RELATIONSHIP OF TOTAL SALIVARY IMMUNOGLOBULINS AND SPECIFIC SALIVARY IMMUNOGLOBULINS TO A FOOD ANTIGEN IN MINOR RECURRENT APHTHOUS STOMATITIS

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ABSTRACT

The onset of minor recurrent aphthous stomatitis (MiRAS) has been associated with the intake of certain foods in some individuals. Hypotheses to explain this observation may include the presence in MiRAS patients of increased levels of salivary IgE leading to a local allergic reaction, or of decreased levels of salivary IgA, IgG, or IgM allowing increased passage of food antigens across oral mucous membrane, thus leading to a local hypersensitivity reaction. The resultant tissue destruction may be represented clinically as MiRAS.

To investigate these hypotheses, total salivary IgA, IgG, IgM, and IgE concentrations of 28 patients suffering from active MiRAS lesions were compared to age- and sex-matched individuals without a history of MiRAS, using a solid-phase radioimmunoassay technique. Levels of salivary IgA antibodies to the food antigen alpha-lactalbumin were also assessed using a double antibody radioimmunoassay method.

No statistical differences in total salivary IgA, IgG, or IgM concentrations were observed between the two study groups. Salivary IgE was detected in minute concentrations in only three of the 56 subjects, two from the control group, and one from the MiRAS group. All saliva samples exhibited IgA antibody activity to alpha-lactalbumin, however, no strongly positive activity was detected in either group.

The results indicated that salivary immunoglobulins were not

altered during the clinically diagnostic ulcerative stage of MiRAS, and most likely did not participate in tissue destruction during this stage.

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ABBREVIATIONS

ADCC - antibody-dependent cellular cytotoxicity

BSA - bovine serum albumin

cpm - counts per minute

DEAE - diethylaminoethylene

HLA - human leukocyte antigen

HU - herpetiform ulcers

IgA - immunoglobulin A

IgD - immunoglobulin D

IgE - immunoglobulin E

IgG - immunoglobulin G

IgM - immunoglobulin M

MiRAS - minor recurrent aphthous stomatitis

MjRAS - major recurrent aphthous stomatitis

mol wt - molecular weight

PBS - phosphate-buffered saline

RAS - recurrent aphthous stomatitis

SDS-PAGE - sodium dodecyl sulfate-polyacrylamide gel electrophoresis

U - units

CHAPTER I

OBJECTIVES

Minor recurrent aphthous stomatitis (MiRAS), the most common form of recurrent aphthous stomatitis (RAS), represents a painful ulcerative condition of the oral cavity which affects a large proportion of the general population.

It has been noted that the intake of certain foods has been associated with the onset of lesions in some MiRAS patients (Alvarez, 1937; Wilson, 1980; Eversole et al., 1982). Clinical studies involving the direct application of suspected foods to oral mucous membranes (Tuft and Ettelson, 1956; Tuft and Girsh, 1958; Kutscher et al., 1958), intradermal and inhalant tests (Barbash et al., 1958), and diet manipulations (Ship, 1960; Ferguson et al., 1976; Ferguson et al., 1980; Wilson, 1980; Walker et al., 1980; Wray, 1981), as well as in vitro tests investigating serum antibodies (Taylor et al., 1964) and cell mediated immune responses (Thomas et al., 1973; Wray et al., 1982) to food antigens have been conducted to investigate a food allergy association with RAS. Although these investigations have given variable results, the involvement of allergic reactions to food antigens in the pathogenesis of MiRAS has not been ruled out.

Few studies have considered the relationship of salivary immunoglobulins and food antigens to MiRAS. Due to the low concentrations of IgG, IgM, and IgE in saliva, and the lack of a sensitive method to assay these immunoglobulins, immunologic studies related to salivary immunoglobulin concentrations in recurrent aphthous stomatitis patients have dealt only with the IgA class of immunoglobulins (Lehner, 1969c; Kakizawa et al., 1973; Ben-Aryeh et al., 1976; Bennet and Reade, 1982). The methods of assay used

in these studies were the single radial immunodiffusion (Mancini et al., 1965) and automated turbidimetric techniques. No modern studies involving RAS and salivary immunoglobulins, using accurate assay techniques of high precision, have been conducted.

The present study addressed the following hypotheses involving salivary immunoglobulins and food antigens with respect to the possible pathogenesis of MiRAS.

1). IgE in saliva participates in an allergic reaction to food antigens leading to local mucosal hypersensitivity tissue destruction.

2). Decreased levels of salivary IgA, IgG, and IgM immunoglobulins result in increased passage of antigens across the oral mucosa leading to local mucosal hypersensitivity tissue destruction.

3). Decreased levels of salivary IgA to specific food antigens result in decreased antigen-IgA antibody complexes and as a consequence, increased passage of such specific food antigens across the oral mucosa leading to local mucosal hypersensitivity tissue destruction.

To evaluate these hypotheses, the following objectives were considered:

1). To select a test group of 28 patients suffering from MiRAS, and a control group of 28 individuals with no history of RAS.

2). To establish the range and mean concentrations of immunoglobulins of the IgA, IgG, IgM, and IgE classes in unstimulated whole human saliva in the 56 subjects utilizing a highly sensitive and highly specific assay technique.

3). To compare the range and mean concentrations of immunoglobulins of the IgA, IgG, IgM, and IgE classes in unstimulated whole human saliva of the test group to the control group utilizing a highly sensitive and highly specific assay technique.

4). To establish the presence of IgE and IgA antibody activity in unstimulated whole human saliva to a purified food antigen in the 56 individuals.

5). To compare the presence of IgE and IgA antibody activity in unstimulated whole human saliva to a purified food antigen of the test group to the control group.

CHAPTER II

LITERATURE REVIEW

(1). Minor Recurrent Aphthous Stomatitis

Minor recurrent aphthous stomatitis is a common condition characterized by painful periodic solitary or multiple ulcerations of the oral cavity. This disorder was first described by Mikulicz and Kummel in 1888, but its classification was not formalized until the studies of Truelove and Morris-Owen in 1958. Subsequently, Lehner (1968) has defined further the classification of this disorder.

(a). Classification of minor recurrent aphthous stomatitis

Recurrent aphthous stomatitis (RAS) is a term applied to three varieties of recurrent oral ulcerations, namely minor recurrent aphthous stomatitis, major recurrent aphthous stomatitis, and herpetiform ulcerations.

Minor recurrent aphthous stomatitis (MiRAS) is the most common form of RAS. This entity is known by a variety of alternate terms, the more common being minor recurrent aphthous ulcers, recurrent Mikulicz's ulcers, and canker sores.

Major recurrent aphthous stomatitis (MjRAS), first described by Sutton in 1911, is a less common form of RAS. This disorder is also known as major recurrent aphthous ulcers, periadenitis mucosa necrotica recurrens, and Sutton's disease. The lesions of MjRAS are larger and remain longer than those of MiRAS, and frequently heal with scarring. It is felt that MjRAS represents a more severe variant of MiRAS (Lehner, 1968).

Herpetiform ulcers (HU), the least common type of RAS, was first described by Cooke in 1960. This entity represents recur-

rent crops of up to one hundred pinhead-sized ulcers occurring in any location in the oral cavity.

Although the criteria for the clinical diagnosis of these variants of RAS are well established (Lehner, 1968), many reports in the literature fail to differentiate the RAS variants. Often the broad term RAS is used without indicating the proportion of MiRAS, MjRAS, or HU lesions. In many cases the term RAS is used synonymously with that of MiRAS.

The topic of this review is concerned with MiRAS, and its features will be discussed in detail. In the following dissertation the term MiRAS will be used only when the lesions are described or identified as minor recurrent aphthous stomatitis lesions. Otherwise, if doubt exists, the broader term RAS will be used.

(b). Epidemiology of MiRAS

The incidence of RAS varies depending on the study and population investigated. Sircus et al. (1957) and Lehner (1968) found approximately 20% of a general population to have a history of RAS. Among hospitalized patients Ship et al. (1967) found a positive history in 13.2% of individuals. A survey involving hospital and dental patients revealed a positive RAS history in 20% of individuals (Spouge and Diamond, 1963). In a mixed Arab population, 27% of persons had experienced RAS (Fahmy, 1976). A study conducted by Embil et al. (1975) involving students and armed forces personnel in 21 countries revealed the incidence of RAS history to be 38.7% for males, and 49.7% for females. Other stud-

ies also involving students showed the presence of a positive RAS history to vary from 41% (Shapiro et al. 1970), 55% (Ship et al. 1960a), 56% (Donatsky, 1973) to 66.2% (Ship et al. 1967). MiRAS was the form of the disorder in approximately 80% of the RAS patients studied by Graykowski et al. (1966) and Lehner (1968).

That RAS tends to be more common in females than males has been reported in numerous studies (Sircus et al., 1957; Farmer, 1958; Ship et al., 1960a; Spouge and Diamond, 1963; Graykowski et al., 1966; Donatsky, 1973; Embil et al., 1975; Fahmy, 1976). Lehner (1968) found the female to male ratio of MiRAS to be 1.3:1.

(c). Age of onset of MiRAS

Many authors state that the age of onset of RAS, in the majority of cases, is during the second and third decades of life (Sircus et al. 1957; Ship et al., 1960a; Cooke, 1961; Graykowski et al., 1966; Donatsky, 1973). In a 1967 study, Ship et al. found that the first decade was the most frequent age of onset. However, Farmer (1958) found the most common age of onset in females to be during the first and second decades, with onset during the first and third decades for males. Lehner (1968) reported the maximum incidence of onset of MiRAS to occur during the second decade of life.

(d). Location of MiRAS lesions

Weathers and Griffin (1970) reported that MiRAS lesions occur almost exclusively on non-keratinized oral mucosa. The labial mucosa, the buccal mucosa, and the tongue are the sites

most often affected by MiRAS, with the floor of the mouth, and the pharynx occasionally being affected (Lehner, 1968).

(e). Clinical features of MiRAS

The natural history of MiRAS lesions has been well documented by Stanley (1972) and is divided into four stages.

During the premonitory stage, which lasts up to 24 hours, a burning, tingling, tense, or hyperesthetic sensation is noted in the future site of the lesion. Clinically there is no evidence of pathologic change.

The preulcerative stage occurs eighteen hours to three days after the previous stage, and is characterized by an erythematous macule or papule with slight induration. Lesions on broad, flat mucosal surfaces such as buccal and labial mucosae are circular in shape, whereas lesions within tissue folds such as buccal and labial sulci tend to be elongated in shape. A central superficial membrane develops with a surrounding red inflammatory halo. During this stage pain in the local area is a common complaint.

The next stage, the ulcerative stage, occurs from day one to day sixteen, and is accompanied initially by severe pain. The membrane sloughs leaving a shallow, well-defined ulcer. Subsequently, the floor of the ulcer is covered by a greyish-yellow membrane. The maximum size of the ulcer, most frequently two to ten millimeters in greatest diameter (Lehner, 1968) is reached within four to six days. Ulcers may coalesce to form larger lesions, and pain tends to decrease two to three days after the maximum size has been reached.

The healing stage takes place anytime from day four to thirty-five, although the majority of MiRAS lesions will heal within one to two weeks (Lehner, 1968). Lehner (1968) found that 8% of MiRAS lesions healed with scarring and suggested that scarring may be a reflection of the size of the lesion, or due to a disturbance in normal healing and repair by a superimposed secondary infection of normal microorganisms.

(f). Histology of MiRAS

The histology of MiRAS lesions has been reported by numerous authors (Graykowski et al., 1966; Francis, 1970; Stanley, 1972). All workers report similar histologic appearances.

During the premonitory and preulcerative stages a focus of mononuclear cells are present in the lamina propria. Subepithelial edema of the lamina propria, liquifactive degeneration of basal cells, and subsequent degeneration of epithelial cells of the lower stratum spinosum occur as the mononuclear cells infiltrate from the lamina propria into the epithelium. Microscopic intraepithelial vesicles may form.

Disintegration of the epithelium occurs in an upward direction and the epithelial surface is eventually breached. This marks the beginning of the ulcerative stage. The mononuclear cell infiltrate predominates during the first six hours of the ulcerative stage after which neutrophils appear at the site of the ulcer. After thirty to sixty hours neutrophils are the predominant cell type throughout the lesion. The neutrophil infiltrate can become so pronounced that it masks the normal architecture of

the lamina propria. As the lesion ceases to enlarge there is a gradual increase in plasma cells, and mast cells and eosinophils are present in the lamina propria.

During the healing stage granulation tissue, characterized by the presence of immature capillaries with fibroblastic and endothelial cell proliferation, is evident. Proliferation, migration, and differentiation of epithelial cells produce an intact surface. The granulation tissue undergoes subsequent collagenization, and a residual mild chronic inflammatory cell infiltrate, composed predominantly of plasma cells may persist in the deep lamina propria.

Lehner (1969a) compared the histology of the MiRAS lesion to that of a non-specific ulcer and noted that the former contained a more marked mononuclear and perivascular infiltrate, a less pronounced neutrophil infiltrate, and a greater number of mast cells. The mast cell counts of MiRAS lesions were more than two times the mast cell counts of normal tissue, and nine times that of a non-specific ulcer.

In contrast to Lehner (1969a), Dolby and Allison (1969) stated that the mast cell counts of early MiRAS lesions did not differ significantly from those of normal mucosa. These authors saw a decrease in the number of mast cells in the lesion after 48 hours, when compared to normal tissue.

(2). Precipitating Factors and MiRAS

The etiology of MiRAS is not known. Various factors, however, have been repeatedly associated with the clinical onset of these lesions and are therefore considered to be precipitating factors in their development. These factors include stress, trauma, and food allergy. Although the factors have been well documented in MiRAS, not all MiRAS patients will associate them with each and every onset of the disorder. Numerous patients, in fact, are never able to identify any unusual event which can be associated with the occurrence of their lesions.

Not surprisingly the studies investigating these diverse factors have yielded inconsistent results.

(a). Association of MiRAS and trauma

Local trauma is often implicated in the formation of MiRAS lesions. Graykowski et al. (1966) reported that 46 of 62 RAS patients (74%) associated trauma with the onset of their lesions. These traumatic episodes included self-inflicted bites, oral surgery, toothbrush trauma, and dental procedures. Eversole et al. (1982) stated trauma was associated with 38% of RAS patients. Wray et al. (1981a) explored this phenomenon and caused minor trauma to the buccal mucosae of control subjects and patients with a history of RAS. Forty-three percent of the RAS patients developed lesions clinically indistinguishable from those of their disorder, whereas such lesions were not produced in the controls. However, Ross et al. (1958) were unable to consis-

tently produce RAS lesions in RAS patients upon puncture, incision, pinching, and toothbrush trauma involving the oral mucosa.

(b). Association of MiRAS and stress

Patients have related stress and emotional disturbances with the appearance of lesions in numerous studies (Sircus et al., 1957; Farmer, 1958; Ship et al., 1960b; Eversole et al., 1982).

A higher incidence of MiRAS lesions was found among a school population during examination periods than during vacations (Ship et al., 1961). Utilizing the Cornell Medical Index to assess emotion and personality traits, Ship et al. (1967) found a higher degree of anger, anxiety, inadequacy, tension, and depression in a hospitalized patient group that suffered from RAS than in a control hospitalized group. However, these findings were not supported by the results in a corresponding group of dental and medical students (Ship et al., 1967). Heft and Wray (1982) scored RAS patients at the onset of lesions using state-trait anxiety tests and concluded that RAS patients exhibited no more anxiety than general medical and surgical patients.

(c). Association of MiRAS and hormonal influences

In numerous studies the onset of MiRAS lesions has been associated with various hormonal changes in females. Strauss (1947) reported that 43 of 45 female patients (96%) suffered from new RAS lesions within 16 days preceding the onset of menstrua-

tion. Sircus et al. (1957) found that 3 of 80 female RAS patients (4%) regularly presented with pre-menstrual lesions, and Dolby (1968a) stated that ulceration was maximal in the post-ovulation period. Eversole et al. (1982) found that 62% of female RAS patients related the appearance of lesions to the onset of the menstrual cycle. In contrast, Segal et al. (1974), observing 104 student nurses over three years, found no association of MiRAS lesions with any specific interval in the menstrual cycle.

Sircus et al. (1957) also observed a decrease of RAS ulcers during pregnancy with a return to a normal incidence post partum. An increased incidence of lesions was also noted after menopause.

Cooke (1961) noted that the peak incidence of onset of MiRAS lesions in females occurred between 10 and 14 years of age, at the time of menarche.

The association of the onset of RAS lesions with various phases of the menstrual cycle prompted investigations to treat RAS with oral contraceptives (Carruthers, 1967), high doses of estrogen (Bishop et al., 1967), and progesterone (Ferguson et al., 1978). In many cases these therapies produced beneficial results.

(d). Association of MiRAS and smoking

Investigators have noted an inverse relationship between smoking and patients suffering from MiRAS. In large studies the percentages of non-smoking RAS patients were 66% (Shapiro et al., 1970), 74% (Sircus et al., 1957), and 94.5% (Sallay and Banoczy, 1968). Various case histories in smokers relate the

onset of RAS ulcers to cessation of cigarette smoking, and the subsequent remission of ulcers upon resumption of smoking (Bookman, 1960; Dorsey, 1964; Sallay and Banoczy, 1968).

