture stomatitis (DS) is a very frequent complication of the wearing of removable dentures or space maintainers in children as well as adults (Budtz-Jørgensen et al. 1975). Several etiological factors such as microbes and allergic response to denture-base materials have been suggested.¹ Bergendal and Holmberg (1982) found an increased humoral antibody response against *Candida albicans* in adult patients with DS, suggesting that these patients are more susceptible to local contamination and infection with *C. albicans*. Recently, inflammatory infiltrate such as macrophages, granulocytes, and lymphocytes has been observed in the inflamed tissues from adult patients with DS (Johannessen et al. 1986). These data indicate that DS is a

complex inflammatory lesion where elements of both humoral and cellular immune responses are seen. We have recently found that there was a significant correlation between the quantitative increase in *Candida* and the severity of clinical condition of DS in children, suggesting that *Candida* infection can be one of the etiologic factors in DS in children.

It is well known that viral and bacterial infection and antigen-antibody complexes can cause the production of IFN which has many biological activities such as antiviral activity, immunoregulatory function (e.g., antibody production), and enhancement of cell functions (Hooks 1982). Therefore, it is possible that IFN can be produced in the patients with DS. To investigate this possibility, we measured the salivary level of IFN in children with DS. In addition, the affected palatal mucosa was studied immunocytochemically.

Materials and Methods

Normal Subjects

Twenty-six healthy nondenture wearers (8 male, 18 female) were recruited from dental outpatients who visited our hospital. The average age was six years, five months. Individuals with a medical condition or those taking drugs were excluded.

Subjects with Palatal Denture

Twenty-four subjects (11 male, 13 female) who wore complete palatal maxillary dentures as a space maintainer and who were diagnosed as having atrophic denture stomatitis were divided into four groups depending on their erythema scores (Table 1, next page) according to Bergendal and Isacson (1980). The same examiner determined the erythema score and recorded it with an oral photograph. The average age of the subjects was seven years, one month.

¹Van Reenen 1973; Budtz-Jørgensen et al. 1975; Arendorf and Walker 1979; Giunta et al. 1979; Holbrook and Russell 1979; Devlin and Watts 1984.

Salivary Samples

Each participant expectorated approximately 1 ml of mixed, whole unstimulated saliva into a sterile plastic tube. The saliva was centrifuged at 1300 G at room temperature to remove the aggregates, dialyzed in phosphate-buffered saline (PBS; pH 7.2) at 4° C, and reconstituted with serum-free Eagle® minimal essential medium (MEM) at 4° C. The samples then were passed through a millipore filter (0.2 µm), and kept at -20° C until assayed.

Interferon Assay

IFN activity in the saliva was determined by its ability to inhibit the cytopathic effect (CPE) of vesicular stomatitis virus (VSV) in the monolayer of a continuous line of human-amnion (WISH) cells or rat-derived lung cells as described previously (Hooks et al. 1979; Shimizu et al. 1985).

In brief, the monolayer of cells in 96 well plates was incubated with each of the diluted samples in a volume of 0.1 ml for approximately 20 hr at 37° C. After the removal of samples, the monolayer was washed with PBS, and then challenged with 50-100 plaque-forming units (PFU) of VSV. After 45 min, the virus inoculum was removed, the cells were washed with PBS, and refed with 0.1 ml of MEM containing 0.75% methylcellulose and 2% heat-inactivated fetal bovine serum. After 24 hr at 37° C, the cells were washed with PBS and stained with Giemsa's solution, and viral plaques were counted. The IFN titer was determined by the reciprocal of the highest dilution that reduced the VSV plaque counts by 50% and expressed as international units (IU)/ml. Saliva that contained antiviral activity greater than 4 IU/ml was considered positive.

Infectivity Assay

VSV propagated in WISH cells was assayed by counting PFU using WISH cells or rat-derived lung cells.

Biopsy Procedure

A tissue biopsy (3 x 3 mm) was taken under local anesthesia from a representative part of affected palatal mucosa. The tissue was immediately put into 10% glycerin-PBS solution and frozen in liquid nitrogen (-190° C) until examined. Consecutive cryostat sections of 10 µm were incubated with antibody for immunohistochemical studies.

