Dextran-Induced Inflammation and Its Effect on Keratinized Gingival Epithelium in Monkeys*

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THE CELL POPULATION PRESENT during dextran-induced inflammation and its effect upon induced keratinization of the sulcular epithelium was investigated in two young adult male Rhesus monkeys. Keratinization of the sulcus epithelium was induced by a combined regimen of scaling, an intravenous injection of achromycin and daily rubber cup prophylaxes. After keratinization was confirmed by means of biopsies, inflammation was induced either by injecting 200 μl of a 5% dextran saline solution or by applying the solution topically on the marginal gingiva for 2 weeks. Clinical grade dextran, molecular weight 70,000, was used. Physiologic saline solution, either injected or topical, was also used. At the same time, the daily prophylaxes were continued. After the 2 weeks, gingival biopsies were taken from each tooth treated with the different regimens. One-half of each biopsy was routinely processed and stained with hematoxylin and eosin or Rhodamine B, while the other half was processed for and stained with alcoholic and aqueous PAS to detect dextran in tissues. Histologic evaluation was carried out in three areas: a crestal zone, a cervical zone and an oral gingival zone. An Inflammatory Index (II) was determined and the width and length of keratin were measured. Dextran, either topical or injected, produced mainly a chronic inflammatory response characterized by lymphocytes (30–35%), monocytes-macrophages (5–10%), plasma cells (10%), polymorphonuclear leukocytes (PMNs) (15%) and unidentified cells (35%). Conversely, the physiologic saline-induced inflammation showed PMNs (75%), lymphocytes (5%) and unidentified cells (20%). The II for injected areas was significantly higher than for those topically treated or for nontreated controls. However, the increased II did not affect the degree of keratinization achieved. The results indicate that chronic inflammation may not necessarily affect tissue keratinization if thorough removal of bacterial plaque is well maintained.

Several recent studies from this laboratory have dealt with keratinization of the sulcular epithelium in Rhesus monkeys.[1-8] One of these studies,[6] designed to evaluate the effect of mechanical stimulation on this epithelium, demonstrated that: (1) systemic antibiotics and local prophylaxes, individually or in combination, were effective in reducing inflammation within the gingival tissues; (2) the reduction in inflammation obtained by either method allowed sulcular keratinization to develop; (3) sulcular keratinization was significantly increased by performing daily subgingival prophylaxes and (4) mechanical stimulation of the sulcular epithelium seemed to promote keratinization. Subsequently, dextran penetration through nonkeratinized and keratinized epithelium was evaluated.[8] This study showed that induced keratinized sulcular epithelium as well as normally keratinized oral gingival epithelium resisted penetration of dextran, but intact nonkeratinized sulcular epithelium apparently lacked a surface layer resistant to penetration. Although not reported, the study also showed that both topically applied and injected dextrans produced inflammation of the gingival tissues. The present study was undertaken to evaluate the cell population in dextran-induced inflammation, and to investigate the extent to which this inflammation modifies the keratinization of the sulcular epithelium induced by daily prophylaxes in monkeys.

MATERIALS AND METHODS

Two young adult male Rhesus monkeys were used. They had a full complement of permanent teeth with

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the third molars erupted, but not yet in occlusion. One week before the experiment, the monkeys were anesthetized with Ketalar* (ketamine hydrochloride) intramuscularly 10 mg/kg body weight, and their teeth were scaled with hand instruments and polished with a rubber cup using Nupro† prophylactic paste. During this preexperimental week, the monkeys also received a daily intravenous (IV) injection of Achromycin‡ (tetracycline hydrochloride) at the maximum safe dosage of 20 mg/kg body weight.

The dental arches of both monkeys were divided into the following six experimental sites, each consisting of four teeth: Site 1, maxillary right bicuspids, first and second molars; Site 2, maxillary incisors; Site 3, maxillary left bicuspids, first and second molars; Site 4, mandibular left bicuspids, first and second molars; Site 5, mandibular incisors; and Site 6, mandibular right bicuspids, first and second molars.