(e). Association of MiRAS with intestinal disorders

MiRAS lesions have been associated with disorders of the intestinal tract, malabsorption states, and nutritional deficiencies.

Wray et al. (1975) reported 23 of 130 RAS patients (17.7%) to be deficient in one or more of vitamin B_{12}, folic acid, or iron, whereas only 11 (8.5%) of the controls were found to be so deficient. Upon replacement therapy, 15 of the 23 vitamin deficient RAS patients showed complete remission of lesions with the remaining eight exhibiting improvement. A later study by Wray et al. (1978) revealed that 47 of 330 RAS patients (14.2%) exhibited the above vitamin and iron deficiencies either singly or in combination. Replacement therapy in 39 deficient patients again yielded complete remission of lesions in 59%, improvement in 28%, and no change in only 13% of these individuals.

In other studies intestinal disease was found to be present in 24% (Ferguson et al., 1976), and 4% (Ferguson et al., 1980) of MiRAS patients, with 28% of the patients in the latter study revealing nutritional deficiencies.

Subsequent studies (Walker et al., 1980; Olson et al., 1982) were unable to establish a correlation between RAS and intestinal disorders. They found no statistically significant differences between RAS patients and control populations.

(f). Familial relationships in MiRAS

The familial tendency of MiRAS has been noted by numerous authors with 46% (Sircus et al., 1957), 41% (Ship et al., 1967), 39% (Farmer, 1958), and 24% (Cooke, 1961) of RAS patients reporting a familial history of similar lesions.

Miller et al. (1977) studied monozygotic and dizygotic twins with respect to RAS. These authors observed 91% concordance of the disease in the identical twin pairings, but only 57% concordance in the non-identical twin pairings.

Ship et al. (1965) investigated the possibility of either an autosomal dominant or autosomal recessive mode of transmission of the disorder. The segregation frequencies for a recessive inheritance pattern were slightly suggestive. A recent study by Miller et al. (1980) was not able to show a precise mode of genetic transmission.

(g). Association of MiRAS with systemic diseases

The three varieties of RAS lesions have been associated to varying degrees with numerous systemic disorders. MiRAS has been reported in cases of gastrointestinal disorders such as coeliac disease, chronic ulcerative colitis, gluten-sensitive enteropathy, and Crohn's disease (Rogers, 1977; Ferguson et al., 1980). Deficiencies of vitamin B_{12}, folic acid, or iron were found in a higher proportion of individuals suffering from RAS lesions than individuals in the general population (Wray et al., 1975).

Recurrent oral ulcerations clinically identical to RAS lesions are a major component of Behcet's syndrome, a disorder also characterized by recurrent genital ulcerations, iridocyclitis, and to lesser extents arthritis, cutaneous lesions, and neurologic manifestations (Chajek and Fainaru, 1975). Much speculation has been generated as to a similar etiopathogenesis of RAS and the oral ulcerations of Behcet's syndrome, yet the etiology of both entities remain uncertain (Haim, 1983).

(h). Association of MiRAS and food allergy

Individuals have long associated the onset of MiRAS with the intake of certain foods including chocolate, eggs, milk, wheat, fruits (particularly tomatoes and strawberries), and nuts (particularly walnuts) (Alvarez, 1937; Eversole et al., 1982). Numerous investigations have studied the relationship of food allergy to RAS with conflicting results.

Tuft and Ettelson (1956) demonstrated the repeated formation of MiRAS lesions in a patient upon direct application of citric acid crystals to the buccal mucosa, whereas such lesions could not be produced in control individuals. A further study revealed the onset of aphthous ulcers in a small number of atopic patients with a positive history of MiRAS upon exposure to citric acid or acetic acid (Tuft and Girsh, 1958). However, Kutscher et al. (1958) were unable to reproduce these findings in RAS patients using crystals of citric acid, potassium citrate, ascorbic acid, or acetic acid.

Intradermal food tests and inhalant tests were performed on

13 RAS patients utilizing 57 substances (Barbash et al., 1958). The results failed to establish food allergy as a cause for the lesions and the results did not differ significantly from those of non-RAS individuals.

Studies have indicated an increase in allergic disorders among RAS patients (Tuft and Girsh, 1958; Spouge and Diamond, 1963; Wilson, 1980) relative to non-RAS individuals. However, a positive relationship of hypersensitivity predisposition and RAS was not observed in the studies of Eversole et al. (1982) and Wray et al. (1982).

Wilson (1980) studied the occurrence of MiRAS lesions in 61 atopic individuals. A positive history of MiRAS was reported in 34 (56%) of the patients. Eleven patients (18%) could relate the onset of ulcers to the intake of specific foods, including tomatoes, milk, eggs, and chocolate. Skin-prick tests utilizing extracts of these foods were positive in the patients, and an intense pain could be elicited upon application of the offending food to the aphthous ulcer base in six of the patients.

Since an allergic reaction to foodstuff has been implicated in the precipitation of RAS, antihistamine drugs and dietary manipulation have been proposed as two possible methods of treatment of the disorder.

Disodium cromoglycate, which inhibits the release of histamine and SRS-A from mast cells, have been shown to be partially effective in the treatment of RAS (Frost, 1973; Dolby and Walker, 1975; Walker and Dolby, 1975; Kowolik et al., 1978). However, Zegarelli et al. (1953) and Dolby (1968b) found no appreciable

improvement of RAS lesions using other antihistamine drugs.

The elimination of gluten from the diet has led to the complete remission of RAS in patients who were also diagnosed as having coeliac disease (Ferguson et al., 1976; Ferguson et al., 1980). Walker and coworkers (1980) placed eight RAS patients with no intestinal disease on a gluten-free diet with a resultant reduction in ulcer number and pain. Wray (1981) found that 25% of RAS patients with no intestinal disease experienced complete remission of lesions when placed on an gluten-free diet. Ship (1960) observed 6 of 7 severe RAS patients undergo remission upon institution of a non-allergenic diet.

(3). Evidence Implicating an Immunologic Basis for RAS

Although considerable investigation has been conducted in an attempt to determine the cause for RAS, no satisfying etiology has been established to date. Numerous studies, however, reveal abnormalities in the cellular and/or humoral immune systems of individuals suffering from RAS. As a result, the literature is replete with evidence implicating an immunopathogenesis for the disorder. The evidence includes data from microscopic observations, HLA associations, and various immunologic studies. These include antigenic studies with microorganisms, food, and some cross-reacting antigens. In addition, toxic immune responses such as mucosal hypersensitivity, immune complexes, and antibody dependent cellular cytotoxicity have been examined. Several workers have quantitated serum and salivary immunoglobulins in RAS

patients. The positive response of RAS to immunosuppressive and immunoenhancing drugs also lends support to an immunopathogenic process.

(a). Microscopic observations

Light microscopic examination of early RAS lesions, as outlined earlier, reveals the presence of a dense lymphomonocytic infiltrate in the lamina propria and lower epithelium (Stanley, 1972). This fulfills the histologic criteria of a delayed-type hypersensitivity reaction as set forth by Coe et al. (1966).

Additional support for such a hypersensitivity reaction was suggested by the electron microscopic findings of Lehner (1969a), Honma (1976), and Schroeder et al. (1983). Lehner (1969a) observed intracytoplasmic phagosome-like bodies within epithelial cells of MiRAS lesions which resembled the "invasive-destructive" reaction pattern described in delayed-type hypersensitivity reactions by Waksman (1962). Honma (1976) noted mononuclear cells within the spinous layer of early RAS lesions which were interpreted to be activated T-lymphocytes in association with phagocytic macrophages. In a recent study, Schroeder et al. (1983) examined the ulcerative stage of MiRAS using stereologic electron microscopic analysis of biopsied material. Again it was found that the cells of delayed-type hypersensitivity reactions were present. Macrophages composed a large proportion of the infiltrate, and blast-forming T-lymphocytes were consistently noted. Blast-forming B-lymphocytes, non-activated lymphocytes, and plasma cells were not a consistent feature of the lesion during this

stage.

The increase in mast cell density noted by Lehner (1969a) in MiRAS lesions when compared with normal tissue was also observed by Schroeder et al. (1983). A similar finding has been reported in aphthous-like ulcers of Behcet's syndrome (Lehner, 1969a; Lichtig et al., 1980; Schroeder et al., 1983) which has led to the speculation that a cutaneous basophil hypersensitivity reaction, a form of delayed type hypersensitivity reaction, occurs.

(b). HLA associations

The strong familial tendency of MiRAS has led to the investigation of HLA antigen association with the disorder. The studies of Platz et al. (1976) and Dolby et al. (1977) failed to show any significant differences in the frequencies of single or shared HLA antigens between patients with and without a history of RAS.

However, subsequent studies have revealed HLA and RAS correlations. Challacombe et al. (1977) observed significant increases in the frequency of HLA-A2, HLA-B12, and HLA-Aw29 in RAS patients when compared to normal individuals. An increased frequency of the former two antigens was confirmed in a study by Lehner et al. (1979). Wray et al. (1981b) observed a significant correlation between HLA phenotypes A2 and Bw4, and RAS. Studying a Finnish population Malmstrom et al. (1983) reported a higher incidence of HLA antigens of the A11, B12, and B13 loci among MiRAS patients than non-affected individuals. Gallina et al. (1985), in a recent study involving Sicilian subjects, demon-

strated a significant increase in HLA-DR7 antigen frequency in MiRAS patients when compared to healthy controls. These authors also reported a significant decrease in HLA-B5 antigen frequency in the MiRAS patients. Such data would suggest a possible immunogenetic susceptibility with respect to RAS.

(c). Immunologic studies related to microorganisms

Much investigation has been conducted with respect to microbial agents and the pathogenesis of RAS. Attempts to implicate viruses, especially the herpes simplex virus, have been unsuccessful. Such studies have been adequately reviewed by Wray (1982).

In the early 1960's speculation surrounded Streptococcus sanguis as an etiologic agent of RAS. Barile et al. (1963) isolated pure cultures of a pleomorphic, transitional L-form of a probable streptococcus from the lesions of three patients suffering from RAS. During a period of severe exacerbation it was possible to culture this organism from the blood of one of the patients. The authors speculated that some cases of RAS might be infectious in nature with the stable L-form variant of the microorganisms representing a dormant carrier state. Under an appropriate predisposing condition, such as trauma, the stable microorganism would undergo change to the pathogenic unstable pleomorphic L-form causing recurrent clinical lesions.

Stanley et al. (1964), examining histologic sections, observed microorganisms morphologically consistent with pleomorphic streptococcus in 93% of RAS lesions that were studied.

Similar organisms were present in only 40% of non-aphthous ulcerative lesions, and 47% of normal oral mucosa obtained from autopsy sources.

Viable and heat-killed transitional L-forms of the pleomorphic streptococcus, isolated from the lesions of a RAS patient, when injected into rabbit skin and guinea pigs produced a delayed skin hypersensitivity reaction which was clinically and histologically similar to a MjRAS lesion (Graykowski et al., 1964). The authors were able to culture the same streptococcal organisms from the animal lesions.

Skin tests were then performed by Graykowski et al. (1966) on patients with RAS and individuals having no history of RAS. All RAS patients exhibited a positive delayed-type skin reaction to a Streptococcus strain 2A23HOT vaccine, whereas only 17% of non-RAS showed a hypersensitivity reaction.

A series of in vitro experiments to establish cellular hypersensitivity were conducted utilizing peripheral leukocytes from RAS and non-RAS patients, and various antigens.

Streptococcal hypersensitivity was studied by Donatsky and Bendixen (1972) with a leukocyte migration technique. Upon exposure to Streptococcus sanguis strain 2A antigen, leukocyte migration inhibition was achieved in leukocytes from 8 of 17 RAS patients, whereas no inhibition was evident with control leukocytes.

A further study by Donatsky (1976a) involving antigenic extracts of Streptococcus sanguis 2A, Streptococcus pyogenes M5, and adult human oral mucosa demonstrated a decreased migration

index in patients with RAS when compared to normal controls. Donatsky (1976b) then showed that the migration index correlated with the clinical activity of RAS when Streptococcus sanguis 2A and adult human oral mucosa antigens were utilized in leukocyte migration tests.

Serum immunoglobulin studies related to Streptococcus sanguis have also been conducted. Donatsky and Dabelsteen (1974a) were able to demonstrate higher levels of serum immunoglobulins to Streptococcus sanguis 2A in RAS patients than non-RAS individuals. However, a correlation between the levels of humoral antibodies to this organism and clinical activity could not be made (Donatsky, 1976b; Donatsky, 1978).

Although these studies suggest a strong case for involvement of Streptococcus sanguis 2A in the pathogenesis of RAS, contradicting findings are present. Francis and Oppenheim (1970), utilizing a leukocyte transformation test, exposed peripheral leukocytes from RAS patients to heat-killed whole Streptococcus sanguis 2A organisms. A significant depression of transformation occurred when patients were compared to controls indicating a decrease, rather than an increase, in cell-mediated responsiveness. Martin et al. (1979) were also unable to demonstrate increased cellular immunity in RAS patients. In their study blastogenic responses of peripheral blood leukocytes to Streptococcus sanguis antigens, including whole heat-killed cells, sterile culture filtrates, and carbohydrate and protein extracts, were similar in RAS patients during exacerbations and remissions, and in normal controls without a history of RAS.

As the results from the above investigations are inconsistent, a definitive cause and effect relationship between an infectious organism and RAS cannot be made.

(d). Immunologic studies related to oral mucosa hypersensitivity

Abnormal immunologic responses to oral mucosa have been associated with RAS and this has led to speculation for the role of an autoimmune reaction in the pathogenesis of this disorder.

Lehner (1964), investigating the humoral aspect of this theory, demonstrated by tanned cell hemagglutination a significantly higher titre of serum antimucosal antibodies to human fetal oral mucosa in patients with RAS than in control individuals. However, a further study (Lehner, 1969b) revealed that the hemagglutinating antibodies were not specific to oral mucosa, and that they cross-reacted with epithelia from the pharynx, larynx, esophagus, conjuctiva, vagina, colon, and skin. A lack of species specificity was also observed. Furthermore, the antibody titres did not correlate with the clinical activity of RAS. High serum antimucosal antibodies in RAS associated with a lack of correlation to clinical activity was also noted by Donatsky (1976b) and Donatsky and Dabelsteen (1974b) with respect to adult human oral mucosa. In a later study, though, Donatsky (1978) was unable to establish an increase in circulating immunoglobulins to adult human oral mucosa in RAS when compared to normal controls.

Indirect and direct immunofluorescent studies by Lehner

(1969a) and Donatsky and Dabelsteen (1974b) revealed specific fluorescent localization of IgG and IgM in the epithelial cytoplasm of the stratum spinosum, the site of epithelial damage observed histologically in early RAS lesions. However, Rogers et al. (1975), utilizing the sera of RAS patients and substrates of human gingiva and monkey esophageal mucosa, were unable to demonstrate a specific immunofluorescent pattern. Bystryn et al. (1973) observed circulating antibodies to the cytoplasm of human epidermal cells in normal individuals, in patients with various dermatoses, and in patients with malignant tumors. This led the authors to suggest that the epidermal anticytoplasmic antibodies may represent a non-specific immunologic reaction due to the release of epidermal cell antigens secondary to chronic or recurrent tissue damage. A similar process may explain the apparent non-specific nature of antimucosal antibodies present in RAS.

Cell mediated immune responses to oral mucosal antigens have also been conducted. Lehner (1967) utilized a lymphocyte transformation test using saline extracts of fetal oral mucosa to demonstrate lymphocyte stimulation in 52% of patients with active MiRAS and MjRAS. A clinical correlation of lymphoblastic activity during exacerbation and remission was noted in one patient who was followed sequentially. Similar results were reported by Donatsky (1976a; 1976b) when adult human oral mucosa instead of human fetal oral mucosa was used as an antigen.

Dolby (1969) employed a typan blue dye exclusion lymphocyte cytotoxicity assay to investigate cell-mediated reactions in RAS. Allogeneic epithelial tissue culture cells were used as targets

and exposed to sera, lymphocytes, and both sera and lymphocytes from patients suffering from MiRAS, and control individuals. No significant difference was noted when serum alone was added, however, exposure to lymphocytes either alone or in patient's serum resulted in decreased survival of the tissue culture. Preincubation of the lymphocytes with rabbit anti-human lymphocyte serum caused a suppression of the cytotoxicity (Dolby, 1970a), and hydrocortisone succinate sodium blocked cytotoxic activity (Dolby, 1970b). A similar lymphocytotoxicity assay by Rogers et al. (1974) revealed a strong cytotoxic reaction in patients with active RAS lesions, a mild reaction in RAS patients during remission, and no reaction in patients with no history of RAS. Findings consistent with those of Rogers et al. were also observed by Donatsky (1976a; 1976b; 1978) utilizing a leukocyte migration assay. Inhibition of leukocyte migration occurred when peripheral lymphocytes from RAS patients were exposed to adult human oral mucosa, whereas there was no migration of control leukocytes tested under the same conditions. It was also noted that inhibition migration indices correlated with the clinical course of the RAS lesions.