Immunocytochemical Method

Avidin-biotin-peroxidase complex (ABC) method was accomplished using rabbit antihuman immunoglobulin A (IgA)^a and ABC kit^b as described by Marty et al. (1982). In brief, cryostat sections were overlaid with rabbit antihuman IgA serum at 4° C overnight, incu-

bated with biotinylated goat antirabbit IgG for 30 min, and then overlaid with ABC for 30 min at room temperature. Thereafter, the sections were incubated with 0.1% diaminobenzidine (DAB) for 10-20 min at room temperature. For background staining, the sections were stained with H&E. In preliminary experiments, optimal condition for ABC method was determined using sections of human liver. Lymphocyte-bearing immunoglobulin is stained brownish.

Results

IFN Activity in Saliva

Figure 1A shows that IFN activity was detected in the saliva from only one patient. This patient had an

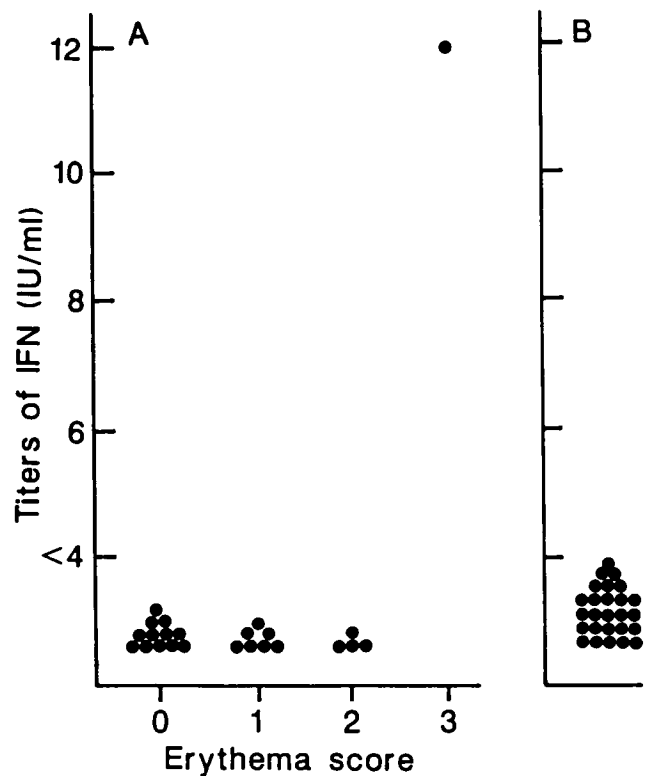


FIG 1. Study on salivary IFN activity. IFN activity in saliva from denture wearers with various erythema scores (A) and nondenture wearers (B). A saliva was considered positive if the IFN titer was greater than 4 IU/ml.

erythema score of 3 and the IFN titer was 12 IU/ml. This activity was confirmed as IFN by several criteria. First, since IFN is known to be species specific, the monolayer of rat-derived lung cells was incubated with the saliva from this patient. As shown in Table 2, the saliva, however, had no IFN activity in the rat-derived lung cells, indicating species specificity. Second, IFN is

^a Rabbit antihuman immunoglobulin A (IgA) — DAKO-immunoglobulin Ltd; Denmark.

^b ABC kit — Vector Lab; USA.

TABLE 1. Erythema Score

0 = Normal pink
1 = Slightly erythematous mucosa
2 = Moderately erythematous mucosa
3 = Pronouncedly erythematous mucosa

TABLE 2. Species Specificity of Antiviral Effect of Saliva*

IFN Activity in	
WISH cells (IU per ml)	Rat Lung Cells (IU per ml)
12	<3

* Obtained from the patient with erythema score 3.

known to be nondialyzable. In our experiments, the saliva from all subjects was dialyzed before the assay. Third, since IFN does not directly inactivate viruses, we determined whether or not the saliva inactivated VSV.

