

## Reduced Inhibition of *Candida albicans* Adhesion by Saliva from Patients Receiving Oral Cancer Therapy

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The effect of saliva on the adhesion of *Candida albicans* to epithelial cells was examined *in vitro* by using saliva from healthy controls and patients with oral squamous cell carcinoma. The adhesion of *C. albicans* to established epithelial tumor cells was reduced by 40% by salivary treatment of the *C. albicans* or epithelial cells. The inhibitory activity of saliva was almost completely abolished by anti-secretory immunoglobulin A antibody, concanavalin A, and mannose. Compared with saliva from healthy individuals, that from patients who had received chemoradiotherapy for oral carcinoma showed reduced suppression of *C. albicans* adhesion, which accompanied decreased salivary secretory immunoglobulin A and lactoferrin concentrations. A greater number of *C. albicans* cells adhered to buccal cells obtained from patients who had received chemoradiotherapy than to those from healthy individuals. Treatment of either epithelial cells or *C. albicans* with anticancer drugs induced an increase in adherence of epithelial cells and yeast cells. In contrast, concanavalin A- and mannose-pretreated *C. albicans* exhibited reduced adhesion to epithelial cells. No further decrease of *C. albicans* adhesion was observed when both epithelial cells and yeast phase *C. albicans* were treated with mannose. In conclusion, the inhibition of *C. albicans* adhesion by saliva depends largely on mannose residues on salivary glycoproteins and mannose is one of the binding ligands on both *C. albicans* and epithelial cells. In addition, anticancer therapy may induce oral *C. albicans* overgrowth by decreasing salivation and the concentrations of glycoproteins in saliva inhibiting *C. albicans* adhesion and by increasing the adhesive properties of both *C. albicans* and oral epithelial cells.

The first step in overgrowth of *Candida albicans* in the oral cavity is the adhesion of its yeast-form cells to the mucosa. Regulation of *C. albicans* adhesion to the oral mucosa appears to largely depend on saliva. The importance of saliva is well recognized when hyposalivation precedes mouth sores. The agents in saliva inhibiting *C. albicans* adhesion have been investigated widely. Secretory immunoglobulin A (sIgA) inhibits *C. albicans* adhesion to keratinocytes (54), and binding of the sugar residues in these glycoproteins to *C. albicans* has recently been demonstrated (8). Mannose and galactose have been identified as *C. albicans* binding ligands, although the latter is associated only weakly with binding (5, 9, 21, 25, 31, 34, 44). Mucins, especially those of lower molecular weight, also bind to *C. albicans*, and they promote yeast adhesion to polymethylmethacrylate (19, 30, 46).

Pseudohyphal forms of *C. albicans* bind to human iC3b (1, 12, 14, 16, 18, 20, 38). Although *C. albicans* complement receptor type 3 (CR3) and human CR3 differ in structure, these two receptors bind identical ligands (11, 12, 14, 16). On the other hand, fucoside-binding adhesin has been separated from yeast phase cells of *C. albicans* (50). However, the addition of L-fucose to assay mixtures caused only a slight inhibition of candida cell adhesion to epithelial cells, suggesting that the natural mucosal receptor is larger than an L-fucose residue or that a particular stereochemical configuration is necessary (8). In addition, fibrinogen- and laminin-binding proteins have been detected in cell wall extracts of *C. albicans* (4, 7, 27, 28). Multiple molecular binding mechanisms such as lectin-carbohydrate binding and also protein-protein binding appear to be collectively responsible for optimal *C. albicans* attachment to

human keratinocytes, depending on the strain and form of *C. albicans* (2, 6, 10, 22, 23, 26, 32, 52).

The pathogen in systemic candidiasis appears to be provided from the oral cavity in many cases. Overgrowth and dissemination of oral candida cells may result when the salivary action against growth and adhesion of candida cells is suppressed. In-depth investigation of the salivary action therefore appears important for prevention of oral candida cell dissemination. In this study, we examined the effects of saliva obtained from healthy individuals and that from patients with oral carcinoma on yeast adhesion.

### MATERIALS AND METHODS

**Salivary flow rate and viscosity.** Unstimulated mixed saliva was collected by placing cotton wool on the floor of the mouth about 5 min after washing of the mouth with sterile water. Ten minutes later, the cotton wool was removed and weighed. The unstimulated salivary flow rate (in milliliters per 10 min) was calculated by converting the weight (in milligrams) to volume (in milliliters). Salivary viscosity was measured at room temperature with a viscosity meter (Vismetron; Toshiba Co., Tokyo, Japan).

**Sampling of saliva.** Unstimulated mixed saliva was collected from 20 healthy individuals and 20 patients with oral squamous cell carcinoma by expectoration into sterilized tubes, and the saliva obtained was centrifuged (10,000 × g) at 4°C for 30 min. The supernatant was used in the experiments. The absence of candida cell or bacterial contamination in the supernatants was microscopically confirmed with periodic acid-Schiff (PAS) and Giemsa stains. The cancer patients were scheduled to receive inductive chemoradiotherapy consisting of 30 to 45 mg of peplomycin sulfate (PLM), 1,500 to 3,000 mg of 5-fluorouracil, and 20 to 30 Gy of irradiation with cobalt 60 (<sup>60</sup>Co). Saliva samples were obtained before the induction therapy, 2 weeks after the finish of chemoradiotherapy, and 3 months and 1 year after the start of therapy. The healthy saliva donors were age matched with the cancer patients.