Although these observations suggest the possible occurrence of cellular immune responses to oral mucosa in RAS, such a concensus is not universal. Reimer et al. (1982) could not confirm lymphocytotoxicity by peripheral blood lymphocytes on syngeneic oral epithelial culture cells using a modified chromium release macro-assay. Peavy et al. (1982) failed to demonstrate activation of peripheral RAS lymphocytes to autologous oral epithelial

cells during periods of RAS exacerbation or remission using a [^{3}H] thymidine incorporation assay. Lymphocyte response was similar to that of normal lymphocytes to their autologous epithelial cells.

Furthermore, Donatsky (1980), conducting an immunoelectrophoretic analysis of the Streptococcus sanguis and adult human oral-mucosa antigen extracts used in previous cell-mediated investigations (Lehner, 1967; Dolby, 1969; Dolby, 1970a; Rogers et al., 1974; Donatsky, 1976a; Donatsky, 1976b; Donatsky, 1978) demonstrated contamination of the mucosal antigen extracts by human serum proteins and hemoglobin. The author inferred that such contaminants could affect in vitro cellular responses and that removal of the serum components be performed in future experiments.

The relationship between oral mucosal antigens and RAS remains unclear.

(e). Immunologic studies related to antigenic cross-reactivity

As studies revealed abnormal immune responses to Streptococcus sanguis 2A, human fetal oral mucosa, and adult human oral mucosa, an antigenic cross-reactivity reaction between oral mucosa and oral bacteria was considered.

Wilton and Lehner (1968) performed immune absorption studies between saline homogenates of human fetal oral mucosae and numerous oral bacteria including Streptococcus sanguis 2A. With the exception of Lactobacillus acidophilus, antigenic cross-reactivity was not demonstrated. However, Donatsky (1975) utilized a double

layer immunofluorescence staining technique to demonstrate that some cross-reacting antigenic determinants were shared between Streptococcus sanguis 2A, human fetal oral mucosa, and adult human oral mucosa. The author also indicated that other antibodies were produced by RAS patients which did not cross-react with the bacterium and mucosae, thus explaining the negative results of Wilton and Lehner (1968).

(f). Immunologic studies related to food antigens

Although a clinical association between RAS and exposure to certain foods has been reported, as outlined earlier, in vitro immunologic studies investigating this relationship have been minimal.

Taylor et al. (1964) utilized a coated tanned erythrocyte test to investigate serum antibodies to bovine milk proteins and gluten in MjRAS patients and control individuals. A significantly higher titre of antibodies to gluten fraction III, alpha-lactalbumin, beta-lactoglobulin, and particularly casein were demonstrated in the RAS patients.

The findings led Thomas et al. (1973) to speculate that the increased serum antibodies were due either to a hypersensitivity to food proteins leading directly to ulceration of oral mucosa, or a normal response to food proteins which were being absorbed in increased amounts through a damaged mucosa. In addition they suggested that a secretory IgA deficiency was allowing increased absorption of food antigens through a normal mucosa. The authors studied the sera of patients with MiRAS, patients with non-

aphthous oral ulcerations, and healthy control individuals for the presence of antibodies to certain food antigens. Total serum immunoglobulins of the IgA, IgG, and IgM classes were also estimated.

Significantly higher titres of serum antibodies to bovine milk antigens were present in MiRAS patients than normal controls, confirming the data of Taylor et al. (1964). However, these increased serum antibody concentrations were similar to those of patients with other non-aphthous oral ulcerations. Total serum IgA, IgG, and IgM immunoglobulin levels in the MiRAS patients were normal. The authors concluded that due to the diversity of the ulcerative disorders studied, primary hypersensitivity to food antigens was probably not the direct cause of the MiRAS ulcerations, and the elevated serum antibodies to food antigens was secondary to increased absorption through already damaged mucosa. Although salivary IgA immunoglobulin levels were not elevated, the normal serum IgA concentrations in the MiRAS patients led the authors to state that increased absorption of food antigens through normal mucosa was not a probable etiologic consideration.

Cell mediated immune responses to food antigens were conducted by Wray et al. (1982). In vitro histamine release by leukocytes from RAS patients and non-RAS patients was observed when the cells were exposed to various environmental and food antigens. Their findings showed that atopic individuals exhibited a similar histamine release to food antigens independent of the RAS history. A higher incidence of histamine release was noted in non-atopic RAS patients than non-atopic non-RAS individuals. However, the

study revealed a poor correlation between food antigen induced histamine release and a history of allergy to these foods, as well as food antigen induced histamine release and the ability of these foods to produce RAS lesions. Furthermore, elimination of suspected RAS precipitating foods from the diet did not completely eliminate the occurrence of the lesions.

Due to the limited in vitro investigations regarding food antigens and RAS, the involvement of food in the pathogenesis of RAS remains unclear.

(g). Immunologic studies related to presence of immune complexes

Circulating and tissue-bound immune complexes may play a role in the tissue damage in RAS.

Williams and Lehner (1977), using plasma fractionation and hemagglutination-inhibition assays, demonstrated immune complexes in 3 of 11 RAS patients studied. Later, Levinsky and Lehner (1978), again by hemagglutination-inhibition, noted increased levels of circulating IgG immune complexes in 12 of 30 RAS patients. Circulating immune complexes were isolated from 11 of 23 RAS patients by Burton-Kee et al. (1981) using a polyethylene glycol precipitation technique.

Direct immunofluorescence microscopic studies of RAS lesions has revealed a high incidence of C3 deposits in subepithelial vessel walls (Ullman and Gorlin, 1978; Malmstrom et al., 1983) and C3 along the basement membrane zone (Donatsky and Dabelsteen, 1977; Ullman and Gorlin, 1978). Similar findings, although to a lesser

degree, have been noted in other studies (Van Hale et al., 1981; Reimer et al., 1983). The presence of IgM (Ullman and Gorlin, 1978; Reimer et al., 1983), IgA, and fibrin (Ullman and Gorlin, 1978) has been reported in subepithelial vessel walls in RAS, and IgG and fibrin have been noted within the basement membrane zone (Van Hale et al., 1981) of RAS lesions.

Although the pattern of immunoglobulins and complement in RAS lesion tissue may vary, the findings suggest that immune complex deposition may be a possible immune mechanism initiating the complement cascade and resulting in tissue destruction.

(h). Immunologic studies related to antibody-dependent cellular cytotoxicity

The possible role of antibody-dependent cellular cytotoxicity (ADCC) with respect to RAS was investigated by Greenspan et al. (1981). Utilizing chicken red blood cells coated with rabbit anti-chicken red blood cell antibodies as targets, cytotoxicity by peripheral leukocytes from patients during various stages of RAS was compared with that from sex- and age-matched paired controls. The leukocytes from patients with early aphthous lesions exhibited significantly higher ADCC than their matched controls, leading the authors to speculate that ADCC plays a role in the early stages of RAS. They proposed that other immune mechanisms such as the formation of immune complexes are involved in later stages.

(i). Immunologic studies related to total serum immunoglobulin levels in RAS

The concentration of serum immunoglobulins of the various classes has been investigated in RAS in order to ascertain if abnormal levels are present in the disorder.

Brody and Silverman (1969), using the single radial immunodiffusion technique of Fahey and McKelvey (1965), noted a decrease of serum IgA in 6 of 8 RAS patients. No quantitative figures were reported, however.

Lehner (1969c) estimated serum immunoglobulin concentrations in 70 patients with RAS lesions, and 30 normal individuals using the radial immunodiffusion technique of Mancini et al. (1963). Although increased levels of serum IgA and IgG were noted in patients with RAS as compared to normal controls, statistically significant increases were observed only in patients suffering from MjRAS. The author stated that the raised IgA and IgG levels were consistent with levels found in such autoimmune disorders as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, and ulcerative colitis. Lehner described the limitations of the immunodiffusion technique and stressed that the values reported should be regarded as estimates.

The study of Thomas et al. (1973) dealing with serum antibodies to food antigens in RAS patients established that the total serum IgG, IgA, and IgM concentrations were within the range of normal controls. The radial immunodiffusion technique described by Mancini et al. (1965) was utilized.

Ben-Aryeh et al. (1976), also using the radial immunodiffusion technique of Mancini et al. (1965), studied IgG and IgA serum levels in RAS patients during the exacerbation and remission of lesions, comparing their sera to healthy controls. The authors found similar levels in all groups, and no correlation between serum levels of IgA and IgG, and the clinical condition of the RAS patient.

Malmstrom et al. (1983) found that total serum IgA, IgG, IgM, and IgE levels in patients with MiRAS and MjRAS patients were within normal limits. Two of the 14 MiRAS patients studied had elevated concentrations of total IgE. The method of determining these results were not stated.

Scully et al. (1983) estimated serum IgE and IgD concentrations in 80 RAS patients, 20 patients with non-aphthous oral ulcerations, and 39 control subjects. A solid-phase radioimmunoassay technique (Ceska and Lundkvist, 1971) was utilized to determine total serum IgE levels, and a single radial immunodiffusion technique (Fahey and McKelvey, 1965) was used for IgD concentrations. Total serum IgE concentrations in RAS patients were significantly higher than those in the other two experimental groups studied. However, the IgE levels were not as great as levels of this immunoglobulin observed in patients with atopic disease (Johansson, 1967). Within the RAS group the serum IgE concentrations were similar in MiRAS, MjRAS, and HU patients. Serum IgD levels were also significantly elevated in the RAS group. Again MiRAS, MjRAS, and HU individuals were found to be similar.

Table I summarizes the findings of the above studies.

TABLE I: TOTAL SERUM IMMUNOGLOBULIN CONCENTRATIONS IN RAS

CONTROL GROUP		RAS GROUP		TECHNIQUE	AUTHORS
NUMBER OF SUBJECTS	CONCENTRATION OF IMMUNOGLOBULIN	NUMBER OF SUBJECTS	CONCENTRATION OF IMMUNOGLOBULIN		
30	Ig_A 175 mg/100 ml Ig_G 1286 mg/100 ml Ig_M 74 mg/100 ml	40	MiRAS Ig_A 242 mg/100 ml Ig_G 1434 mg/100 ml Ig_M 84 mg/100 ml	SINGLE RADIAL IMMUNODIFFUSION	LEHNER (1969c)
		15	MjRAS Ig_A 305 mg/100 ml Ig_G 1589 mg/100 ml Ig_M 86 mg/100 ml		
		15	HU Ig_A 264 mg/100 ml Ig_G 1369 mg/100 ml Ig_M 77 mg/100 ml		

50	MEAN ± S.D. I_gA 210 ± 82 mg/100 ml I_gG 1250 ± 280 mg/100 ml I_gM 143 ± 58 mg/100 ml	25	RAS MEAN ± S.D. I_gA 245 ± 168 mg/100 ml I_gG 1560 ± 469 mg/100 ml I_gM 165 ± 86 mg/100 ml	SINGLE RADIAL IMMUNODIFFUSION	THOMAS ET AL. (1973)
22	RANGE MEAN I_gA 120-460 mg/100 ml 280 mg/100 ml I_gG 830-1580 mg/100 ml 1210 mg/100 ml	21	RAS RANGE MEAN I_gA 180-410 mg/100 ml 300 mg/100 ml I_gG 900-1600 mg/100 ml 1280 mg/100 ml	SINGLE RADIAL IMMUNODIFFUSION	BEN-ARYEH ET AL. (1976)
NOT SPECIFIED	RANGE I_gA 90-450 mg/100 ml I_gG 800-1800 mg/100 ml I_gM 60-240 mg/100 ml I_gE <110 U/ml	20	RAS MEAN ± S.D. I_gA 238 ± 72 mg/100 ml I_gG 1308 ± 321 mg/100 ml I_gM 158 ± 110 mg/100 ml I_gE 68.65 ± 55.53 U/100 ml	NOT SPECIFIED	MALMSTROM ET AL. (1983)

39	HEALTHY CONTROLS IgE 15.2 U/ml	80	RAS GROUP IgE 31.2 U/ml	SOLID-PHASE RADIOIMMUNOASSAY	SCULLY ET AL (1983)
20	NON-APHTHOUS ULCERATION GROUP IgE 17.6 U/ml	(57) (17) (6)	(MiRAS IgE 30.9 U/ml) (MjRAS IgE 32.3 U/ml) (HU IgE 32.1 U/ml)		
39	HEALTHY CONTROLS IgD 6.0 ug/ml	80	RAS GROUP IgD 14.3 ug/ml	SINGLE RADIAL IMMUNODIFFUSION	
20	NON-APHTHOUS ULCERATION GROUP IgD 6.7 ug/ml	(57) (17) (6)	(MiRAS IgD 14.9 ug/ml) (MjRAS IgD 14.0 ug/ml) (HU IgD 13.8 ug/ml)		

(j). Immunologic studies related to total salivary immunoglobulin levels in RAS

As a means of evaluating the local immunologic status of the oral cavity in RAS, total salivary immunoglobulin concentrations have been studied.

Lehner (1969c) attempted to estimate total salivary immunoglobulin concentrations of the IgA, IgG and IgM classes in 22 MiRAS patients and 16 control patients. Stimulated whole saliva was concentrated 20 times and immunoglobulin levels were assessed by the radial immunodiffusion technique of Mancini et al. (1963). Salivary IgA concentrations in RAS patients were similar to the concentrations in normal controls. Lehner detected the presence of IgG in about 90% of whole saliva samples but comparisons were not made. IgM was not detected in any of the saliva samples.

Kakizawa et al. (1973) performed longtitudinal studies on salivary IgA concentrations in healthy subjects and patients suffering from RAS during various stages of the RAS lesions. Unstimulated whole saliva was evaluated for IgA levels using the radial immunodiffusion technique of Mancini et al. (1965). The authors were able to demonstrate, in RAS males, an increase in salivary IgA during early stages of the lesions, and a decrease during the later stages. In RAS females, salivary IgA concentrations decreased during recovery if the attacks were related to their menstrual cycle. If the lesions were not related to the menstrual cycle, no relationship between stages of ulceration and IgA in saliva was noted. Kakizawa and coworkers, however, did not

subject their results to statistical analysis, and exact quantities of salivary IgA in the RAS patients were not stated.

Ben-Aryeh et al. (1976) were unable to confirm the findings of Kakizawa et al.. Whole unstimulated saliva of 21 RAS patients, 10 in a remission stage, 11 in an acute exacerbation stage, were compared to whole unstimulated saliva of 22 healthy controls. Salivary IgA concentrations were assessed using the radial immunodiffusion techniques of Mancini et al. (1965). All three groups exhibited similar salivary IgA levels. Furthermore, a longtitudinal study involving RAS patients and healthy control subjects was conducted. No correlation between the clinical condition and salivary IgA concentrations was found.

Bennet and Reade (1982) assayed whole unstimulated saliva for IgA concentrations using an automated turbidimetric technique with the antibody-antigen reaction enhanced with polyethylene glycol. Unstimulated whole saliva was collected from 12 males suffering from MiRAS during stages of both exacerbation and remission, and 12 healthy control individuals. Levels were comparable in all three clinical situations.

Table II summarizes the findings of the above studies.

(k). Immunoenhancing and immunosuppressive drugs in the treatment of RAS

The majority of individuals experiencing MiRAS lesions have infrequent episodes, with solitary lesions. Since the lesions are self-limiting and run their course in 10 to 14 days, treatment is rarely instituted. However, in individuals who have

TABLE II: TOTAL SALIVARY IMMUNOGLOBULIN CONCENTRATIONS IN WHOLE UNSTIMULATED SALIVA IN RAS

CONTROL GROUP		RAS GROUP		TECHNIQUE	AUTHORS
NUMBER OF SUBJECTS	CONCENTRATION OF IMMUNOGLOBULIN	NUMBER OF SUBJECTS	CONCENTRATION OF IMMUNOGLOBULIN .		
16	IgA 13 mg/100 ml	22	MiRAS IgA 14.4 mg/100 ml	SINGLE RADIAL IMMUNODIFFUSION	LEHNER (1969c)
		10	MjRAS IgA 15 mg/100 ml		
		10	HU IgA 13 mg/100 ml		
	RANGE MEAN		RANGE MEAN		
22	IgA 2-19 mg/100 ml 8.5 mg/100 ml	10	RAS IN REMISSION STAGE IgA 3-16 mg/100 ml 9.3 mg/100 ml	SINGLE RADIAL IMMUNODIFFUSION	BEN-ARYEH ET AL (1976)
		11	RAS IN ACUTE EXACERBATION STAGE IgA 1.5-15 mg/100 ml 7.8 mg/100 ml		
12	IgA 7-21 mg/100 ml	12	MiRAS IN REMISSION STAGE IgA 6.0-26 mg/100 ml	AUTOMATED TURBIDIMETRIC	BENNET AND READE (1382)
		12	MiRAS IN ACUTE EXACERBATION STAGE IgA 6.0-26 mg/100 ml		

frequent attacks of MiRAS ulcers, or multiple lesions during any one episode, treatments have been attempted. Due to the numerous studies implicating an immunologic basis in the pathogenesis of RAS, clinical trials involving immunoenhancing and immunosuppressive drugs have been carried out.