The saliva from the patient with erythema score of 3 was incubated with VSV for 45 min at 37° C and residual titers of VSV were determined. No decrease in the residual titer of VSV was obtained, indicating no direct effect of the saliva on the infectivity of VSV (Table 3).

We also studied the saliva from 26 normal subjects who had not worn a denture. No IFN activity was detected in that group (Fig 1B).

TABLE 3. Effect of Saliva on VSV Infectivity

Samples	Residual Titers (PFU/0.1 ml/Well)
VSV* + saliva†	45 ± 6‡
VSV + medium	50 ± 9

* VSV (approximately 130 PFU/0.1 ml) were incubated with an equal volume of saliva or medium for 45 min at 37°C. Residual titers of VSV then were assayed in rat lung cells in 96 well plates (N = 5).

† Obtained from the patient with erythema score 3.

‡ Standard deviation (SD).

Histological and Immunocytochemical Studies

The group with an erythema score of 0 showed a pale, healthy mucosa. An orthokeratotic epithelium with a distinct stratum granulosum was observed histologically. The rete pegs showed a regular wave pattern sharply separated from a dense collagenous lamina propria (Fig 2A). On the other hand, tissue sections from subjects with an erythema score of 3 showed a mucosa generally covered with a nonkeratinized epithelium with deeply penetrating rete pegs (Fig 2B). To quantify IgA-producing cells in mucosa, tissue sections from patients with erythema score of 3 were examined by the

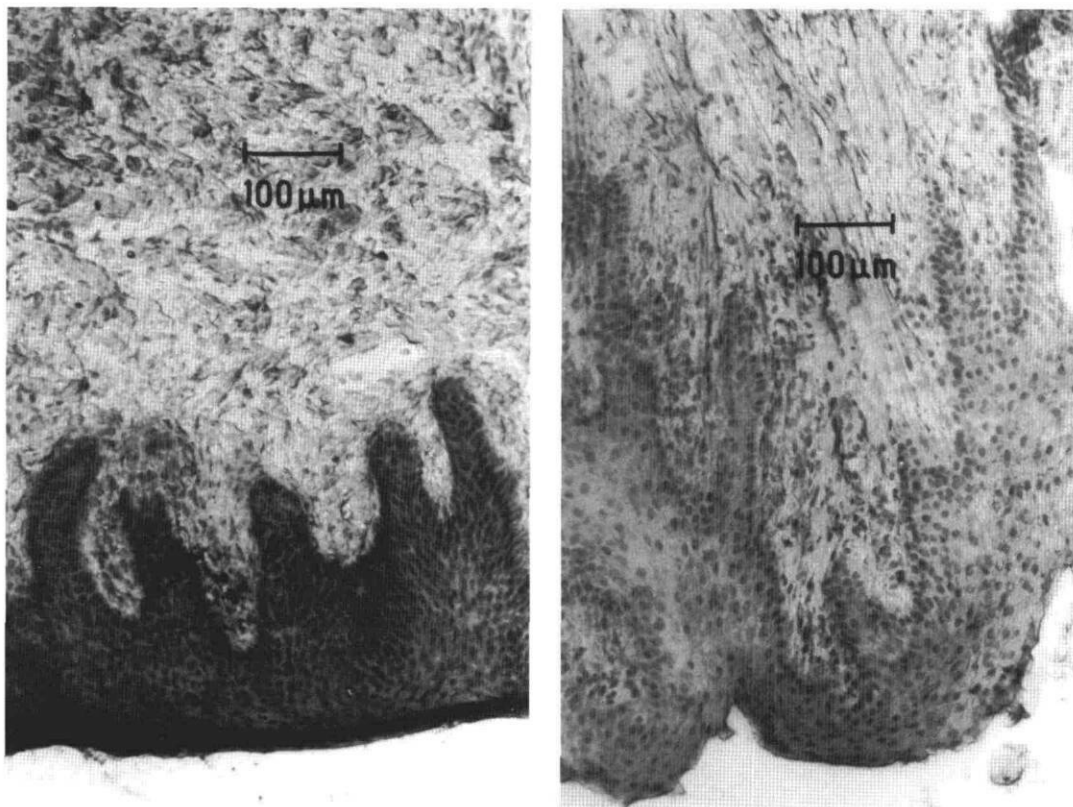


FIG 2. The palatal mucosa stained with the ABC method and H&E. Fig 2A (left): The palatal mucosa from a patient with an erythema score of 0 showing orthokeratosis, regular wave pattern of the rete pegs, and a dense lamina propria. Fig 2B (right): The palatal mucosa from a patient with an erythema score of 3 with a nonkeratinized epithelium with deeply penetrating rete pegs. Very little leukocyte penetration was observed in the epithelial and papillary layers of the lamina propria.