**Cell cultures.** *C. albicans* (TIMM0134) was kindly supplied by the Department of Microbiology at Kochi Medical School, Kochi, Japan, and *C. albicans* KSC1, KSC2, and KSC3 were isolated from the oral cavity of a patient with candidiasis and classified serotype A according to the criteria of Fukazawa et al. (13). All strains were grown in Sabouraud's dextrose agar (Difco, Detroit, Mich.) at 37°C. The candida cells were then planted in the Sabouraud's broth medium and cultured for 24 h, and yeast-form cells in the exponential growth phase were used

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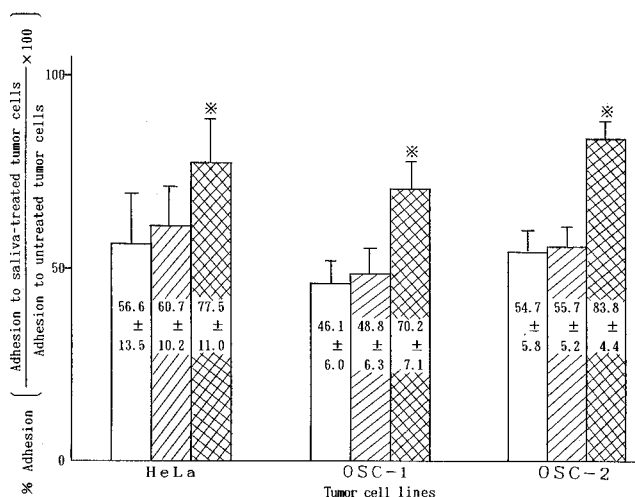


FIG. 1. Inhibitory effects of saliva on adhesion of *C. albicans* to tumor cells. *C. albicans* cells received no pretreatment or were pretreated with 50% saliva (diluted twofold with PBS) for 1 h at 37°C and were washed twice with PBS before being suspended in PBS. The suspended cells ( $10^5$  cells per ml) were poured into dishes with monolayers of tumor cells and were allowed to adhere to the tumor cells for 1 h. Tumor cells were treated with saliva from 10 healthy persons (□), saliva from 20 patients before therapy (▨), and saliva from the patients after chemoradiotherapy (▩). \*,  $P < 0.001$  (versus values for saliva from healthy persons and from patients before chemoradiotherapy; by *t* test).

in all experiments in order to promote plateau adhesion and avoid different adhesion and growth rates due to the plateau phase. The cells were washed by centrifugation and suspended in pyrogen-free saline. Viability was confirmed by planting serially diluted suspensions on Sabouraud's dextrose agar plates.

HeLa cells and other tumor cells, OSC-1 and OSC-2 cells, which had been

established from oral squamous cell carcinoma lines in our laboratory (39) were cultured in Dulbecco's modified minimum essential medium (Nissui, Tokyo, Japan) containing 10% fetal bovine serum and 1% glutamic acid. Confluent monolayered cells were trypsinized and washed in phosphate-buffered saline (PBS) before being adjusted to the desired concentrations for the adhesion assay.

After vigorous mouth washing with 3% amphotericin B, buccal epithelial cells were collected from healthy individuals and cancer patients by gently rubbing the mucosal surface with sterile swabs. The cells thus obtained were agitated in 10 ml of Hanks' balanced salt solution (HBSS) and collected by centrifugation. The collected epithelial cells were washed twice in PBS and resuspended in HBSS at appropriate concentrations.

**Adhesion assay.** The adhesion of *C. albicans* to the HeLa or tumor cell monolayer was assayed by the in vitro assay system described by Samaranyake and MacFarlane (43). Briefly, HeLa cells were suspended in Dulbecco's modified minimal essential medium ( $2 \times 10^5$  cells per ml) and 1 ml of the cell suspension was poured into each 35-mm-diameter dish (Corning Glass Works, Corning, N.Y.) containing a square glass coverslip (22 by 22 mm) on the bottom. To obtain a confluent monolayer, the dishes of cells were cultured in an incubator under 5%  $CO_2$  at 37°C for 3 days. The monolayers of HeLa cells on the coverslips were washed with PBS and either received no pretreatment or were pretreated with saliva, lectins, or other agents for 1 h at 37°C. After being washed with PBS, the monolayers of cells were incubated with  $10^5$  yeast phase *C. albicans* cells which either received no pretreatment or were pretreated with saliva, lectins, or other agents for 1 h at 37°C. The coverslips were then removed and washed in PBS for 45 s before being dried and stained with PAS. The yeast cells adhering to HeLa cells were counted microscopically in five high-power fields of different areas. The examination was performed in duplicate, and the average number of attached yeast cells per square millimeter of monolayered cells was calculated. In the above-described adhesion assay, the saliva was pretreated with concanavalin A (ConA), phytohemagglutinin, *Ulex europaeus* agglutinin-1, peanut agglutinin, wheat germ agglutinin, and monosaccharides such as mannose, fucose, galactose, and glucose as well as mannosidase. In some experiments, the saliva was pretreated with EDTA (10 mM) or DETAPAC (diethylenetriaminepentaacetic acid) (10 mM) and was heated at 100°C for 30 min and 5 and 10% high-molecular-weight dextran in PBS was used for confirming the influence of viscosity.

The level of adhesion of *C. albicans* to the buccal cells was assessed by the method of Sandin et al. (44). Briefly, 0.2 ml of a mixed suspension of buccal and yeast cells at a ratio of 1:5 was pipetted into tubes (12 by 75 mm) with a polycarbonate filter (12- $\mu$ m pore size; Nuclepore Corp., Pleasanton, Calif.) and