The immunoenhancing drug levamisole has been investigated extensively with respect to RAS lesions, and its use has been reviewed by Miller (1980). Numerous open studies have revealed a high degree of beneficial results among patients (Verhaegen et al., 1973; Symoens and Brugmans, 1974; Olson et al., 1976; Van de Heynig, 1978).

However, double-blind placebo controlled studies have yielded conflicting results. Some investigations revealed no statistical improvement, or subjective improvement which could not be supported by statistical analysis (Drinnan and Fischman, 1978; Gier et al., 1978; Miller et al., 1978; Olson and Silverman, 1978). Other double-blind studies were able to establish positive improvement of lesions with the use of levamisole (Lehner et al., 1976; De Meyer et al., 1977; De Cree et al., 1978; Zissis et al., 1983).

However, the use of levamisole carries with it the biologic penalty of possible side-effects, the most common being dysgeusia, hyperosmia, and nausea (Symoens and Brugmans, 1974).

Immunosuppressive drugs have also been tested clinically due to their anti-inflammatory effect. Systemic and topical steroids have been shown to decrease the duration of RAS lesions and in some cases to decrease the incidence of lesions (Cooke and

Armitage, 1960; Zegarelli et al., 1960; Graykowski et al., 1966; Merchant et al., 1978; Yeoman et al., 1978; Pimlott and Walker, 1983).

Treatments of MiRAS, including those mentioned above, have been adequately reviewed by Antoon and Miller (1980). No universally effective therapy has yet been identified, and treatment often remains empirical.

(4). Quantities of Immunoglobulins in Normal Whole Human Saliva

The immunoglobulin content of whole saliva is a reflection of locally produced immunoglobulins transported and secreted by major and minor salivary glands. Serum immunoglobulins and locally produced mucosal immunoglobulins entering the oral cavity through surface and crevicular epithelium as transudative and exudative proteins also contribute, although to a lesser extent.

Numerous investigators have estimated the content of the various immunoglobulin classes in normal whole human saliva with varying results. Surveys of the literature indicate that the variation in results may derive in part from technical factors. There has been no standardization of the collection, concentration, and storage of saliva. In addition, differences in the quantitation techniques, and the use of different types of standard proteins in assessing the immunoglobulins (Lehner, 1969c; Brandtzaeg et al., 1970; Gronblad, 1982) contribute to the variation in results. Table III summarizes the methodology and findings of the studies reporting immunoglobulin concentrations of the IgA, IgG, IgM, and

TABLE III: IMMUNOGLOBULIN CONCENTRATIONS REPORTED FOR NORMAL WHOLE HUMAN SALIVA

IgA mg/100 ml		IgG mg/100 ml		IgM mg/100 ml		IgE			
RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	TECHNIQUE	AUTHORS
	14.2		11.1					a)	LEHNER AND CARDWELL (1967)
0-31.0	5.95	0-9.25	0.5	0-1.3	0.078			a)	ROWE ET AL (1968)
	16.0		0		0			a)	WALDMAN ET AL (1968)
	13							a)	LEHNER (1969c)
	4.5		0.31		1.06			b)	SALMON ET AL (1969)
	1.64							a)	SHKLAIR ET AL (1969)
14.2-29.3	19.4	0.48-2.46	1.44	0-0.46	0.21			a)	BRANDTZAEG ET AL (1970)
						0-240 ng/ml		b)	SALMON (1970)
						1-10 ng/ml		?	BENNICH AND JOHANSSON (1971)
						0-500 U/ml	47.2 U/ml	b)	OZKARAGOZ ET AL (1972)
0.42-5.02	1.86	0.48-2.21	1.36					a)	SIMS (1972)
7.8-240.0								a)	KAKIZAWA ET AL (1973)
3.3-32								a)	AARLI (1976)
2.0-19.0	8.5							a)	BEN-ARYEH ET AL (1976)
4.0-19.0	7.7							a)	MACH ET AL (1976)
7.4-28.7	14.0	0.2-2.5	1.04					a)	ARNOLD ET AL (1977)
0-45.2	14.5							a)	EVERHART ET AL (1977)
2.5-13.0	7.96							a)	BEN-ARYEH ET AL (1978)
	6.5		1.9					c)	COLE ET AL (1978)
	10.3							a)	DEMETRIOU ET AL (1978)
	6.4							a)	SMITH ET AL (1979)
	17.2							a)	SMITH ET AL (1979)
	7.7					0-3.50 U/ml	0.29 U/ml	b)	HYYPPA (1980)

IgA mg/100 ml		IgG mg/100 ml		IgM mg/100 ml		IgE		TECHNIQUE	AUTHORS
RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN		
	11.5							a)	RICHTER ET AL (1980)
5.2-35.0	12.0							a)	ALALUUSUA ET AL (1981)
5.2-26.0	9.0							c)	ALALUUSUA ET AL (1981)
3.8-30.0	10.0							b)	ALALUUSUA ET AL (1981)
5.0-48.0	13.7	0.72-3.7	1.60	0.17-0.78				b)	GRONBLAD (1981)
1.0-5.0								c)	MODEER ET AL (1981)
	2.12							c)	MORRIS ET AL (1981)
	3.3							c)	TWETMAN ET AL (1981)
3-26								e)	BENNET AND READE (1982)
	4.44							d)	BRATTHALL AND ELLEN (1982)
	2.27							a)	BRATTHALL AND ELLEN (1982)
	6.03							a)	D'AMELIO ET AL (1982)
1.5-23.9								d)	JOHNSON AND LIU (1982)
	7.7							d)	SORENSEN (1982)
1.35-8.5	4.4							a)	GUVEN AND DEVISSCHER (1983)
1.78-11.60	4.83							d)	WIDERSTROM AND BRATTHALL (1984)

a) SINGLE RADIAL IMMUNODIFFUSION
b) SOLID-PHASE RADIOIMMUNOASSAY
c) IMMUNOELECTROPHORESIS
d) IMMUNOBEAD ENZYME-LINKED IMMUNOSORBENT ASSAY
e) AUTOMATED TURBIDIMETRIC TECHNIQUE

IgE classes in normal whole human saliva.

(5). Factors Affecting Salivary Immunoglobulin Concentration

The concentration of salivary immunoglobulins are influenced by numerous physiologic and pathologic conditions.

Mandel and Khurana (1969) and Brandtzaeg (1971) observed a decrease in salivary IgA concentration after stimulation of salivary flow. This was confirmed by Gronblad (1982) who noted that IgG levels in stimulated whole saliva did not change significantly as compared to unstimulated whole saliva, whereas IgA and IgM levels decreased after stimulation.

Widerstrom and Bratthall (1984) studied salivary IgA concentrations during pregnancy and the various phases of the menstrual cycle. IgA levels in saliva were found to be increased in pregnant females as compared to non-pregnant controls, with markedly elevated levels during the third trimester and immediate post partum periods. No significant differences in salivary IgA concentrations were noted during the three phases of the menstrual cycle. This latter finding contrasted that of Kakizawa et al. (1973) who found both increases and decreases of salivary IgA in females during the menstrual cycle.

Richter et al. (1980) found significant changes in salivary IgA levels during the course of a day with a minimum average at 11 a.m., and a maximum at 5 p.m.. However, a longtitudinal study performed by Bennet and Reade (1982) could not show any relationship between time of day and salivary IgA concentrations in whole

unstimulated saliva. This latter study also investigated salivary IgA levels in tobacco smokers and non-smokers. No statistical difference in salivary IgA concentrations was detected between a group of individuals that smoked more than 20 cigarettes a day for 20 years and a matched control group. However, a highly defined tobacco smoking group of individuals that smoked in excess of 20 cigarettes a day for at least 40 years was found to have a significant decrease in salivary IgA levels when compared to a matched control group.

The use of medications may affect the concentration of salivary immunoglobulins. Phenytoin (diphenyldantoin), an antiepileptic compound, has been found in certain studies to reduce IgA levels in saliva (Aarli and Tonder, 1975; Seager et al., 1975; and Aarli, 1976). However, more recent investigations (Smith et al., 1979; Modeer et al., 1981) were unable to confirm this IgA decrease during treatment with phenytoin.

The relationship of salivary immunoglobulin levels to oral and systemic diseases has been investigated by numerous authors. Normal, decreased, and elevated levels of salivary IgA in whole saliva have been reported in studies involving caries susceptible individuals (Brandtzaeg, 1975; Everhart et al., 1977; Twetman et al., 1981). Elevated levels of IgA in whole saliva, directly related to the severity of periodontal inflammation, have been observed in the vast majority of studies involving periodontitis, as reviewed by Guven and De Visscher (1983). The studies of Waldman et al. (1973) and Hyyppa (1980) demonstrated that elevated salivary IgE levels occurred more frequently in individuals

suffering from extrinsic asthma than in normal healthy control subjects.

(6). Role of immunoglobulins in saliva

The secretion of immunoglobulins into saliva is only one component of the body's secretory immune system. The majority of studies undertaken on secretory immunity have concentrated on secretions into the gut, and stimulation of secretory immune responses through Peyer's patches (Bienenstock and Befus, 1980; Tomasi, 1982). Relatively few studies have placed specific emphasis on the control of secretion and activities of salivary immunoglobulins, and tend to concentrate on salivary IgA and its role in the etiology of caries and periodontal disease.

In saliva IgA and IgM are selectively transmitted through, and actively secreted by salivary glands predominantly as 11S IgA (secretory IgA) and 19S IgM molecules (Brandtzaeg, 1971). These polyvalent antibodies containing a secretory piece which confers resistance to proteolytic degradation, represent a first line of defense against exogenous antigens (Brandtzaeg, 1966; Brandtzaeg et al., 1970; Tomasi, 1972).

IgA, in a monomeric 7S form, IgM without a secretory piece, and IgG are also present in saliva as exudative and transudative serum proteins passively transmitted in small amounts through glandular, surface, and gingival crevicular epithelium (Brandtzaeg, 1965). An increase in the quantities of IgA, IgG, and IgM in whole saliva can be observed in patients suffering from

gingivitis and periodontitis due to an increase in the inflammatory exudate through the gingival crevice (Lindstrom and Folke, 1968; Brandtzaeg et al., 1970).

Whether IgE and IgD are transferred into saliva remains uncertain. Although an active glandular transfer and secretion of IgE has been suggested by the studies of Bennich and Johnson (1971), and Ozkaragoz et al. (1972), the mechanism is not comparable to that for secretory IgA and IgM (Waldman et al., 1973). The presence of IgD in whole saliva has only recently been demonstrated (Sewell et al., 1979). No theory on the transfer mechanism of this immunoglobulin into saliva has been proposed.

Secretory IgA and, to a lesser extent, IgM, represent the principal mediators of humoral immunity in saliva. Numerous protective roles have been attributed to these immunoglobulins in saliva and other secretions including antiviral activity, antibacterial activity, and inhibition of absorption of non-viable antigens (Tomasi, 1982).

The virus-neutralizing ability of secretory IgA and IgM is well established (Brandtzaeg et al., 1970; Tomasi, 1972; Hanson and Brandtzaeg, 1973).

Antibacterial activity by secretory immunoglobulins, although not as well documented, is also recognized. The coating and agglutination of bacteria by secretory IgA have been reported by numerous authors (Brandtzaeg et al., 1968; Newcomb and de Vald, 1969; McClelland et al., 1972; Williams and Gibbons, 1972). Secretory IgA is able to complex with bacterial glycoproteins, thus impairing attachment and colonization of the bacteria to

mucosal and hydroxyapetite surfaces (Bratthall and Carlen, 1978). Secretory IgA may complex with proteins on the surface of oral epithelium and teeth giving rise to a protective immunoglobulin coat (Heremans et al., 1966). It is not certain that secretory IgA can promote phagocytosis of bacteria through opsonization. Girard and de Kalbermatten (1970), Knop et al. (1971), and Tomasi (1972) reported opsonization of Escherichia coli in the gut by secretory IgA with subsequent phagocytosis, however, other authors (Eddie et al., 1971; Wilson, 1972; Zipursky et al., 1973) were unable to attribute such activity to IgA. The phagocytosis promoting effect of IgM antibodies has long been recognized (Rowley and Turner, 1966). Although IgA is unable to fix complement via the classic pathway (Ishizaka et al., 1966; Vaerman and Heremans, 1968), Adinolfi et al. (1966) and Burdon (1973) were able to demonstrate that secretory IgA, in the presence of salivary enzymes, can exhibit a bactericidal effect. This has led to the speculation that activation of complement via the alternate pathway may be involved. IgM in conjunction with complement and lysozyme can result in the lysis of bacteria (Glynn, 1969). In saliva, complement factors are available from gingival crevicular fluid (Brandtzaeg, 1966; Shillitoe and Lehner, 1972; Larsson et al., 1973).

Immunoglobulins in saliva may also inhibit the absorption of non-viable antigens across mucous membranes. It has been proposed that secretory antibodies with specificity for food antigens limit absorption and access of these antigens to the general circulation through the formation of non-absorbable antigen-antibody complexes

(Tomasi, 1972). Hanson and Brandtzaeg (1973) have reviewed the studies which indicate that some individuals with IgA hypogammaglobulinemia exhibit an increased permeability to and absorption of food and milk proteins with subsequent increases in circulating immunoglobulins specific to these antigens.

IgG immunoglobulins in secretions are not afforded the protection of the secretory piece against protease degradation. However, the relatively high concentrations of IgG in whole saliva, and the presence of complement factors has led Brandtzaeg (1975) to speculate on the potential for biological amplification of IgG-antigen reactions in whole saliva, especially within the gingival crevice. These protective reactions include virus and toxin neutralization, enzyme inhibition, allergen blocking, bacteriolysis, chemotaxis and opsonization.

The roles of IgE and IgD in saliva are uncertain. It has been shown that salivary IgE concentrations are elevated in asthmatics (Hyyppa, 1980), and that gingivitis scores are greater in asthmatics than healthy controls when plaque indices are comparable (Hyyppa et al., 1979). This has led to the speculation that the presence of IgE in saliva may be associated with immediate type allergic reactions involving the oral mucosa and gingiva (Hyyppa, 1980). The function of IgD in saliva is unknown.

(7). Proteases affecting immunoglobulins in saliva

Enzymes abound in whole human saliva and originate, in the most part, from oral microorganisms (Nakamura and Slots, 1983).

Plaut et al. (1974) demonstrated that Streptococcus sanguis produces an enzyme, present in saliva, which is capable of cleaving IgA in the hinge region. Kilian et al. (1983) were able to identify numerous other bacteria, including Streptococcus mitior and the suspected bacterial etiologic agents in periodontal disease, which produce an enzyme with a similar IgA protease activity. Most strains of the oral organisms Bacteroides gingivalis, and all strains of Bacteroides intermedius possess a particularly potent IgA protease activity (Kilian et al., 1983). Furthermore, the proteases from certain strains of Bacteroides gingivalis and Bacteroides melaninogenicus are capable of complete degradation of IgA1, IgA2, secretory IgA, and polyclonal IgG (Kilian, 1981).

Kilian and coworkers (1983) were able to inactivate all IgA_1 proteases from the numerous bacteria studied with either 0.125 M EDTA, or 0.01 M bathocuproine disulfonate. Traditional serine protease inhibitors such as phenylmethylsulfonylfluoride, diisoproprylfluorophosphate, and aprotinin were found to be ineffective.

Proteases in saliva which cleave IgM, IgE, or IgD have not been studied.

CHAPTER III

METHODS AND MATERIALS

(1). Subject Selection

Twenty-eight MiRAS patients, exhibiting one or more MiRAS lesions in the ulcerative stage, were selected to participate in this study. Twenty-eight individuals with a negative history of RAS, matched to the MiRAS patients with respect to age and sex, were selected as control subjects.

(2). Clinical Examination

Certain physiologic and pathologic phenomena have been associated with increased levels of salivary IgA including pregnancy (Wilderstrom and Bratthall, 1984), tobacco smoking (Bennet and Reade, 1982), the presence of caries (Brandtzaeg, 1975; Everhart et al., 1977; Twetman et al., 1981), and the presence of periodontal disease (Guven and De Visscher, 1983). As a consequence clinical examination and subject history verified the presence or absence of these factors. The oral cavities of all participants were examined, and the number of carious teeth, and the Gingival Index (Loe, 1967) score were recorded for each subject. In the test group the presence of MiRAS lesions were verified.

(3). Subject History

Each subject was asked to give details regarding any allergies, systemic diseases, tobacco smoking habits, use of medications, and pregnancy status. Suspected precipitating factors involving the onset of MiRAS lesions were also noted.

(4). Collection of saliva

Whole unstimulated saliva was collected from each subject using the following protocol. The subject, seated at a table, swallowed all saliva present in the mouth. With the head tilted forward and the mouth open very slightly, saliva was allowed to drip from the lower lip into a graduated tube, the lower half of which was immersed in ice water. Upon collection of 3 ml of saliva in this manner, the subject forcefully expectorated the saliva remaining in the mouth in order to collect the viscous minor salivary gland secretions. Since one group of investigators (Richter et al., 1980) found changes in salivary IgA levels during a 24 hour period, the saliva from the paired age- and sex-matched individuals were collected at the same time of day.