ABC method. Neither IgA-producing cells nor other leukocytes, however, were seen in the epithelial and the papillary layers of lamina propria (Fig 2B). Almost similar findings were observed in the tissue sections from patients with an erythema score of 2.

Discussion

IFN was first recognized as a soluble antiviral glycoprotein induced by viruses (Barron and Dianzani 1977). It is now recognized that under certain circumstances, IFN has immunoregulatory actions such as antibody production and cell-mediated immunity (Hooks 1982). Not only viruses but also bacterial products, polynucleotids, antigens, antigen-antibody complexes, and several other substances, under appropriate conditions, can induce cells to synthesize IFN (Hooks 1982). The observation that DS could be closely associated with infections by oral microbes, especially *Candida*,² suggested that IFN might be produced in patients with DS. In our studies, no IFN activity was found in saliva from patients except one. At least three possibilities may be suggested to explain this result.

First, IFN can be produced in patients with DS, but in most patients, it is diluted to undetectable level by saliva flow. Second, IFN detected in our patient might be induced by another factor. Third, the presence of IFN in saliva may depend on disease activity, and the period and stage of its development. Indeed, it was suggested that in acute aphthous or herpetic stomatitis, the presence of salivary IFN depends on the severity of the disease and the stage of its development (Melnichenko and Scherbakova 1971). It was also shown that IFN level in serum of patients with autoimmune diseases (e.g., systemic lupus erythematosus) fluctuates with the disease activity (Hooks et al. 1979).

When the denture of the patient with erythema score of 3 was treated with an antifungal agent and his erythema score reduced from 3 to 1, we were not able to detect IFN activity in his saliva (data not shown). So far, we do not know exactly what possibility is probable, but the third one is the most likely. To make this point clearer, more cases of DS with an erythema score of 3 need to be studied.

Since: (1) secretory IgA (sIgA) may act as an initial barrier in mucosa to the entry of invasive antigens such as viruses and bacteria (Waldman and Ganguly 1982), and (2) DS may be associated with *Candida* infection,³ it is possible that higher concentration of sIgA exists in saliva of patients with DS than in healthy subjects. Indeed, IgA and IgG in the total saliva were reported to be twice as high in adult patients with DS as in a control

group (Schröder and Maravíc 1980). It is known, however, that in children, concentration of sIgA in saliva greatly differs between individuals, ages of patients, and the time of day (Alaluusua 1983). Therefore, we tried to quantify the numbers of IgA-producing lymphocytes in the lesion.

Unexpectedly, leukocyte penetration was not observed in the lesion. This result is quite different from that of adult patients with DS; that is, in both cases of atrophic and hyperplastic stomatitis in adult patients, leukocyte penetration was observed in the epithelial and the papillary layers of lamina propria (Bergendal and Isacsson 1983; Johannessen et al. 1986). So far, we have no explanation for this difference, but it might be possible that immunological response against DS is different between children and adults.

Of particular clinical interest is the observation that in contrast with adult patients, children with DS usually have no complaint of pain, although the disease is severe.

Because IFN may be found in the saliva and the peripheral blood of the adult patients with an atrophic or hyperplastic denture stomatitis, further study is merited.

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Drs. Fumio and Keiko Shimizu are assistant professors and Dr. Kamiyama is a professor, pedodontics, and Dr. Itoh is a senior scientist, surgery, Tohoku University. Reprint requests should be sent to: Dr. Fumio Shimizu, Dept. of Pedodontics, Tohoku University School of Dentistry, Sendai 980, Japan.

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