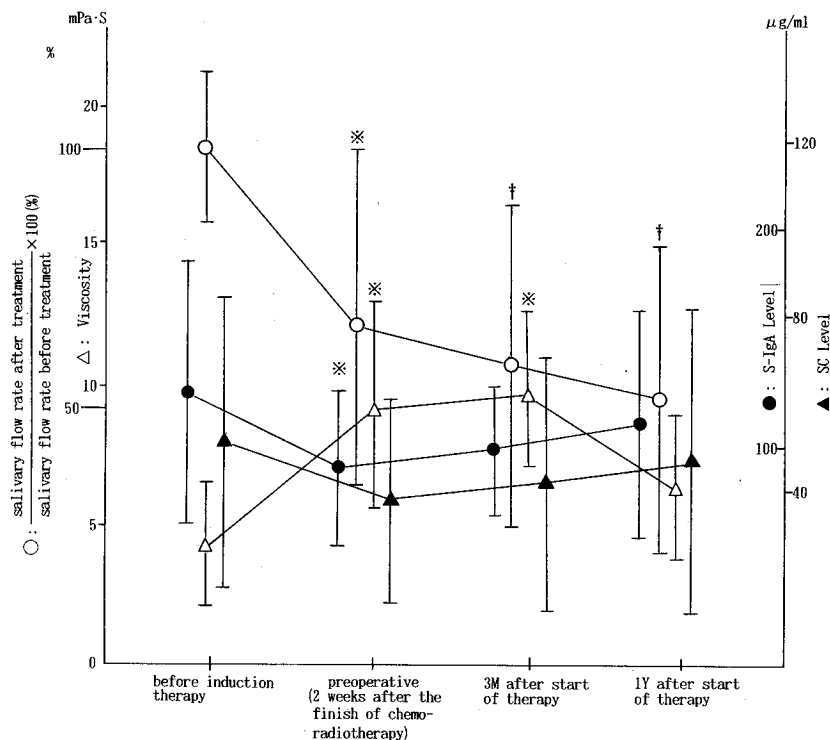


FIG. 2. Salivary flow rate, salivary viscosity, and sIgA and SC levels at various times before and during oral cancer therapy. Unstimulated whole-saliva samples were collected at the times indicated from 20 patients with oral carcinoma, and sIgA, SC, and viscosity were measured as described in Materials and Methods. \*,  $P < 0.05$ ; †,  $P < 0.01$  (versus values before therapy; by *t* test).

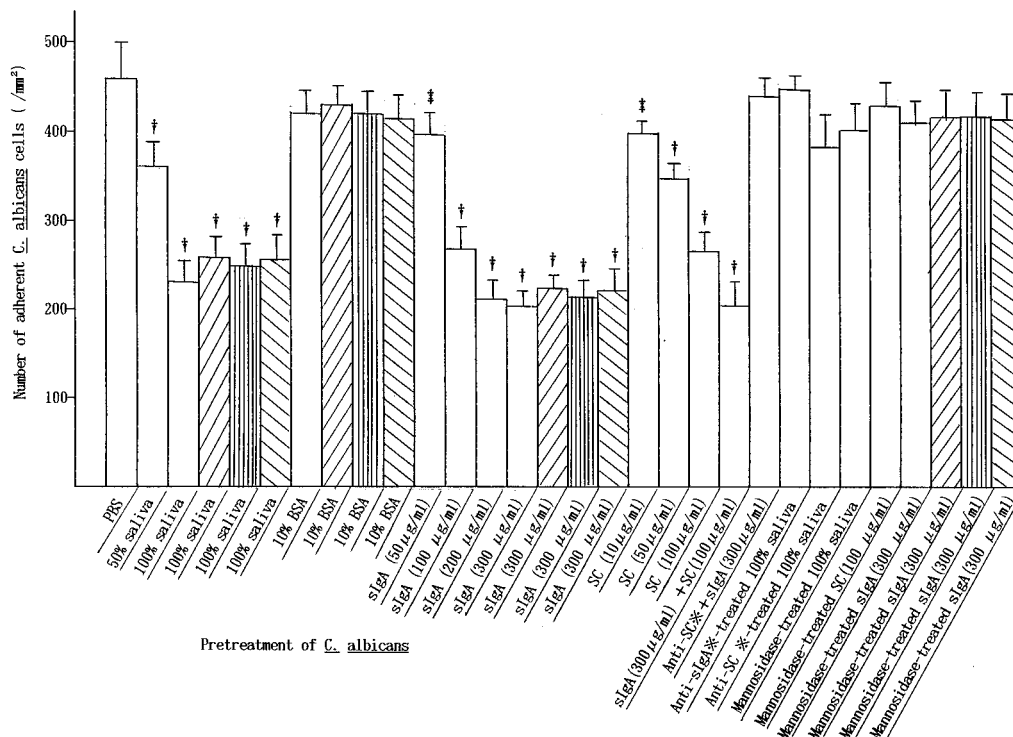


FIG. 3. Influences of saliva, sIgA, and SC on adhesion of *C. albicans* to HeLa cells. *C. albicans* cells were treated with each agent for 1 h at 37°C. After being washed twice, the cells were floated ( $10^5$  cells per ml) in PBS and 1.0 ml of the yeast cell suspension was added to each dish of HeLa cell monolayers. The cells in the dish were incubated for 1 h, and adherent *C. albicans* cells were counted microscopically in five high-power fields. Saliva samples were obtained from 10 healthy individuals, and the examination was performed in duplicate. □, TIMM0134; ▨, KSC1; ▤, KSC2; ▩, KSC3. \*, anti-SC antibody (200  $\mu$ g/ml) or anti-sIgA (300  $\mu$ g/ml). †,  $P < 0.001$ ; ‡,  $P < 0.01$  (versus values with PBS; by *t* test).

incubated with shaking at 180 rpm for 1 h at 37°C. The buccal cells and adherent yeast cells were collected on the filters and washed with 100 ml of PBS under continual agitation to remove nonadherent yeast cells. The filters were stained with PAS, and the number of yeast cells adhering to 200 buccal cells was microscopically determined.

**Measurement of salivary sIgA and SC.** Salivary sIgA was measured by an enzyme immunoassay using an sIgA test kit (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Briefly, anti-human secretory component (SC) antibody-bound polystyrene balls were mixed with 40-fold diluted saliva at 37°C for 1 h. After a washing with HBSS, the balls were treated with peroxidase-labeled anti-human IgA antibody at 20°C for 1 h. After another washing, *O*-phenylenediamine was added and allowed to react for 30 min and the reaction was stopped with 24% sulfuric acid. The production of 2,2'-diaminoazobenzol was measured at  $A_{492}$  with a Beckman Du-50 spectrophotometer. The sIgA level was calculated by comparison with the curve obtained from the standard samples.

The SC level in saliva was determined by a sandwich enzyme-linked immu-

(Jackson ImmunoResearch Laboratory, West Grove, Pa.) was added to each well. After incubation for 90 min at 37°C and a wash, a phosphatase substrate (Sigma, St. Louis, Mo.) was added and the color developed was read at 405 nm on an ELISA reader. The results were calculated from sigmoidal standard curves.