At the end of the preexperimental week, both monkeys began a two-stage experimental regimen. The first stage consisted of rubber cup prophylaxes as described above. While the experimental teeth of Site 5 received only one prophylaxis weekly (Friday), the other five experimental sites received daily prophylaxes during the 5 week-days. This sequence of prophylaxes was followed for 10 weeks.

At the end of the 8th week, one gingival biopsy from each of the two monkeys was taken to check for sulcular induced keratinization. Since the biopsies showed keratinization, the second experimental stage was started. The gingival tissues in experimental Site 1 were injected with 200 μl of a 5% dextran saline solution, buccally and lingually. The gingiva of the maxillary incisors, Site 2, received no treatment, to serve as a positive control of induced keratinized sulcular epithelium. The gingival tissues of teeth in Site 3 were injected with 200 μl of physiologic saline solution, buccally and lingually. To the gingiva of teeth in Site 4, a 5% dextran saline solution was applied topically. The gingival tissues of the mandibular incisors, Site 5, received no treatment, to serve as a negative control of intact nonkeratinized sulcular epithelium. The gingiva of teeth in Site 6 received topical applications of normal saline solution. These treatments were given to both monkeys, daily, for 2 weeks, at which time gingival biopsies were taken from all experimental sites. Clinical grade dextran,§ molecular weight 70,000, was used. Injections were made with a tuberculine syringe. Topical applications were made dropwise with disposable Pasteur pipettes over the marginal gingiva of each tooth. Five applications, one every 3 minutes, were made on each gingival test site. Precautions were exercised to avoid cross-over between test sites.

The procedure used for obtaining gingival biopsies has already been published.[8] One-half of each biopsy was fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5-micron intervals and stained with Harris's hematoxylin and eosin,[9] or Rhodamine B.[10] The other half of the biopsy was fixed in 100% ethanol to preserve water-soluble dextran, and subsequently embedded in paraffin, sectioned and stained with alcoholic periodic acid Schiff (PAS) and aqueous PAS to detect dextran in tissue according to the technique of Mowry and Millican.[11]

For histologic evaluation the gingiva was divided into three zones: A (crestal zone), B (cervical zone) and C (oral gingival zone), as previously described.[4] The degree of inflammation in the connective tissue subjacent to these zones was evaluated. An Inflammatory Index (II) was obtained for each zone by counting the inflammatory cells within a microscopic field.[4] Separate inflammatory indices for Zones A, B and C were obtained both buccally and lingually for the different experimental regimens. For each tooth within a particular treatment regimen, determinations from four individual microscopic sections were summed and averaged both buccally and lingually. An overall buccal or lingual II value for a given regimen was then determined by averaging the means of the individual teeth within that regimen. The inflammatory cells counted were: lymphocytes, plasma cells, polymorphonuclear leukocytes, macrophages, monocytes and unidentified cells. Cell identification was based on previously reported morphologic criteria relating to nuclear shape and cell size.[12]

A histologic determination was made of the distribution of induced keratin within the sulcular area. A quantitative analysis of keratin width and keratin length was performed using a Filar Micrometer Eyepiece,‖ at 100× magnification. Width measurements were made separately for the buccal and lingual sulcular areas at the approximate midpoint of the sulcular keratin band. Length measurements were also made separately for the buccal and lingual sulcular areas. Mean keratin length and mean keratin width values for the various regimens were determined like those for inflammatory indices. A total of 384 microscopic sections were evaluated. To obtain the inflammatory indices, 192 sections stained with hematoxylin and eosin were evaluated, 96 per monkey and 16 per experimental regimen. Similarly, 192 sections stained with Rhodamine B were evaluated for the determination of the keratin values.

RESULTS

Sections from experimental Site 5, untreated negative control, exhibited a para- and ortho-keratinized oral gingival epithelium, with the keratin stopping abruptly

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at the crest of the marginal gingiva. The sulcular epithelium had a generally flat basement membrane with gentle undulations. No evidence of keratinization was observed in the sulcular epithelium. A minimal inflammatory infiltrate was evident approximating the sulcular and junctional epithelium.