(5). Treatment and Storage of Saliva

Each saliva sample was vortexed for one minute. Five-hundred microlitre aliquots of saliva were each diluted with 400 ul of PBS buffer (0.008 M Na_2HPO_4, 0.0018 M KH_2PO_4, 0.137 M NaCl, and 0.1% NaN_3 pH 7.4) with 0.281 M EDTA. The saliva solutions were stored at -70°C in 1.5 ml polypropylene eppendorf tubes (Sybron/Brinkman Instruments, Rexdale, Ontario) for less than 6 months.

(6). Preparation of a Secretory IgA Standard

(a). Materials:

Human colostrum

BioRad protein macro-assay kit

0.45 um filter, Sybron/Nalge Nalgene Labware

Amicon Diaflo ultrafilter system with 100,000 mol wt filter

Sephadex G-200 (fine)

DEAE Sephacel

Rabbit immunoglobulin to human colostrum IgA (α chains and secretory component) Lot: 0198 DAKO-immunoglobulin Ltd.

Rabbit immunoglobulin to human colostrum IgA specific for α chain Lot: 095 DAKO-immunoglobulin Ltd.

Rabbit immunoglobulin to human IgM (μ chains) Lot: 013A DAKO-immunoglobulin Ltd.

Rabbit immunoglobulin to human IgG (γ Fc fragment) Lot: 111A DAKO-immunoglobulin Ltd.

Antiserum (goat) to human serum Lot: 8030F003A Hyland Diagnostics

Molecular weight protein standards for SDS gel electrophoresis Lot: 28037 BioRad Laboratories.

(b). Method:

The secretory IgA standard was isolated from human colostrum using a modification of the procedure set forth by Johnstone and Thorpe (1982) which describes the isolation of secretory IgA from rabbit colostrum. A secretory IgA standard with no contamination was the primary objective during the isolation procedure. Therefore, high yield of the immunoglobulin was sacrificed to a certain extent to ensure a product of high purity.

(i). Clarification of human colostrum

Human colostrum from three subjects was collected less than three days post partum and pooled, as the concentration of secretory IgA in human colostrum decreases rapidly after 72 hours (Ogra and Ogra, 1978).

The pooled colostrum was mixed with a one-half volume of 0.9% NaCl and centrifuged at 100,000 g for 1 hour at 4°C. The clear aqueous layer between the floating fatty layer and the pellet was removed. In order to remove milk proteins such as casein, the solution was stirred, brought to a pH of 4.0, and centrifuged at 30,000 g for 30 min. at 4°C. The pellet was discarded and the supernatant neutralized with 2 M tris. The solution was again centrifuged in this same manner and the supernatant passed through a 0.45 um filter.

The protein concentration of the filtered solution was established using the BioRad protein macro-assay technique and concentrated to an approximate protein concentration of 20 mg/ml using

an Amicon Diaflo ultrafiltration system with a 100,000 mol wt filter. The concentrated solution was centrifuged at 650 g for 10 minutes at 4°C, and the pellet was discarded. This supernatant, clarified colostrum, represented the source of secretory IgA to be separated by gel filtration and ion exchange chromatography. Its optical density at 280 nm, and its protein concentration determined by the BioRad protein macro-assay technique, were established. The presence of secretory IgA and other immunoglobulins was tested by the Ouchterlony double diffusion technique. The antisera used included rabbit antisera to human colostrum IgA and secretory component, human IgA α chains, human IgM μ chains, human IgG γ Fc fragments, and goat antiserum to human serum.

(ii). Gel filtration chromatography of clarified colostrum

A 2.6 cm (internal diameter) x 100 cm column was packed at 4°C with 500 ml wet settled volume of Sephadex G-200 (fine) in 0.1 M sodium phosphate buffer pH 6.8 with 0.1% sodium azide. Calibration of the column using blue dextran, partially purified IgG, bovine serum albumin, ovalbumin, and RNAase at various flow rates, volumes, and loads was conducted to establish a maximum flow rate, sample volume, and sample protein load. Five millilitres of the clarified colostrum was applied to the G-200 column and eluted with 0.1 M sodium phosphate buffer pH 6.8 with 0.1% sodium azide at 4°C. The flow rate was 18 ml/hr and 4 ml fractions were collected. The absorbance of the eluate was monitored at 280 nm and the proteins were collected as a series of

four peaks. Various fractions within peaks 1, 2, 3 and 4 were tested for the presence and identification of immunoglobulins using the Ouchterlony method described above. The fractions of peak 1 containing secretory IgA were pooled. Peak 1 was dialyzed against 0.01 M sodium phosphate buffer pH 7.5 using dialysis buffer volume 50 times the sample volume. The dialysis buffer was changed three times over three days. The dialyzed sample was centrifuged at 650 g for 10 min at 4°C and the pellet was discarded. This supernatant, treated G-200 peak 1, was characterized by optical density (at 280 nm), protein concentration (BioRad), and presence of immunoglobulins using the Ouchterlony method described above.

(iii). Preparation of ion exchange column

Twenty millilitres of wet settled volume of DEAE Sephacel was activated with 30 ml of 0.50 M sodium phosphate buffer pH 7.5 for 8 hours at room temperature. The activating buffer was replaced with a starting buffer of 0.01 M sodium phosphate buffer pH 7.5. This ion exchange gel was packed into a column measuring 1.5 cm (internal diameter) X 21 cm. The ion exchanger was washed with the starting buffer for 16 hours at room temperature at a flow rate of 20 ml/hr to equilibrate the gel.

To ensure that both the treated G-200 peak 1 sample and the ion exchanger were fully equilibrated, the electrical conductivity and pH of the sample, the eluting buffer, and the eluate buffer were measured. Electrical conductivity values and pH values were verified to be the same for all solutions.

(iv). Ion exchange chromatography of treated G-200 peak 1 sample

Forty millilitres of equilibrated treated G-200 peak 1 sample, at a concentration of 0.46 mg/ml, was applied to the DEAE Sephacel column. The column was eluted with the starting buffer at room temperature at a flow rate of 20 ml/hr. Fractions of 2.5 ml were collected and absorbance was monitored until no further proteins were eluted.

This procedure was repeated with eluting buffer of increasing molarities of sodium chloride in phosphate buffer pH 7.5. The molarities of NaCl were 0.05, 0.10, 0.15, 0.25, and 0.50. All peaks obtained from elutions with optical density values at 280 nm greater than 0.20 were tested for the presence of immunoglobulins using the Ouchterlony method as outlined above.

(v). Treatment of DEAE fractions containing secretory IgA

The fractions from DEAE peaks produced by NaCl molarities of 0.10, 0.15, and 0.25 were pooled and labelled DEAE peak 2, DEAE peak 3, and DEAE peak 4 respectively. These solutions were lyophilized and resuspended in 2.5 ml PBS buffer pH 7.4. The three solutions were dialyzed against PBS buffer pH 7.4 as described above. The three samples were then centrifuged at 650 g for 10 min at 4°C and the pellets were discarded. Optical density values and protein concentrations were recorded for the three peaks. A generalized scheme of the secretory IgA isolation

procedure is outlined in Table IV.

(vi). SDS-PAGE analysis of clarified colostrum, G-200 and DEAE fractions

To further assess the purity of the secretory IgA isolated from human colostrum, an SDS-PAGE analysis of the various substrates and fractions collected during the gel filtration and ion exchange chromatographies was performed. Molecular weight protein standards for the SDS-PAGE analysis (BioRad) consisted of lysozyme (mol wt 14,400), soybean trypsin inhibitor (mol wt 21,500), carbonic anhydrase (mol wt 31,000), ovalbumin (mol wt 45,000), bovine serum albumin (mol wt 66,200), and phosphorylase B (mol wt 92,500). Human monoclonal IgM derived from the serum of a Waldenstrom's macroglobulinemic patient, and purified pooled human IgG, were also used as protein standards.

The SDS-PAGE analysis employed a 6% stacking gel and a 12% separation gel.

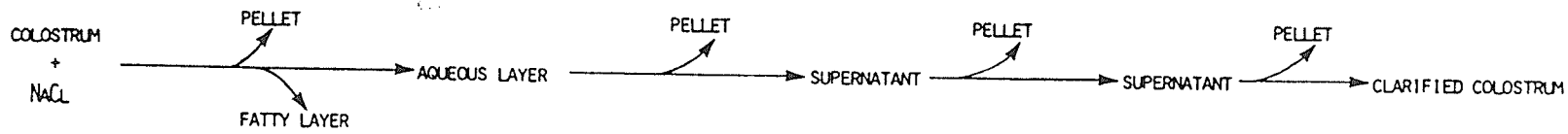
(7). Assay of Immunoglobulins in Saliva

(a). Treatment of saliva

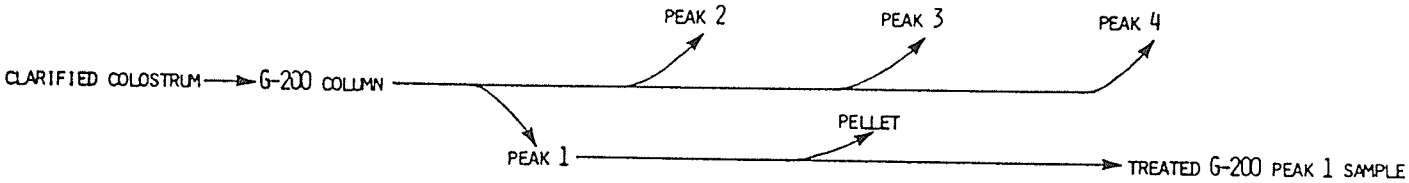
The saliva samples to be assayed were thawed and centrifuged at 6,500 g for 15 minutes. To 450 ul of the saliva sample supernatants were added 50 ul of PBS buffer pH 7.4 containing 1% gelatin and 0.125 M EDTA. The resulting saliva samples represented one-half dilutions of centrifuged whole human saliva in PBS

TABLE IV: ISOLATION OF SECRETORY IgA FROM HUMAN COLOSTRUM: GENERALIZED SCHEME

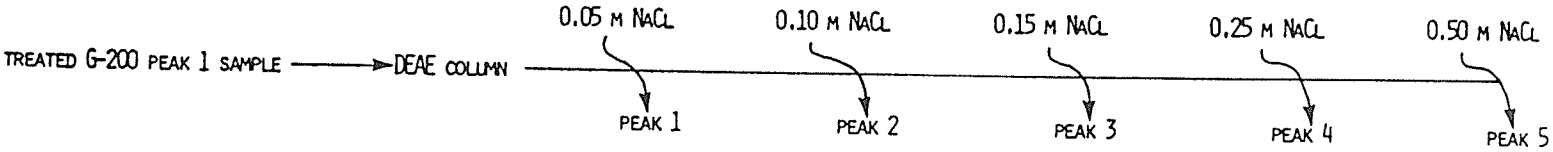
CLARIFICATION OF HUMAN COLOSTRUM



GEL FILTRATION CHROMATOGRAPHY



ION EXCHANGE CHROMATOGRAPHY



SPECIFIC DETAILS CAN BE OBTAINED BY REFERENCE TO TEXT

buffer pH 7.4 containing 0.1% gelatin and 0.125 M EDTA.

(b). Solid-phase radioimmunoassay technique

The saliva samples were tested in duplicate for their content of IgA, IgG, IgM, and IgE immunoglobulins by a solid-phase radioimmunoassay technique (Delespesse et al., 1983). Briefly, for IgA and IgG assays, polyvinyl microtitre plates (Dynatech Diagnostics, S. Windham, Maine) were soaked in 75% acetic acid for 1 hour and thoroughly washed with double distilled water. The wells were then coated overnight with the IgG fraction of sheep anti-human γ or α chains at 50 ug/ml in PBS buffer pH 7.4. IgM and IgE were assayed by coating overnight the polyvinyl microtitre plates with the IgG fraction of sheep anti-human μ chains, or a mouse monoclonal antibody specific to human IgE (clone CIA/E/7.12, a gift from Dr. A. Saxon, UCLA, Los Angeles, California) at 50 ug/ml 0.01 M sodium bicarbonate buffer pH 9.0.

After the overnight incubation at room temperature, the anti-human immunoglobulins were discarded and any uncoated well sites were blocked with 1% gelatin in PBS buffer pH 7.4 for 2 hours. The wells were then thoroughly washed with PBS buffer pH 7.4. Two-hundred microlitres of purified human secretory IgA (Materials and Methods (6)), purified polyclonal serum IgG, purified polyclonal serum IgM (a gift from Dr. F. Paraskevas, University of Manitoba, Winnipeg, Manitoba), and IgE from a pool of reaginic human sera calibrated against the standards included in the Pharmacia kit for serum IgE determination (P.R.I.S.T., Pharmacia Diagnostics, Uppsala, Sweden) were used as standard immunoglobu-

lins. These standard immunoglobulins were diluted in an assay buffer (0.1% gelatin and 0.125 EDTA in PBS buffer pH 7.4) and placed in the wells at different concentrations to establish standard curves. Two hundred microlitres of the saliva samples in assay buffer at dilutions appropriate to the level of immunoglobulins in saliva (1/500 and 1/2000 for IgA, 1/200 and 1/800 for IgG, 1/100 and 1/550 for IgM, and 1/2 for IgE) were placed in the wells in duplicate.

After an overnight incubation at room temperature, the wells were thoroughly washed with PBS buffer pH 7.4 and then incubated overnight with 200 ul of ^{125}I-labelled affinity chromatography-purified sheep antibodies to human γ, α, or μ chains, or with 200 ul of ^{125}I-labelled anti-human IgE (anti Dϵ2, Pharmacia Diagnostics, Uppsala, Sweden), at 58,000-64,000 cpm/well, radioiodinated by the chloramine-T method (Klinman and Taylor, 1969). The wells were thoroughly washed with PBS buffer pH 7.4 and counted in a gamma counter.

With the exception of the standard secretory IgA, IgM, and IgE immunoglobulins, the sources of which have been noted above, all other immunoglobulins and specific antibodies were supplied by Dr. G. Delespesse. These immunoglobulins and antibodies had been prepared using methods described by Gausset et al. (1976), with the antibodies having been produced in sheep rather than rabbits.

(c). Specificity of solid-phase radioimmunoassay

The specificity of the radioimmunoassays was tested by demonstrating the absence of reactivity (in terms of bound

radioactivity) with unrelated purified immunoglobulins employed at a concentration of 10 ug/ml.

(d). Determination of valid region of linear portion of standard curve

A dilution curve of centrifuged normal whole human saliva, diluted in the assay buffer, was performed with the IgA, IgG, and IgM assays. Only the linear portion of the standard curve which was parallel with the linear portion of the saliva dilution curve was considered to be valid for immunoglobulin concentration calculations.

(e). Calculation of immunoglobulin concentrations in saliva

The counts per minute detected in the duplicate saliva sample wells were related to the valid portion of the standard curve to determine the diluted saliva immunoglobulin content. The mean value of the duplicates was multiplied by the dilution factor to yield the immunoglobulin concentration of the saliva sample. Two different dilutions of each sample in the IgA, IgG, and IgM assays were performed to ensure a greater probability that one dilution would fall on the valid portion of the standard curve. Only one dilution of each sample (1/2) was performed in the IgE assay due to its minimal presence in saliva.

(8). Assay of Specific Antibody Activity in Saliva

(a). Treatment of saliva

The saliva samples to be assayed were thawed and centrifuged at 6,500 g for 15 minutes. To 90 ul of the saliva sample supernatants were added 110 ul of PBS buffer pH 7.4 with 0.125 M EDTA. The resulting saliva samples represented 1/4 dilutions of centrifuged whole human saliva in PBS buffer pH 7.4 containing 0.125 M EDTA.

(b). Double antibody radioimmunoassay technique

Duplicate saliva samples were tested by a double antibody radioimmunoassay technique, as described by Platts-Mills et al. (1978) with modification, for their content of IgA antibodies specific to alpha-lactalbumin.

Briefly, 50 ul of centrifuged saliva supernatant at a 1/4 dilution in PBS buffer pH 7.4 with 0.125 M EDTA were placed in the wells of polyvinyl microtitre plates. Fifty microlitres of a ^{125}I-labelled bovine alpha-lactalbumin (Sigma Chemical Co., St. Louis, MO., Lot 52F-80751) solution, radioiodinated by the chloramine-T method (Klinman and Taylor, 1969) and diluted in an assay buffer (0.02 M phosphate, 0.14 M NaCl, 0.1% NaN_3, 0.05 M EDTA, 0.5% tween 20, and 0.1% BSA at pH 7.4) were added to the wells. This solution contained unlabelled carrier alpha-lactalbumin at an excess concentration sufficient to ensure complete saturation of all antibody sites in the saliva samples. The plates were incubated, with agitation, at room temperature over-

night.

Preliminary experiments involving test and control saliva samples with maximum and minimum total IgA levels established the concentration range of anti-human α chain antibody necessary to produce maximal precipitation of the salivary IgA within the zone of equivalence. Therefore, 50 ul of an IgG fraction of sheep anti-human α chains, supplied by Dr. G. Delespesse, at dilutions of 12.62, 6.31, 3.15, and 1.58 mg/ml in the assay buffer were added to the wells. These antibodies had been prepared using methods described by Gausset et al. (1976) in sheep rather than rabbits. The plates were agitated for 4 hours at room temperature and then set still overnight at 4° to allow precipitates to form. The use of four dilutions of sheep anti-human α chain antibody ensured maximal precipitation of the double antibody complex per saliva sample. The precipitates were washed three times with the assay buffer and transferred to new tubes for counting in a gamma counter.