***C. albicans* growth inhibition.** *C. albicans* ( $10^4$  cells per ml) cells were suspended in HBSS containing 1% fetal bovine serum, and 50  $\mu$ l of the suspension was added to each microwell before saliva (50  $\mu$ l) was added. The microplates were incubated for 20 h at 37°C, and the medium was discarded prior to the addition of 50  $\mu$ l of [ $^3$ H]glucose [10  $\mu$ Ci/ml; Glucose D-(5,6- $^3$ H); New England Nuclear, Boston, Mass.] to each well. After an additional incubation for 3 h in a 5% CO<sub>2</sub> incubator at 37°C, 50  $\mu$ l of 5.25% sodium hypochlorite was added to each well and the proliferating fungi that had incorporated the radiolabeled glucose were transferred and harvested onto glass fiber filters after being washed with distilled water to remove any nonincorporated isotope. The triplicate results were averaged, and the standard errors were found to be within 5% of the mean value. The percent growth inhibition of *C. albicans* was calculated as follows:

$$\% \text{ growth inhibition} = \frac{\text{cpm of } C. \text{ albicans in control medium} - \text{cpm of } C. \text{ albicans in saliva}}{\text{cpm of } C. \text{ albicans in control medium}} \times 100$$

nosorbent assay (ELISA) (53). Briefly, microwells were coated with 10  $\mu$ g of anti-SC antibody (ICN, Costa Mesa, Calif.) per ml and left overnight at 4°C. After blocking of nonspecific reactions with PBS containing 0.5% bovine serum albumin (BSA), samples and serially diluted standard SC were added to the wells and allowed to react with the antibody for 2 h at room temperature. After three washes, peroxidase-conjugated anti-SC antibody was added to each well and incubated for 2 h at room temperature. After the washes, *O*-phenylenediamine was added and the developed color was read at 492 nm on an ELISA reader.

**Titration of lactoferrin in saliva.** The lactoferrin level in saliva was also determined by sandwich ELISA (40). Briefly, microwells were coated overnight with rabbit anti-human lactoferrin serum diluted to 400-fold with bicarbonate buffer (pH 9.6). After blocking of nonspecific reactions with PBS containing 0.5% BSA, the samples and serially diluted standard lactoferrin were poured into the microwells and left overnight at 4°C. After a thorough washing, alkaline phosphatase-conjugated, affinity-purified rabbit anti-human lactoferrin antibody

## RESULTS

The adhesion of *C. albicans* organisms to epithelial tumor cells was inhibited by saliva obtained from the healthy individuals to less than 60% of the adhesion level of the control without saliva (Fig. 1). The saliva obtained from the oral cancer patients before treatment showed only a slightly lower level of suppression of *C. albicans* adhesion than saliva from healthy individuals. However, the saliva obtained from the patients after induction therapy showed statistically significantly reduced suppression of cellular adhesion of *C. albicans* to the monolayers of cells of all tumor cell lines examined.