The results of the study are summarized in Tables 1–5. The mean values of the inflammatory indices, keratin widths and keratin lengths in the remaining five treatment groups were compared using analysis of variance. When significant differences were found, the means of every pair of groups were analyzed further using Scheffe's method of multiple comparisons. The results of this procedure are indicated by using brackets to connect pairs of means that do not differ significantly ($P > 0.05$).

Tables 1 and 2 show the differences between the treatments with respect to inflammation. In both the sulcular and gingival regions, the differences between topical saline and topical dextran proved nonsignificant, as did the comparison of topical saline with the positive control in the sulcular area. All other pairwise comparisons were significantly different ($P < 0.05$). Injecting either saline or dextran produced the most inflammation while topical application of these agents reduced inflammation relative to the control mean value.

Table 1
Inflammatory Index, Zones A and B (Sulcular)*

Treatment	N	Mean†	SD
Positive control	16	73.50	5.89
Topical saline	16	68.19	3.07
Topical dextran	16	67.41	4.44
Injected saline	16	103.24	4.48
Injected dextran	16	167.90	6.74

* Analysis of variance $P < 0.0001$.
† The bars indicate means not significantly different using Scheffe's method of multiple comparisons.

Table 2
Inflammatory Index, Zone C (Gingival)*

Treatment	N	Mean†	SD
Positive control	16	33.40	2.56
Topical saline	16	28.22	4.07
Topical dextran	16	26.04	2.10
Injected saline	16	44.26	4.92
Injected dextran	16	111.60	5.35

* Analysis of variance $P < 0.0001$.
† The bar indicates means not significantly different using Scheffe's method of multiple comparisons.

Table 3
Keratin Width (Sulcular)*

Treatment	N	Mean†	SD
Positive control	16	0.378	0.013
Topical saline	16	0.367	0.005
Topical dextran	16	0.345	0.018
Injected saline	16	0.380	0.013
Injected dextran	16	0.353	0.016

* Analysis of variance $P < 0.0001$.
† The bars indicate means not significantly different using Scheffe's method of multiple comparisons.

Table 4
Keratin Width (Gingival)*

Treatment	N	Mean†	SD
Positive control	16	0.412	0.007
Topical saline	16	0.388	0.008
Topical dextran	16	0.386	0.018
Injected saline	16	0.445	0.034
Injected dextran	16	0.370	0.018

* Analysis of variance $P < 0.0001$.
† The bars indicate means not significantly different using Scheffe's method of multiple comparisons.

Table 5
Keratin Length (Sulcular)*

Treatment	N	Mean	SD
Positive control	16	6.85	0.44
Topical saline	16	6.67	0.84
Topical dextran	16	6.42	0.75
Injected saline	16	6.23	0.63
Injected dextran	16	6.38	0.92

* Analysis of variance $P = 0.13$.

The inflammatory cell population in the dextran-induced inflamed gingiva was estimated to be: lymphocytes (30–35%), monocytes/macrophages (5–10%), plasma cells (10%), polymorphonuclear leukocytes (15%) and unidentified cells (35%). Inflammatory cells in the normal saline-induced inflamed gingiva were estimated to be: polymorphonuclear leukocytes (75%), lymphocytes (5%) and unidentified cells (20%). However, the overlapping variability in cell size and nuclear characteristics made it difficult to distinguish between these cells. For this reason, the cells were grouped into a single category for the purpose of obtaining the inflammatory indices. Furthermore, since the resulting buccal and lingual mean values for each regimen were very close, these means were averaged and the resulting averages used for statistical analysis of the data.

Keratin widths are compared in Tables 3 and 4. Despite the fact that injecting saline produced high inflammatory indices, the injected saline group had the highest keratin width mean values in both the sulcular and oral gingival regions. Additionally, injected dextran, which produced the highest inflammatory indices, did not differ significantly from either the topical saline or the topical dextran groups with respect to keratin width. These results, taken together with those given in Table 5, where no differences between the groups with respect to keratin length were found ($P = 0.13$), imply that inflammation does not significantly impede the keratinization process.