Control IgA was derived from the screening of human sera obtained from the Clinical Immunology Laboratory at Brussels University Hospital. The sera from fifty-nine individuals, at a 1/40 dilution, were tested for the presence of alpha-lactalbumin specific IgA using the method described above. The serum exhibiting the greatest cpm value was used as the control in the saliva assay.

(c). Specificity of double antibody radioimmunoassay

The specificity of the radioimmunoassay was tested by

the employment of a competitive inhibition test. The assay was performed with a 100-fold increase in unlabelled alpha-lactalbumin added to the radiolabelled antigen solution before the solution was incubated with the saliva and serum samples. The absence of radioactive counts indicated a specific competitive inhibition by the unlabelled alpha-lactalbumin within the precipitates formed.

(d). Assessment of antibody activity to alpha-lactalbumin

The mean counts per minute detected in the duplicate serum and saliva precipitates were recorded at the various anti-IgA dilutions. The greatest mean cpm value for each sample was assumed to be within the zone of equivalence of the antigen-antibody precipitin curve. From this value the background counts per minute were subtracted resulting in a numerical representation, in counts per minute, of the antibody activity in each sample to alpha-lactalbumin.

CHAPTER IV

STATISTICAL TREATMENT OF DATA

The range, mean, standard deviation and standard error were determined for the total IgA, total IgG, and total IgM concentrations in the unstimulated whole saliva of the individuals involved in the study.

An unpaired Student's t-test was used to compare the total IgA, total IgG, and total IgM concentrations in the unstimulated whole saliva of the MiRAS group to the control group.

A paired Student's t-test was used to compare the total IgA, total IgG, and total IgM concentrations in the unstimulated whole saliva of the MiRAS individuals to the control individuals paired by age, sex, and time of day saliva collection.

CHAPTER V

RESULTS

(1). Subject Clinical Examination and History

The 28 MiRAS patients selected for this study consisted of 7 males and 21 females. This group was matched, by age and sex, with 28 control individuals without a history of RAS.

All subjects, test and control, had less than five carious teeth each, and a Loe's Gingival Index score of less than 1.0. Subjects with a tobacco habit smoked less than 20 cigarettes a day. None of the females in this study were, to their knowledge, pregnant, and none were within a six month postpartum period. Table V summarizes additional information gathered from the subjects' histories.

(2). Isolation of Secretory IgA from Human Colostrum

The results of secretory IgA isolation from human colostrum using gel filtration chromatography and ion exchange chromatography are summarized in graphs 1 and 2, and plates 1, 2, 3, 4 and 5.

The profile and locations of significant fractions from the separation of the clarified colostrum on a Sephadex G-200 column are shown in graph 1. The clarified colostrum and various fractions from the gel filtration chromatography were subjected to Ouchterlony double diffusion and SDS-PAGE analyses in order to ascertain the presence and identity of immunoglobulins.

Double diffusion analysis (Plate 1) showed that the clarified colostrum contained immunoglobulins of the IgG, IgM, and IgA classes (Plate 1(a)), whereas the fractions 40, 41, 44, and 52

TABLE V: ADDITIONAL INFORMATION FROM THE HISTORIES OF STUDY SUBJECTS

	CONTROL GROUP		RAS GROUP	
	MALES (7)	FEMALES (21)	MALES (7)	FEMALES (21)
SUSPECTED FACTORS PRECIPITATING MiRAS			STRESS 5 FATIGUE 1	STRESS 14 FOODS 8 (TOMATOES 4) (ORANGES 3) (PICKLES 1) FATIGUE 2 TRAUMA 2 MENSTRUATION 2
TOBACCO SMOKING HABIT	1	5	1	12
MEDICATIONS		ORAL CONTRACEPTIVES 7		ORAL CONTRACEPTIVES 7
ALLERGIES		DETERGENTS 3 PENICILLIN 2 CODEINE 1 A.S.A. 1 POLLEN 1 SULFONAMIDES 1	POLLEN 2	SULFONAMIDES 4 PENICILLIN 2
SYSTEMIC DISEASES		ANEMIA 1		RHEUMATOID ARTHRITIS 1

GRAPH 1: SEPARATION OF CLARIFIED COLOSTRUM USING SEPHADEX G-200 GEL FILTRATION CHROMATOGRAPHY

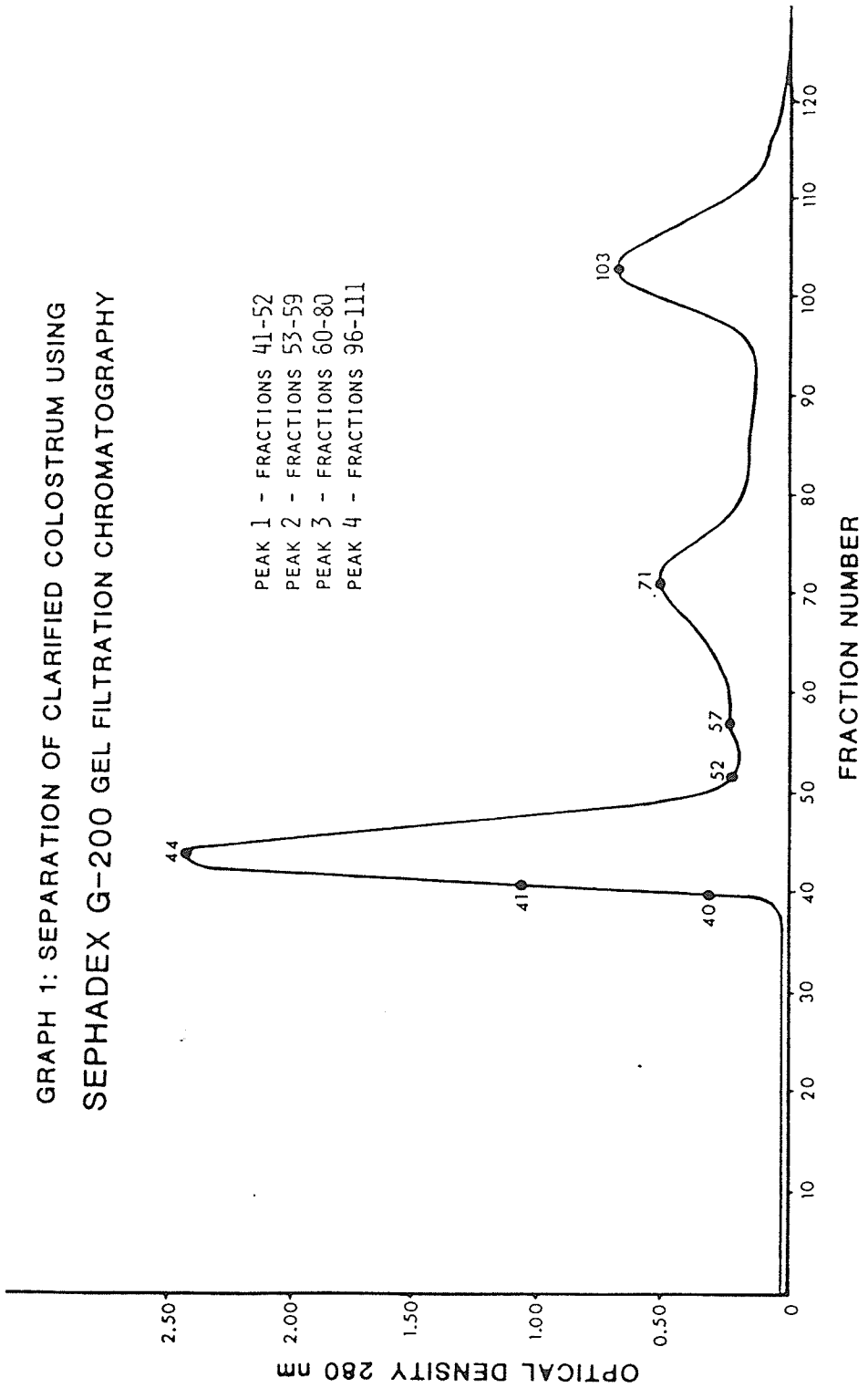
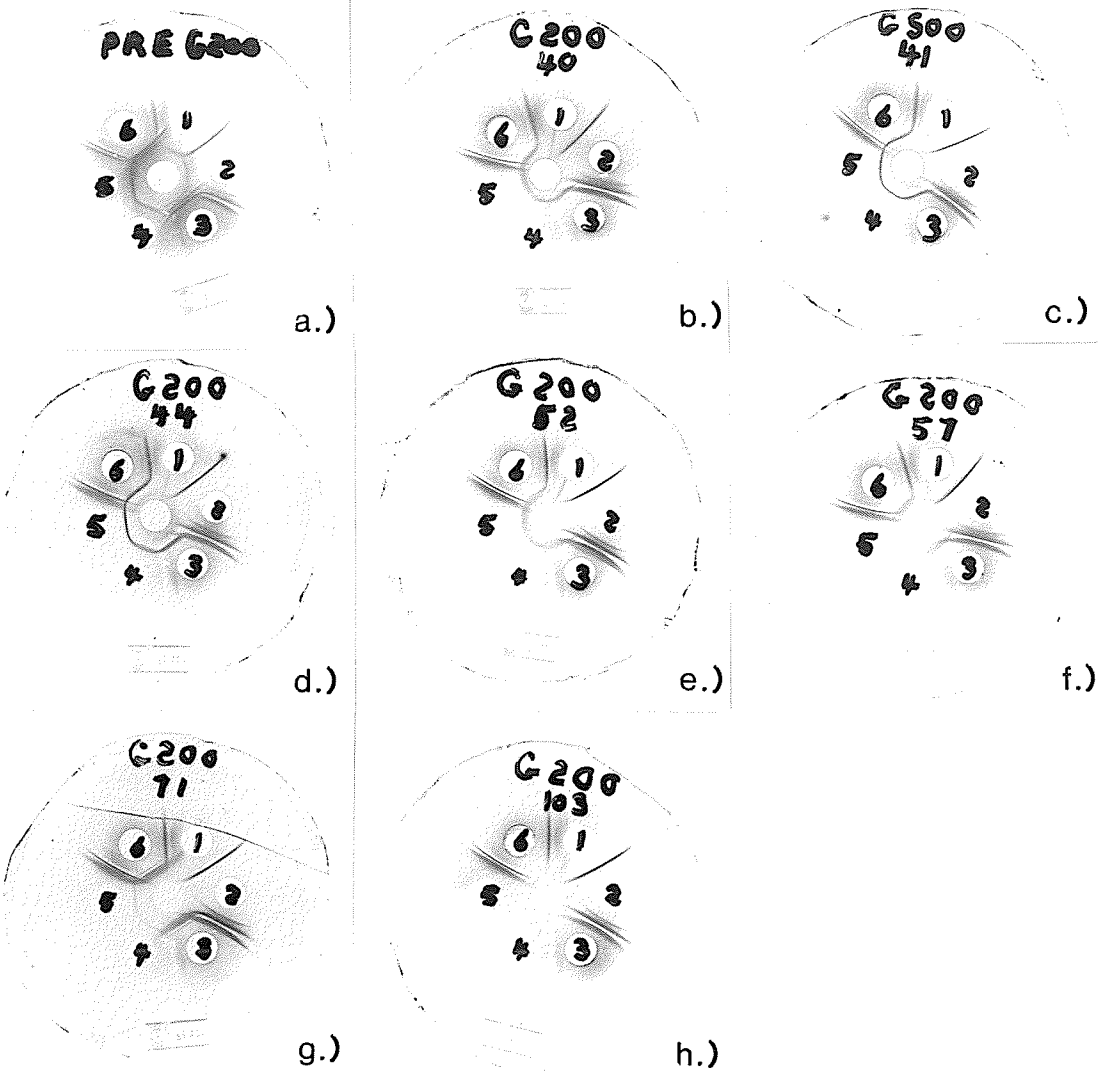


PLATE 1: OUCHTERLONY DOUBLE DIFFUSION ANALYSIS OF CLARIFIED COLOSTRUM AND VARIOUS G-200 FRACTIONS



WELL 1 - ANTISERA TO HUMAN IgG
WELL 2 - ANTISERA TO HUMAN IgM
WELL 3 - ANTISERA TO WHOLE HUMAN SERUM
WELL 4 - ANTISERA TO HUMAN IgA
WELL 5 - ANTISERA TO HUMAN IgA AND SECRETORY COMPONENT
WELL 6 - ANTISERA TO WHOLE HUMAN SERUM

CENTRE WELLS:

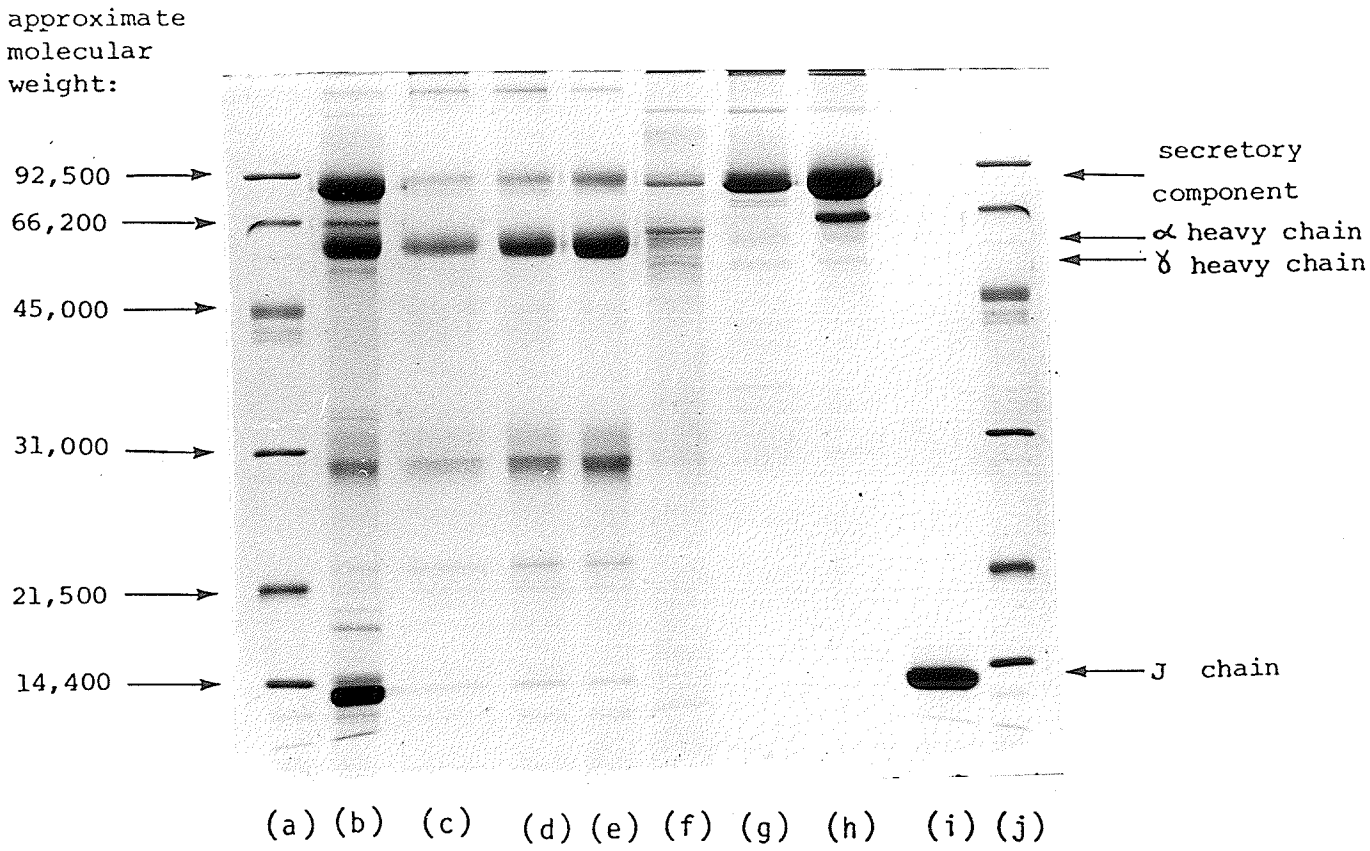
(a) CLARIFIED COLOSTRUM SOLUTION
(b) G-200 FRACTION 40
(c) G-200 FRACTION 41
(d) G-200 FRACTION 44
(e) G-200 FRACTION 52
(f) G-200 FRACTION 57
(g) G-200 FRACTION 71
(h) G-200 FRACTION 103

(portions of G-200 peak 1) contained only IgA (Plate 1, (b), (c), (d), (e), (f)). Calibration of the G-200 column indicated that the G-200 peak 1 would contain molecules of the appropriate molecular weight for dimeric IgA. Specific antiserum against secretory component only was not available. Fractions 57 and 71 (portions of G-200 peaks 2 and 3 respectively) contained both IgG and IgA (Plate 1, (g), (h)). Fraction 103 of G-200 peak 4 failed to exhibit the presence of any immunoglobulins (Plate 1, (i)). IgM, which was present in the pre G-200 solution (Plate 1, (a)), was not detected in any of the selected fractions. This was due to the early elution of IgM prior to the dimeric IgA G-200 peak 1.