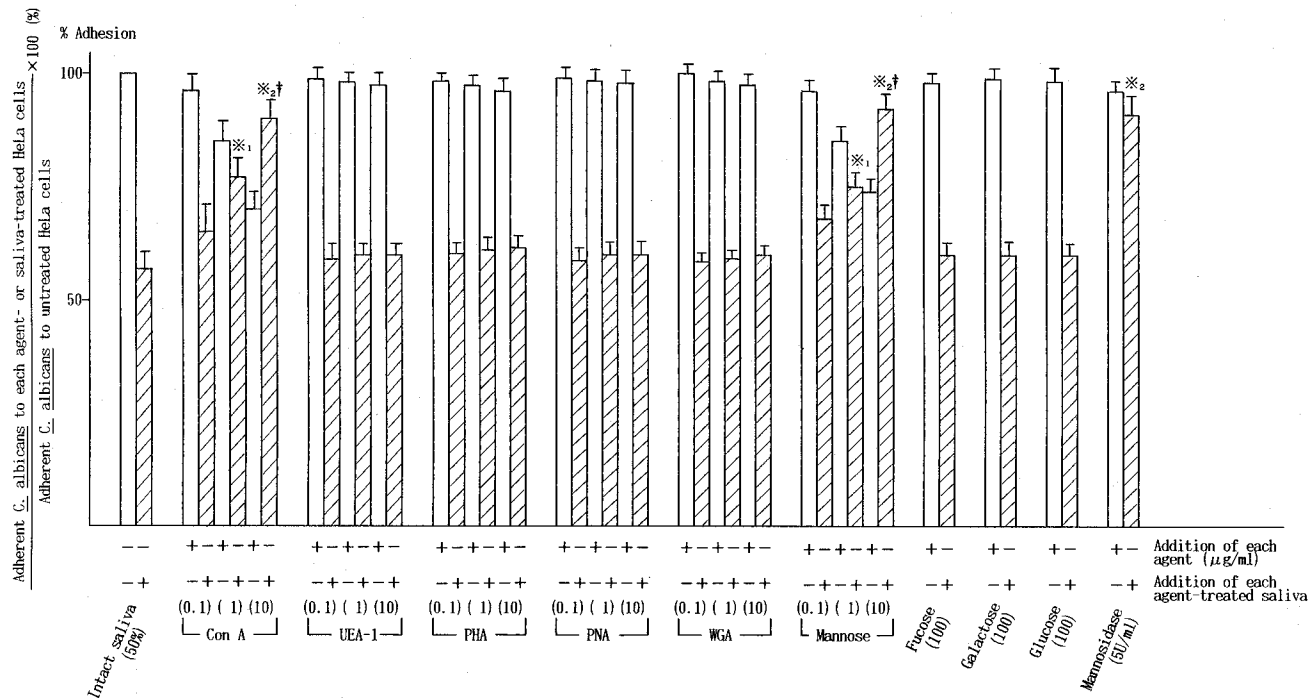


FIG. 4. Influences of lectins and sugars on adhesion of *C. albicans*. Monolayers of HeLa cells were treated for 1 h each with lectin, monosaccharide, and mannosidase or with aliquots of saliva pretreated with each agent, from eight healthy individuals. The dishes were washed twice, and 1.0 ml of *C. albicans* suspension ( $10^5$  cells per ml) was added to each HeLa cell monolayer dish. After being cultured for 1 h, adherent *C. albicans* cells were counted microscopically. The final concentration of saliva was 50%. \*,  $P < 0.01$ ; \*\*,  $P < 0.001$  (versus values for intact 50% saliva). †,  $P < 0.05$  (versus values for ConA at 1  $\mu\text{g}/\text{ml}$  and mannose at 1  $\mu\text{g}/\text{ml}$ ; by  $t$  test). UEA-1, *Ulex europaeus* agglutinin-1; PHA, phytohemagglutinin; PNA, peanut agglutinin; WGA, wheat germ agglutinin.

Salivary secretion was greatly suppressed by cancer therapy, and the viscosity of saliva was increased after chemoradiotherapy (Fig. 2). The flow rate of unstimulated saliva was greatly decreased following chemoradiotherapy. Following inductive chemoradiotherapy, simultaneous decreases of sIgA and SC levels in saliva were also observed, although the SC level was not greatly decreased.

sIgA inhibited *C. albicans* adhesion in a dose-dependent manner; however, BSA did not suppress adhesion of any candida cells (Fig. 3). Treatment of *C. albicans* cells with 300  $\mu\text{g}$  of sIgA per ml resulted in a decrease in the number of adherent cells to less than half that in the control. SC also dose dependently inhibited adhesion of *C. albicans* to HeLa cells, and inhibition of the adhesion to slightly above 60% of the control level was observed with 100  $\mu\text{g}$  of SC per ml. When 100  $\mu\text{g}$  of SC per ml was added to the coculture system containing 300  $\mu\text{g}$  of sIgA per ml, no further additive inhibition of *C. albicans* adhesion was observed. Similar levels of inhibition of adhesion by saliva in all candida cell strains were observed. When saliva was pretreated with anti-sIgA or anti-SC antibody, its inhibitory action on *C. albicans* adhesion was almost completely abrogated. In addition, these inhibitory activities of saliva, sIgA, and SC were almost completely eliminated by mannosidase.

ConA reduced the inhibitory activity of saliva on *C. albicans* adhesion dose dependently, *Ulex europaeus* agglutinin-1, phytohemagglutinin, peanut agglutinin, or wheat germ agglutinin negligibly affected the inhibitory activity of saliva (Fig. 4). When saliva was treated with mannose, fucose, galactose, or glucose, only mannose showed almost complete abolishment of salivary inhibitory activity, while the other sugars had no effect on this activity. Similarly, ConA- and mannose-treated *C. albicans*

showed reduced adhesion to HeLa cells but no effect of other lectins or monosaccharides on adhesion was observed (data not shown).

Inhibition of candida cell adhesion by saliva was not reduced by chelators; however, boiling of the saliva decreased its inhibitory action slightly (Fig. 5). When candida cells were cultured with EDTA (10 mM)-pretreated or DETAPAC (10 mM)-pretreated saliva, their adhesion to HeLa cells was suppressed to nearly the same degree as those cultured with intact saliva. However, 5 or 10% dextran intended to increase viscosity did not influence candida cell adhesion. The treatment of HeLa cells with dextran or each type of pretreated saliva resulted in almost the same level of adhesion as observed for the treatment of candida cells.

Treatment of HeLa cells with sIgA (200  $\mu\text{g}/\text{ml}$ ) or saliva from healthy individuals reduced *C. albicans* adhesion to the cells to about 40 to 50% of the control level (Fig. 6). In addition, pretreatment of HeLa cells with SC (100  $\mu\text{g}/\text{ml}$ ) decreased the level of *C. albicans* adhesion to about 60% of the control level. When both HeLa cells and *C. albicans* were treated with saliva, sIgA, or SC, the degree of inhibition of *C. albicans* adhesion was similar to that observed when either HeLa cells or *C. albicans* cells were treated (Table 1).

Pretreatment of *C. albicans* with anti-CD11b antibody suppressed *C. albicans* adhesion to one-third of that with untreated *C. albicans* (Table 2). The degree of adhesion inhibition of *C. albicans* by ConA was less marked than that by anti-CD11b, and no additional inhibition was observed when combined pretreatment with ConA and anti-CD11b antibody was used.

The buccal cells obtained from the cancer patients after chemoradiotherapy showed higher levels of adhesion of *C.*