DISCUSSION

The inflammatory cell population in the dextran-induced inflamed gingiva consisted of lymphocytes, plasma cells, polymorphonuclear leukocytes (PMNs),

macrophages and unidentified cells. The identification of plasma cells and PMNs was easy. Plasma cells were distinguished by an ovoid shape with an eccentrically placed nucleus, radial pattern of chromatin and a clear perinuclear zone, whereas PMNs had granular multilobed nuclei and basophilic cytoplasm. However, there was difficulty in differentiating the lymphocyte and the macrophage. Similar difficulties have been reported by other investigators.[13–15] The significantly greater percentage of unidentified cells in the inflamed gingiva has also been reported by others.[15,16] These findings are consistent with the fact that infiltrated areas of connective tissue contain a greater density of inflammatory cells and increased numbers of degenerating cells, thereby making cell identification more difficult.

In the dextran-induced inflamed gingiva, inflammatory cells were scattered throughout the inflamed gingiva. It is of interest that in humans[17] and monkeys[18] lymphocytes and plasma cells are the predominant cells in inflamed tissues of established chronic periodontal lesions.[17,18] Immune responses associated with lymphocyte and plasma cell populations are believed to play a major role in these periodontal lesions.[19–21] However, although the humoral and cell-mediated immune reactions are largely effected by these cells, the tissue destruction associated with their presence occurs principally through the chemotactic attraction of PMNs and macrophages into the area.[22,23] Both PMNs and macrophages constitute a line of defense against all forms of injury and microbial challenge around the teeth, and throughout the body. However, PMNs carry potent acid hydrolases, collagenases, prostaglandins and other substances having the capacity to destroy collagen and other connective tissue substances, and to induce bone resorption.[24,26] There is also ample evidence that macrophages mediate collagen resorption by secreting enzymes, including collagenases and lysosomal hydrolases, into the extracellular compartment.[27] Macrophages also release prostaglandins which are chemotactic for inflammatory cells,[28] affect collagen synthesis[29] and induce osteoclastic bone resorption.[30] The tissue destruction and pathologic bone resorption associated with PMN and macrophage activities may be at least in part a prostaglandin-driven phenomenon.[31]

The present study also showed that topically applied dextran permeated intact nonkeratinized sulcular epithelium, widened the intercellular epithelial spaces and disorganized the connective tissue ground substance. Dextran penetrated the intact sulcular lining; however, most of the penetration took place through the junctional epithelium. The study confirmed our previous report[8] and other reports[32,33] that the junctional epithelium may be the predominant pathway for entry of foreign substances from the sulcus to the underlying connective tissue. Greater penetration in the junctional epithelium may be due to larger intercellular spaces and a lower desmosome density than the sulcular epithelium.[34]

However, when dextran was applied topically to induced-keratinized sulcular epithelium as well as to normally keratinized oral gingival epithelium, it failed to permeate, remaining on the outer surface of the keratinized epithelium. Minimal penetration was seen at the bottom of the sulcus through the junctional epithelium and also in spots of lacerated keratinized epithelium.[8] Consequently, dextran-induced inflammatory cell infiltration was not observed in these specimens.

When dextran was injected into the gingival tissues, it produced the inflammatory reaction described above. However, this inflammation did not modify the keratinization process of the induced keratinized sulcular epithelium, and that of the normally keratinized oral gingival epithelium. It is evident, then, that the degree of inflammation elicited in the gingival tissues by dextran did not affect the degree of keratinization, when plaque was removed by daily prophylaxes. This may suggest that the control of inflammation within the tissues is not as critical as the removal of bacterial plaque. Several recent reports indicate that this may be the case. Well documented longitudinal and cross-sectional evaluations in adults and children have determined that no minimal zone of keratinized gingiva is necessary to maintain clinical signs of periodontal health in regions where plaque control was well maintained.[35–37] Furthermore, in a study of the role of keratinized gingiva in plaque-associated gingivitis in beagle dogs, it was found that the character of the marginal tissue had little effect upon the histologic location or extension of an inflammatory reaction, and it was concluded that the amount of inflammatory response from plaque was the same regardless of the width of keratinized gingiva.[38] Therefore, these studies seem to strongly indicate that little, if any, keratinized gingiva is necessary to maintain periodontal health if plaque control is adequate. Chronic inflammation may not necessarily affect tissue keratinization if thorough plaque removal is maintained.

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