SDS-PAGE analysis (Plate 2), a more sensitive method to evaluate the presence of proteins than that of the double diffusion method, showed the same results as the double diffusion analysis with the additional findings of the presence of proteins with the approximate molecular weight of γ heavy chains in fraction 52 (a portion of G-200 peak 1) (Plate 2, (f)). It was also noted that proteins with the approximate molecular weight of J chains were found in greatest amount in fraction 103 (of G-200 peak 4) in a free, unattached form (Plate 2, (i)).

The profile and locations of significant fractions from the separation of the treated G-200 peak 1 on a Sephacel DEAE ion exchange column are shown in graph 2. Double diffusion analysis (Plate 3) revealed that IgA was present in the G-200 peak 1, and all fractions tested (Plate 3, (a), (b), (c), (d), (e)). No immunoglobulins of the IgG or IgM classes were detected using this method of analysis.

PLATE 2: SDS-PAGE ANALYSIS OF CLARIFIED COLOSTRUM AND VARIOUS G-200 FRACTIONS



LANE:
(a) MOLECULAR WEIGHT STANDARDS
(b) CLARIFIED COLOSTRUM SOLUTION
(c) G-200 FRACTION 40
(d) G-200 FRACTION 41
(e) G-200 FRACTION 44
(f) G-200 FRACTION 52
(g) G-200 FRACTION 57
(h) G-200 FRACTION 71
(i) G-200 FRACTION 103
(j) MOLECULAR WEIGHT STANDARDS

GRAPH 2: SEPARATION OF THE TREATED G-200 PEAK 1
USING SEPHACEL DEAE ION EXCHANGE CHROMATOGRAPHY

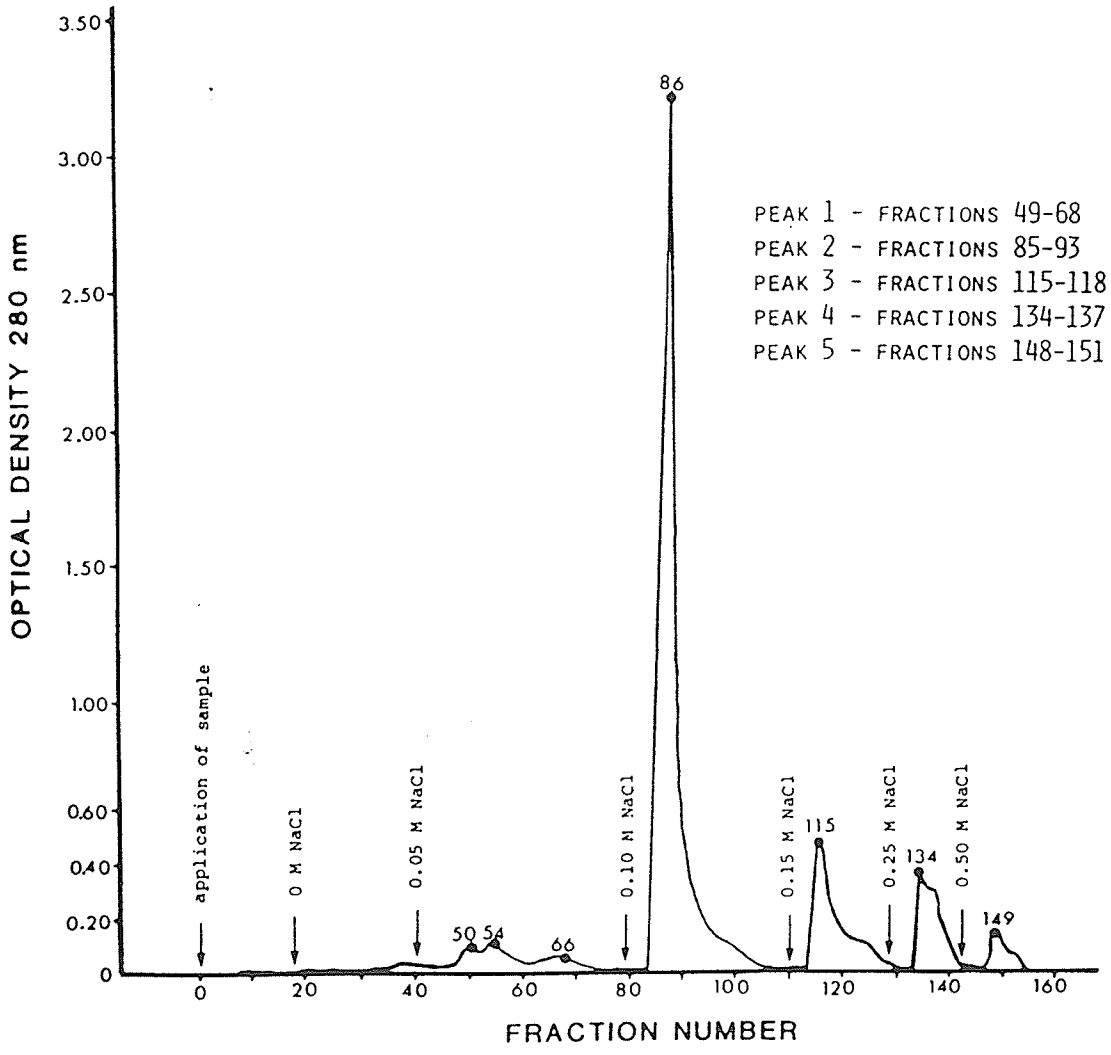
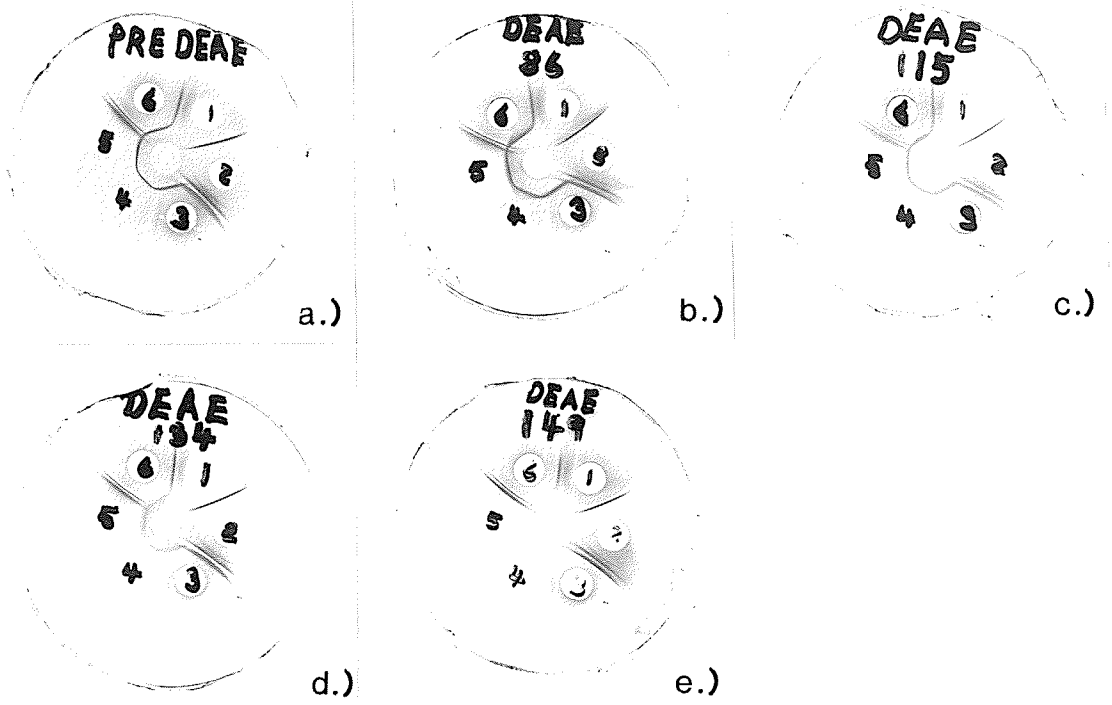


PLATE 3: OUCHTERLONLY DOUBLE DIFFUSION ANALYSIS OF TREATED G-200 PEAK 1 AND VARIOUS DEAE FRACTIONS



WELL 1 - ANTISERA TO HUMAN IgG
WELL 2 - ANTISERA TO HUMAN IgM
WELL 3 - ANTISERA TO WHOLE HUMAN SERUM
WELL 4 - ANTISERA TO HUMAN IgA
WELL 5 - ANTISERA TO HUMAN IgA AND SECRETORY COMPONENT
WELL 6 - ANTISERA TO WHOLE HUMAN SERUM

CENTRE WELLS:

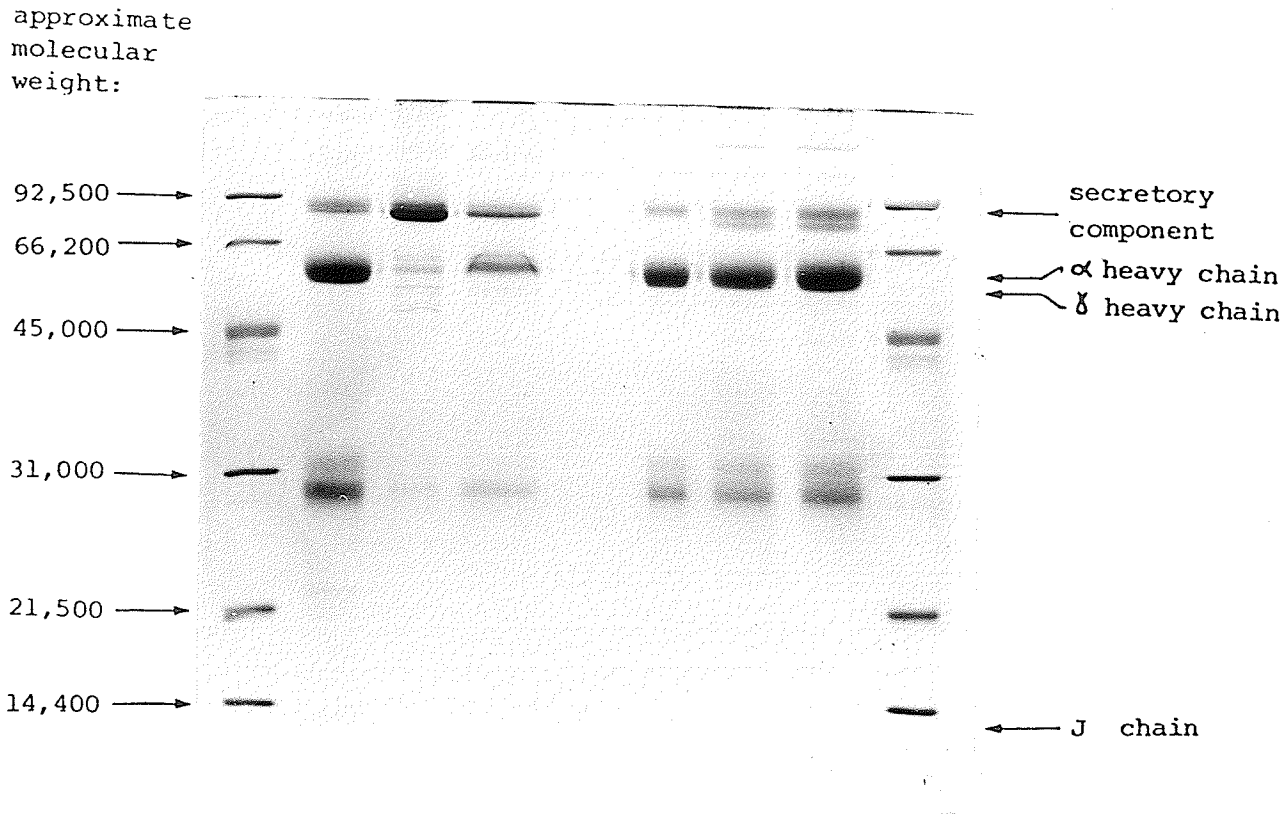
(a) TREATED G-200 PEAK 1 SOLUTION
(b) DEAE FRACTION 86
(c) DEAE FRACTION 115
(d) DEAE FRACTION 134
(e) DEAE FRACTION 149

However, the SDS-PAGE analysis (Plate 4) revealed the presence of proteins with approximate molecular weights of γ chains in fraction 50 of the DEAE peak 1 (Plate 4, (c)). Proteins of the approximate molecular weight of J chains were present in the treated G-200 peak 1 (Plate 4, (b)), but were not noted in any of the other fractions tested.

The pooled DEAE peaks 2, 3, and 4 were also subjected to SDS-PAGE analysis and the results are presented in plate 5. The pooled DEAE peak 2 (Plate 5, (d)) contained bands corresponding to the molecular weights of secretory component (approximately 70,000), α heavy chains (approximately 58,000) and λ and κ light chains when compared to those of the human purified IgG standard and human IgM myeloma standard. The band corresponding to the molecular weight of the J chain (approximately 14,000) was not present in the pooled DEAE peak 2. The minimal amount of attached J chain evident in the treated G-200 peak 1 solution was eventually located, with other contaminants, in the pooled DEAE peak 4 (Plate 5, (f)). Other contaminants, revealed by SDS-PAGE analysis, were also present in the pooled DEAE peaks 3 and 4 (Plate 5, (e), (f)).

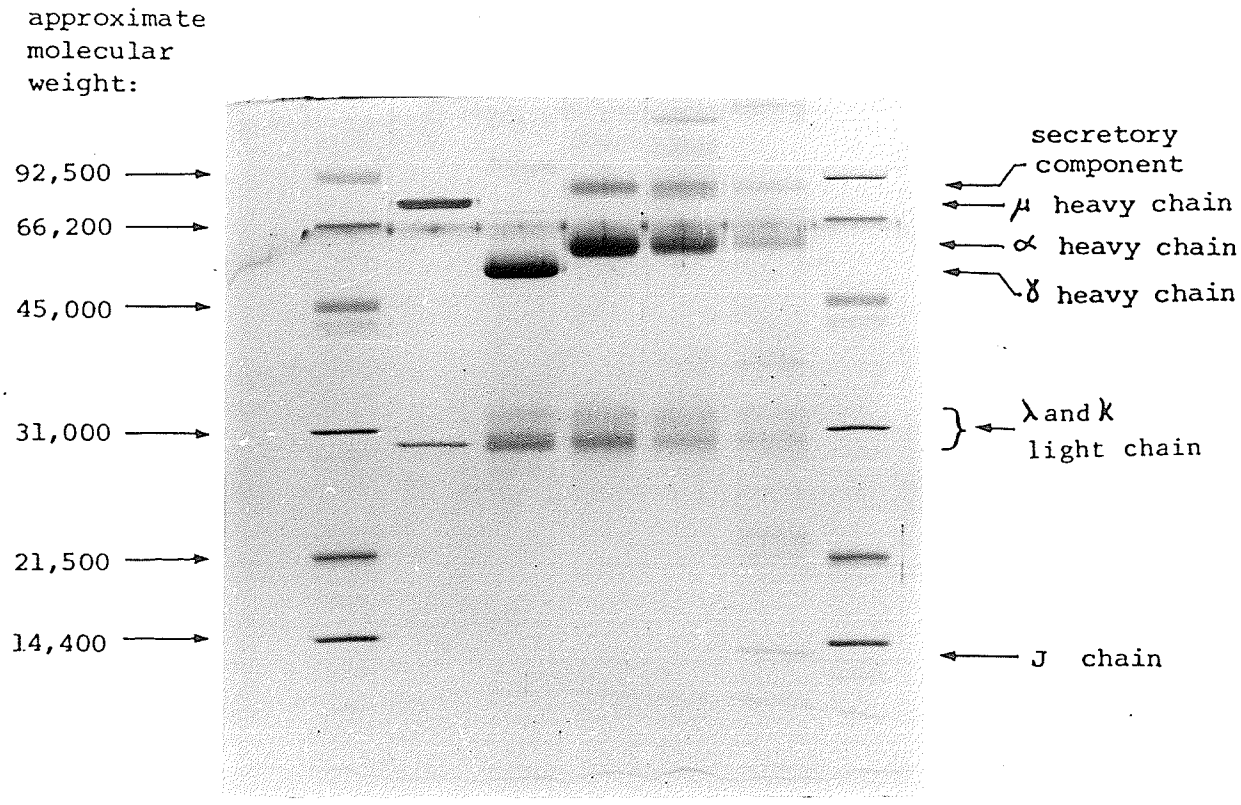
The pooled DEAE peak 2 was free from contaminants including μ and γ heavy chains, and this pool was used as the secretory IgA standard derived from a human secretory source in the solid phase radioimmunoassay methods.