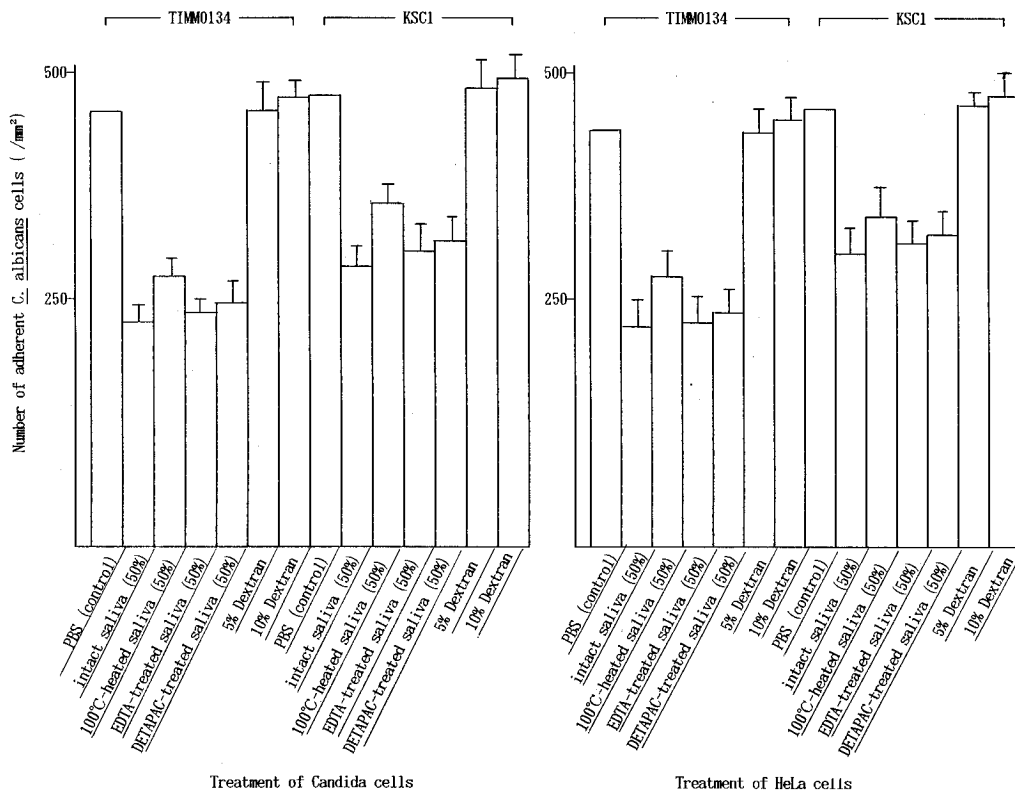


FIG. 5. Influences of dextran and chelators on inhibitory activity of saliva against candida cell adhesion. Candida cells or HeLa cells were treated with 5 and 10% dextran or saliva which was pretreated with each chelator, and both types of cells were cocultured for 1 h at 37°C before adherent candida cells were counted microscopically.

*albicans* than those from healthy individuals (Fig. 7). More than 10 organisms of *C. albicans* adhered to each buccal cell obtained from the patients after treatment, while fewer than 8 cells of *C. albicans* adhered to each buccal cell from the healthy controls and the patients before treatment.

Adhesion of *C. albicans* to epithelial cells was found to be enhanced by pretreatment with PLM or <sup>137</sup>Cs (Fig. 8). The levels of adhesion of *C. albicans* cells to PLM (50 µg/ml)-treated HeLa cells, OSC-1 cells, and OSC-2 cells were about 120, 130, and 160%, respectively, of the level of adhesion to untreated cells, while the *C. albicans* adhesion was slightly increased in PLM-treated buccal cells. The magnitude of the increase of *C. albicans* adhesion to irradiated cells was almost the same as that for PLM-treated cells.

Pretreatment of *C. albicans* with the anticancer agents resulted in increased adhesion to HeLa cells or buccal cells from healthy individuals, and the increased adhesion was dependent on the doses of these agents (data not shown). Likewise, HeLa cell treatment with the anticancer agents increased candida cell adhesion in a dose-dependent manner.

The data shown in Fig. 9 indicate that cell growth of *C. albicans* was inhibited by saliva and the inhibition was significantly correlated with the concentration of lactoferrin ( $Y = 0.224X + 0.56$ ;  $\gamma = 0.873$ ;  $R^2 = 0.761$ ; and  $P < 0.0001$ , where  $X$  is lactoferrin concentration and  $Y$  is percent *C. albicans* growth inhibition). Compared with the saliva samples from healthy controls, those from the patients who had received chemoradiotherapy showed lower lactoferrin concentrations; the mean lactoferrin levels  $\pm$  standard errors were  $28.1 \pm 16.9$  µg/ml in the former and  $15.4 \pm 12.1$  µg/ml in the latter. The growth inhibition of *C. albicans* by the patient saliva was markedly weaker than that by control saliva.

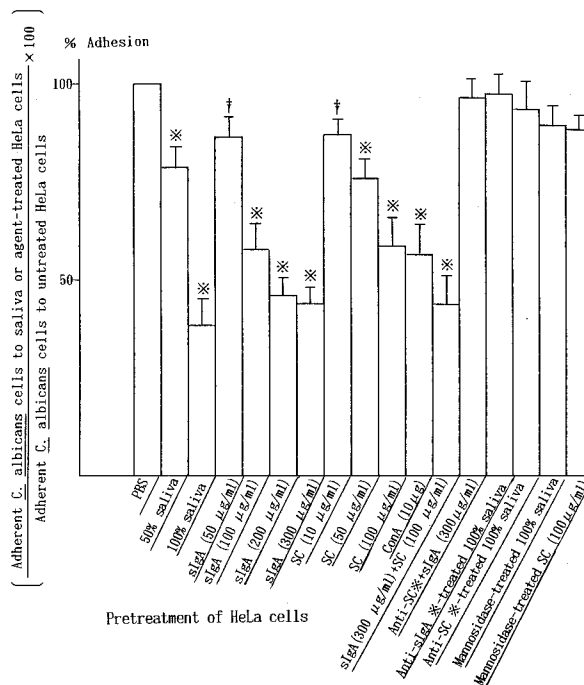


FIG. 6. Influences of HeLa cell treatment with saliva, mannosidase, sIgA, SC, or their antibodies on adhesion of *C. albicans*. Monolayers of HeLa cells were cultured in PBS containing each agent indicated for 1 h. After being washed twice with PBS, they were cocultured with a high concentration of yeast cells. After incubation for 1 h and two washes, the adhesion of *C. albicans* was observed microscopically in five high-power fields. Saliva samples were obtained from 10 healthy individuals. The means  $\pm$  standard deviations for adherent *C. albicans* cells are indicated. \*,  $P < 0.001$ ; †,  $P < 0.01$  (versus values with PBS; by  $t$  test).

TABLE 1. Influence of sIgA, SC, mannose, ConA and saliva on adhesion of *C. albicans* to HeLa cells

Treatment of cells <sup>a</sup>		Inhibition (%) after treatment with:				
HeLa	<i>C. albicans</i>	sIgA (100 µg/ml)	SC (100 µg/ml)	Mannose (100 µg/ml)	ConA (10 µg/ml)	Intact saliva <sup>b</sup>
-	-	100	100	100	100	100
-	+	45.7	58.7	59.8	62.4	57.1
+	-	72.0	78.1	75.7	70.6	61.4
+	+	46.7	57.5	56.3	60.4	55.7

<sup>a</sup> Monolayers of HeLa cells or *C. albicans* cells were left untreated (-) or treated (+) with the indicated agent for 1 h at 37°C and were mixed together after being washed with PBS. The adhesion of *C. albicans* was assayed by the method described in Materials and Methods. The examination was performed in triplicate. The mean control levels of adhesion (number of adherent *C. albicans* cells) ± standard deviations were 438.3 ± 18.0 for sIgA and SC, 450.3 ± 23.5 for mannose and ConA, and 467.3 ± 16.9 for intact saliva.

<sup>b</sup> Obtained from four individuals.

DISCUSSION

The incidence of fungal infections, especially those caused by *C. albicans*, has been increasing with the prevalence of organ transplantation and immunosuppressive virus infection (3, 17, 33, 42). The oral cavity is one of the most common habitats of *C. albicans*, and oral candida species frequently become opportunistic pathogens in immunocompromised patients. The first step in oral candidiasis is the enhanced adhesion of the cells to the mucosal surfaces. Their adhesion to the oral mucosa is regulated in a complex fashion by numerous factors. It is well known that saliva is the major agent suppressing *C. albicans* adhesion in the oral cavity. However, the inhibitory action has not yet been sufficiently clarified.

Adhesion of *C. albicans* was suppressed by saliva from healthy persons, putatively with mediation by mannose residues (5, 9, 21, 34, 44). In other words, mannose appears to be a major ligand for binding to the mucosal surfaces. Salivary sIgA has also been found to inhibit adhesion of *C. albicans* to mucosa (54). However, the binding ligand in sIgA remains to be clarified. The carbohydrate portion constitutes 10 to 20% of the molecular weight of sIgA and SC (48, 49). The sugar chains of SC and the J chain possess an extraordinarily high multiplicity; α- or β-linked mannose residues may be important (24, 35, 37).

Salivary inhibition of *C. albicans* adhesion was negligibly affected by pretreatment of saliva with *Ulex europaeus* agglutinin-1, phytohemagglutinin, peanut agglutinin, wheat germ agglutinin, or chelators but was almost completely abrogated by ConA pretreatment. Likewise, ConA-treated SC did not suppress the adhesion of *C. albicans*. In addition, mannose-treated *C. albicans* and epithelial cell adhesion levels were reduced, while dextran did not influence candida cell adhesion. After

treatment with mannose of either *C. albicans* or epithelial cells, the inhibition of yeast-epithelial cell adhesion by saliva was minimal. Furthermore, mannosidase-treated saliva did not inhibit *C. albicans* adhesion. These results appear to indicate that mannose residues bind to their ligands, which have not however been identified, on both *C. albicans* and epithelial cells. Judging from the fact that mannosidase-treated SC did not possess any adhesion-inhibitory activity, it appears likely that SC inhibits candida-epithelial cell adhesion via competitive binding to these cells.

The binding of *C. albicans* with epithelial cells via CD11b has been explored previously (15). Anti-CD11b antibody showed greater suppression of *C. albicans* adhesion to tumor cells and healthy keratinocytes than did ConA. When both agents were added together to the coculture medium, however, further inhibition was not observed. Therefore, mannose appears to be one of the binding sites of CD11b.

In cancer patients, especially those with oral malignancies, overgrowth of oral *C. albicans* occurs frequently. Systemic candidiasis is understood to be induced by cellular immunosuppression, especially the hypofunction of phagocytic cells (45, 47). Even in the oral cavity, phagocytic cells appear responsible for yeast growth control. We have reported hypofunction of salivary polymorphonuclear leukocytes which had been induced by therapy for oral cancers (51). In addition to the

TABLE 2. Influence of ConA and anti-CD11b on adhesion of *C. albicans* to epithelial cells<sup>a</sup>

Pretreatment of cells		<i>C. albicans</i> adhesion to:			
ConA	Anti-CD11b	HeLa	OSC-1	OSC-2	Buccal cells
-	-	124.0 ± 13.0	86.0 ± 7.9	97.4 ± 9.6	45.8 ± 11.3
+	-	53.0 ± 14.0	48.3 ± 18.0	52.1 ± 15.4	28.2 ± 4.3
-	+	38.7 ± 11.0	33.7 ± 11.5	35.8 ± 13.2	15.8 ± 4.3
+	+	38.3 ± 10.1	30.0 ± 5.7	31.4 ± 12.9	13.8 ± 4.0

<sup>a</sup> *C. albicans* cells were left untreated (-) or were pretreated (+) with 10 µg of ConA and/or anti-CD11b antibody per ml for 1 h, and the number of *C. albicans* cells adherent to the line of epithelial cells indicated was assayed microscopically. Each examination was performed in triplicate, and each value indicates the mean number of adherent *C. albicans* cells per square millimeter ± the standard deviation.

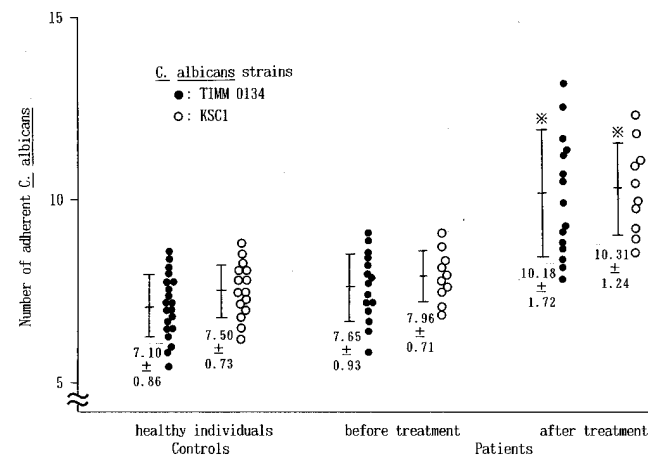


FIG. 7. Increased adhesion of *C. albicans* to buccal cells obtained from oral cancer patients who received chemoradiotherapy. Buccal cells obtained from healthy individuals and oral cancer patients before and after chemoradiotherapy were cocultured with *C. albicans* cells for 1 h, and adherent yeast cells were counted microscopically to determine the number of *C. albicans* cells per buccal cell. \*, *P* < 0.001 (versus values for healthy controls and patients before treatment; by *t* test).

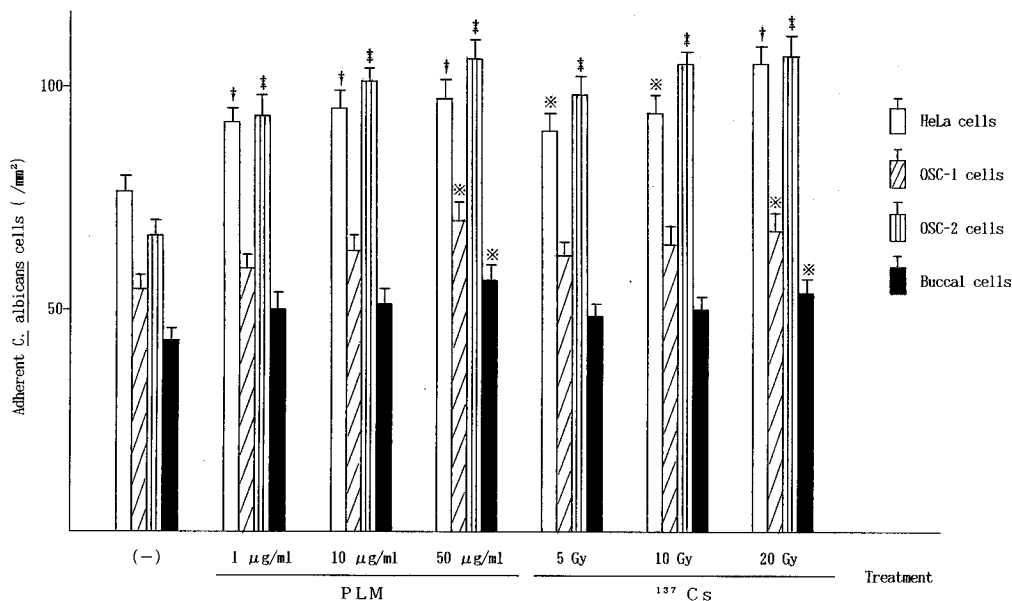


FIG. 8. Influences of treatment of epithelial cells with PLM or  $^{137}\text{Cs}$  on adherence of *C. albicans*. The three tumor cell lines indicated and the buccal cells from 10 healthy individuals were cultured in medium containing the indicated concentrations of PLM for 48 h or irradiated with  $^{137}\text{Cs}$  at the indicated doses. After the treatment, the epithelial cells were cocultured with *C. albicans* for 1 h, and adherent yeast cells were counted microscopically. Each bar indicates the mean  $\pm$  standard deviation of duplicates. \*,  $P < 0.05$ ; †,  $P < 0.01$ ; ‡,  $P < 0.001$  (versus values for *C. albicans* cells adhering to untreated epithelial cells; by *t* test).

cellular hypofunction, a reduction of the hormonal factors was ascertained in the present study. The decrease of *C. albicans* adhesion-inhibitory activity of saliva after chemoradiotherapy seems to partially depend on lactoferrin because this glycoprotein possesses mannose residues (29).

Anticancer agents dose dependently increased adhesion between HeLa cells and *C. albicans*. Judging from the results in the present study, it is likely that the levels of expression of mannose residues on both the epithelial cells and *C. albicans* are increased by treatment with anticancer agents. Interestingly,  $^{137}\text{Cs}$ -irradiated *C. albicans* adhesion to epithelial cells did not increase, while irradiation of epithelial cells caused increased *C. albicans* adhesion. Compared with the radiation

dose needed for epithelial cell death, the lethal dose for candida cells is extremely high (41). The different effects of  $^{137}\text{Cs}$  on the two types of cells may depend on the difference in cell wall stability, although this issue remains to be clarified.

For candida cell growth-inhibiting factors in the oral cavity, lactoferrin appears to play a critical role. This iron-binding protein is thought to inhibit candida cell growth by taking iron which is necessary for candida cell proliferation (36). The high correlation between the lactoferrin level in saliva and candida cell growth inhibition observed in this study suggests that the significant decrease of salivary lactoferrin levels during cancer therapy is a serious risk factor which allows overgrowth of oral candida cells. The decreased levels of the anti-candida molecules in saliva as well as the decrease in secretion of saliva itself should be taken into consideration in efforts to prevent candida cell overgrowth in cancer patients.

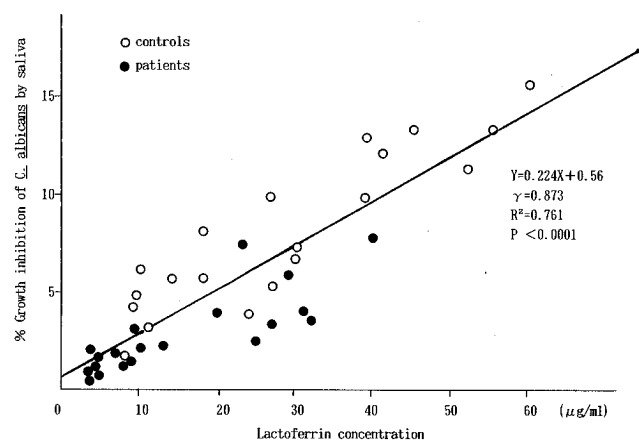


FIG. 9. Correlation between salivary lactoferrin level and *C. albicans* growth inhibition by saliva. *C. albicans* (TIMM0134) was treated with 50% saliva from healthy donors (controls) or oral cancer patients who received chemoradiotherapy. After 20 h, the cells were washed and were then incubated in the presence of  $^3\text{H}$ -labeled glucose for 3 h and the isotope incorporated was measured. The lactoferrin concentration in saliva was measured by ELISA.

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