PLATE 4: SDS-PAGE ANALYSIS OF TREATED G-200 PEAK 1 AND VARIOUS DEAE FRACTIONS



LANE:
(a) MOLECULAR WEIGHT STANDARDS
(b) TREATED G-200 PEAK 1
(c) DEAE FRACTION 50
(d) DEAE FRACTION 54
(e) DEAE FRACTION 66
(f) DEAE FRACTION 86
(g) DEAE FRACTION 115
(h) DEAE FRACTION 134
(i) MOLECULAR WEIGHT STANDARDS

PLATE 5: SDS-PAGE ANALYSIS OF POOLED DEAE PEAKS 2, 3, AND 4



LANE:
(a) MOLECULAR WEIGHT STANDARDS
(b) HUMAN I_gM MYELOMA STANDARD
(c) PURIFIED HUMAN I_gG STANDARD
(d) POOLED DEAE PEAK 2
(e) POOLED DEAE PEAK 3
(f) POOLED DEAE PEAK 4
(g) MOLECULAR WEIGHT STANDARDS

(3). IgA, IgG and IgM Concentrations in Whole Unstimulated Saliva

The minimum sensitivities of the solid-phase radioimmunoassays were 0.4 ng/ml for IgA, 0.8 ng/ml for IgG, and 0.4 ng/ml for IgM. The concentrations of immunoglobulins of the IgA, IgG, and IgM classes in whole unstimulated saliva of the 28 paired individuals, as determined by the solid phase radioimmunoassays, are shown in Table VI. The concentration of IgA in the saliva of control subject 19, and the concentrations of IgG in the saliva of control subjects 9 and 10, and MiRAS subject 20 were not determined as the diluted saliva sample counts per minute values did not fall on the valid linear portion of the standard curves (Materials and Methods (7)(d)).

In Table VII the range, mean, standard deviation, and standard error of the immunoglobulin assays of the saliva samples are shown.

Statistical analysis of salivary immunoglobulin concentration in the MiRAS patient group and the control group was made using an unpaired Student's t-test. Table VIII summarizes these findings. The t-values indicate no statistical difference between the two groups for all three classes of immunoglobulins.

A paired Student's t-test was also employed to compare salivary immunoglobulin concentrations of the IgA, IgG and IgM classes in the MiRAS and control individuals paired by age, sex, and time of day collection. Table IX summarizes these results. The t-values indicate no statistical differences in any immunoglobulins between the paired subjects in the two groups.

TABLE VI: TOTAL IMMUNOGLOBULIN CONCENTRATIONS IN WHOLE UNSTIMULATED SALIVA OF PAIRED CONTROL AND M1RAS INDIVIDUALS (mg/100 ml)

	CONTROL GROUP			M1RAS GROUP		
	IgA	IgG	IgM	IgA	IgG	IgM
1	22.40	3.20	1.76	7.40	3.12	0.73
2	13.60	0.68	0.15	6.30	0.74	0.64
3	26.60	5.04	1.21	7.00	1.02	0.92
4	6.96	0.06	0.62	16.70	0.55	1.60
5	18.50	0.72	0.76	10.50	1.55	0.88
6	7.80	0.35	0.86	13.30	0.55	0.48
7	11.80	0.70	1.20	5.20	0.29	0.68
8	7.80	0.68	1.58	26.60	2.04	2.97
9	7.20	——	1.08	17.60	1.17	5.61
10	4.86	——	0.42	17.40	2.66	0.65
11	14.76	1.14	0.76	11.80	0.84	1.34
12	10.36	1.20	0.77	7.56	4.80	0.66
13	10.40	0.57	1.54	11.84	5.00	1.28
14	7.90	7.60	1.34	7.80	0.46	0.28
15	22.80	6.76	1.32	8.76	0.44	0.79
16	9.40	1.87	1.00	4.16	0.28	0.54
17	16.00	2.62	3.51	9.76	0.28	0.70
18	5.40	0.35	0.35	11.16	2.46	0.63
19	——	2.37	1.34	20.60	4.80	0.64
20	8.76	0.88	1.21	5.56	——	0.76
21	2.61	0.63	0.55	12.84	3.10	1.72
22	5.10	0.43	4.20	12.76	2.08	0.47
23	15.16	1.42	0.92	7.16	0.62	0.76
24	15.40	2.40	5.72	4.76	0.94	0.53
25	11.80	6.40	3.58	5.36	1.30	1.07
26	8.76	0.29	0.60	12.80	0.64	2.22
27	16.36	2.22	0.54	11.24	0.42	0.16
28	12.50	0.48	1.18	13.92	1.88	0.71

TABLE VII: STATISTICAL ANALYSIS OF TOTAL IMMUNOGLOBULIN CONCENTRATIONS IN WHOLE UNSTIMULATED SALIVA OF ALL SUBJECTS

	IgA	IgG	IgM
NUMBER OF SUBJECTS	55	53	56
RANGE (mg/100 ml)	2.61-26.60	0.06-7.60	0.15-5.72
MEAN (mg/100 ml)	11.434	1.7944	1.2586
STANDARD DEVIATION	5.5687	1.8160	1.1823
STANDARD ERROR	0.75088	0.24945	0.15799

TABLE VIII: STATISTICAL ANALYSIS OF TOTAL IMMUNOGLOBULIN CONCENTRATIONS IN WHOLE UNSTIMULATED SALIVA OF CONTROL AND MiRAS INDIVIDUALS

	IgA		IgG		IgM	
	CONTROL GROUP	MiRAS GROUP	CONTROL GROUP	MiRAS GROUP	CONTROL GROUP	MiRAS GROUP
NUMBER OF SUBJECTS	27	28	26	27	28	28
RANGE (mg/100 ml)	2.61-26.60	4.76-26.60	0.06-7.60	0.28-5.00	0.15-5.72	0.47-5.61
MEAN (mg/100 ml)	11.889	10.994	1.9640	1.6310	1.4312	1.0860
STANDARD DEVIATION	5.9180	5.2808	2.1485	1.4495	1.2816	1.0690
STANDARD ERROR	1.1389	0.99798	0.42135	0.27896	0.24221	0.20203
t - VALUE	0.593		0.664		1.095	

TABLE IX: STATISTICAL ANALYSIS OF TOTAL IMMUNOGLOBULIN CONCENTRATIONS IN WHOLE UNSTIMULATED SALIVA IN PAIRED CONTROL AND MiRAS SUBJECTS

	I_gA	I_gG	I_gM
NUMBER OF PAIRS	27	25	28
t- VALUE	0.703	0.690	1.035

(4). IgE Concentrations in Whole Unstimulated Saliva

The minimum sensitivity of the solid phase radioimmunoassay for IgE was 0.1 ng/ml. IgE was detected in two saliva samples of control individuals at concentrations of 1.06 ng/ml (control subject 12), and 0.59 ng/ml (control subject 5), and in one MiRAS saliva sample at a concentration of 0.78 ng/ml (MiRAS subject 10). None of these subjects had any history of allergy and the MiRAS patient could not identify any specific precipitating factor associated with the onset of the lesions. No significant increase or decrease in salivary levels of IgA, IgG, or IgM were present in these three subjects as these levels fell within two standard deviations of the mean salivary level of their respective subject groups. Table X indicates the standardized score (Z value) for each of these subjects with respect to their IgA, IgG, and IgM salivary concentrations.

(5). Antibody Activity to Alpha-lactalbumin in Whole Unstimulated Saliva

Since immunoglobulins of the IgE class were detected in the saliva of only three subjects, and then only in very low concentrations, it was considered of no value to assay specific IgE antibody activity to a purified food antigen.

Immunoglobulins of the IgA class were found to be the most abundant immunoglobulins in saliva. Secretory IgA is capable of forming non-absorbable antigen-antibody complexes with exogenous

TABLE X: STANDARDIZED SCORE (Z VALUE) FOR SUBJECTS EXHIBITING DETECTABLE LEVELS OF SALIVARY I_gE, WITH RESPECT TO THEIR I_gA, I_gG, AND I_gM SALIVARY CONCENTRATIONS

	I_gA Z VALUE	I_gG Z VALUE	I_gM Z VALUE
CONTROL SUBJECT 5	1.1171	-0.57901	-0.52327
CONTROL SUBJECT 12	0.26294	0.28438	-0.51592
MiRAS SUBJECT 10	1.2131	0.70990	-0.40786

antigens (Tomasi, 1972; Hanson and Brandtzaeg, 1973). It can be proposed that the reaction of certain food antigens with the IgA antibody in saliva would reduce the exposure of these exogenous antigens to the oral mucosa. This in turn could prevent the occurrence of localized hypersensitivity reactions within oral mucosa, which has been suggested as a possible pathogenic mechanism of RAS (Taylor et al., 1964; Thomas et al., 1973).

In order to explore the possibility that specific antibodies to a food antigen may vary significantly in the saliva of control and MiRAS patients, a suitable food substance was sought. Since the intake of milk has been associated with the onset of MiRAS (Alvarez, 1937; Eversole et al., 1982), and alpha-lactalbumin, a major protein component of milk, represents a single antigen which is commercially available in a highly purified form, bovine alpha-lactalbumin was selected as the test antigen to monitor specific IgA antibody activity in saliva in a double antibody assay.

Table XI shows the antibody activity to alpha-lactalbumin measured in counts per minute in the unstimulated whole saliva of the paired test and control subjects. The counts per minute value for the positive serum control (Materials and Methods (8)) is also included in the table.

The assay demonstrated that all the saliva samples contained low levels of IgA antibodies to alpha-lactalbumin. However, the extremely low counts per minute values of the saliva samples, and the lack of a strongly positive counts per minute signal in the serum control did not make possible accurate quantitative evaluations and comparisons between the test and control saliva samples.

TABLE XI: IgA ANTIBODY ACTIVITY TO ALPHA-LACTALBUMIN IN WHOLE UNSTIMULATED SALIVA OF PAIRED CONTROL AND MiRAS INDIVIDUALS (COUNTS PER MINUTE)

	CONTROL GROUP	MiRAS GROUP
1	81	128
2	82	14
3	49	106
4	46	19
5	46	139
6	54	27
7	59	62
8	34	29
9	63	83
10	109	50
11	52	61
12	62	61
13	68	9
14	111	45
15	83	251
16	39	11
17	26	27
18	99	53
19	63	101
20	28	52
21	14	22
22	83	58
23	19	70
24	64	22
25	32	26
26	44	31
27	37	77
28	17	23
RANGE	14-111	9-251
MEAN	55.86	59.20
SERUM CONTROL VALUE	401	

CHAPTER VI

DISCUSSION

Although considerable time and effort have been spent by numerous investigators in attempting to establish an etiopathogenesis for minor recurrent aphthous stomatitis (MiRAS), to date none has been identified. A number of precipitating factors, based on clinical observations, have been noted to be associated with the onset of MiRAS. One such factor, the intake of certain foods, results in the development of lesions in some RAS patients. Along with this clinical observation, limited in vitro immunologic studies have led investigators to consider that an immunologic response to food antigens may play a role in the formation of MiRAS lesions.

It can be hypothesized that certain food antigens may penetrate oral mucosa and generate a local mucosal reaction clinically identified as MiRAS. As saliva immunoglobulins represent a first line of mucosal defense against exogenous antigens, including food, the present thesis explored the relationship of the concentrations of immunoglobulins of the IgA, IgG, and IgM classes in the saliva of MiRAS patients to those without a history of this disorder.

Total salivary IgE concentrations were determined in both groups to investigate the hypothesis that IgE in saliva participates in a type I allergic reaction to food antigens leading to mucosal tissue destruction resulting in MiRAS lesions.

In addition, salivary IgA antibody levels to a MiRAS-associated food antigen was recorded. A significant reduction of specific antibody in the MiRAS group could support the concept that these subjects were not protected from antigen pene-

tration of the mucosa by its previous interaction with IgA antibody.

It was found that immunoglobulin levels in both groups were similar for all immunoglobulin classes studied. IgA antibodies to the food antigen were present in low levels in both populations and were not significantly increased in the non-MiRAS group.

One important aspect of the present study was that considerable care was taken to evaluate those factors of both physiology and the environment which could influence the levels of immunoglobulins or antibodies in the saliva. In many of the previous studies relating the immune system to MiRAS, these factors had not been considered.

Whole saliva contains contaminants including bacteria. Several of these bacteria produce proteases, some of which are specific for IgA. Consequently, the proteolytic enzyme inhibitor EDTA was added to all saliva samples immediately after collection to prevent any protease activity of bacteria reducing the levels of immunoglobulins. The possibility that bacterial proteases could reduce the level of specific immunoglobulins in saliva has apparently not been considered previously.

In past studies various methods have been utilized to establish immunoglobulin concentrations in saliva and include single radial immunodiffusion, solid-phase radioimmunoassay, immunoelectrophoresis, immunobead enzyme-linked immunosorbent, and automated turbidimetric techniques (see Table III).

Since secretory IgA is mainly dimeric in structure, errors can occur in estimating secretory IgA if the assay technique uti-

lizes monomeric serum IgA as a standard. The production of a standard secretory IgA is essential in any assay method in which molecular size and configuration is crucial. These are those assays which utilize a gel diffusion stage and include the single radial immunodiffusion and immunoelectrophoresis techniques. To compensate for the discrepancy of molecular size when serum IgA is used as a standard in those techniques, most authors have multiplied values obtained by the factor 3.25 (Brandtzaeg et al., 1968). Mandel and Khurana (1979), however, have shown that standard curves for immunodiffusion based on serum IgA and secretory IgA are not parallel and recommend that a range of correction factors from 1.2 to 4.6 be used depending on the relative proportion of dimeric to monomeric IgA present in the test samples.

Other authors have treated salivary samples and standards with reducing agents to eliminate differences in molecular size. This has led to less than satisfactory results since a variable resistence to reduction of salivary IgA exists due to the presence of secretory component (Alaluusua et al., 1981), and monomeric IgA molecules not containing secretory component are more readily reduced to smaller subunits than are polymeric IgA molecules (Peppard, 1979).

A solid-phase radioimmunoassay technique was chosen in the present study to quantitate salivary immunoglobulin concentrations, thus eliminating the sources of error associated with immunoassays incorporating a gel diffusion stage. To minimize the differences between the standard IgA and the salivary IgA in the test samples, the standard used in the IgA assays was secretory in

origin, derived from pooled human colostrum. The radioimmunoassay technique proved to be highly sensitive and highly specific, and allowed an accurate measurement of immunoglobulins of the IgA, IgG, IgM, and IgE classes.

The present study, with a carefully selected subject population, a stringent saliva sampling technique, and an accurate assay method confirms and adds support to the previous studies of Lehner (1969c), Ben-Aryeh et al. (1976), and Bennet and Reade (1982).

Kakizawa et al. (1973) also reported on the relationship of salivary IgA immunoglobulin content in control and RAS subjects, yet their work is difficult to interpret. Their study revealed variations in salivary IgA levels during different stages of the RAS lesions. However, the exact quantities of salivary IgA concentrations in the RAS patients were not stated, and their results were not subjected to statistical analysis. Furthermore, their findings were not borne out by the subsequent work of Ben-Aryeh et al. (1976) and Bennet and Reade (1982).

The present study is the first to realistically compare salivary IgG, IgM, and IgE levels in MiRAS patients and control individuals. The only previous study to report on salivary IgG and IgM levels in such groups was conducted by Lehner in 1969. In that investigation IgG immunoglobulins, although detected, were not quantitated, and no IgM immunoglobulins were detected in any saliva samples. Undoubtedly, this was due to limitations of the single radial immunodiffusion technique utilized in the study.

The present study also represents the first attempt to investigate the presence of antibody activity in the saliva of MiRAS

patients to a specific food antigen. Some aspects of the assay for antibody of this type should be noted.

It is imperative when conducting a double antibody assay that strongly positive immunoglobulins to the test antigen are identified as a positive control. In the present study, although 59 human sera samples were screened for the presence of alpha-lactalbumin-specific IgA, only two samples exhibited any significant activity, and in both instances this was minimal. Given sufficient time and facilities, positive control immunoglobulins could have been obtained through the raising of serum antibodies to alpha-lactalbumin in animals.

Upon testing the saliva, alpha-lactalbumin IgA activity was present in all samples, but the activity was of a very low magnitude. As qualitative rather than quantitative results were obtained, statistical analysis comparing the control and MiRAS group could not be performed. Nevertheless, the finding of antibody activity, even at minimal levels, suggests that the screening of saliva for antibody to food antigens is possible.

From the data obtained in the present thesis, it can be stated that salivary immunoglobulin concentrations are not altered during the ulcerative stage of MiRAS and do not appear to participate in tissue destruction during this stage. Differences in immunoglobulin levels may contribute to the etiopathogenesis of MiRAS during the earlier premonitory and preulcerative stages, a hypothesis which could be evaluated by means of a longtitudinal experimental study involving MiRAS patients and control subjects. However, in light of the present findings, and those of previous

studies, it would seem reasonable to suggest that salivary immune mechanisms play no role, or at a most a very obscure role in MiRAS. The etiopathogenesis of this disorder remains an enigma.

CHAPTER VII

SUMMARY AND CONCLUSIONS

In order to investigate the possibility that differences in the various classes of salivary immunoglobulin concentrations are involved in the etiopathogenesis of MiRAS, the saliva from 28 patients suffering from active MiRAS lesions was compared to the saliva of 28 age- and sex-matched individuals without a history of the disorder.

Total immunoglobulin concentrations of the IgA, IgG, IgM, and IgE classes in whole unstimulated saliva were quantitated using a solid-phase radioimmunoassay technique. Salivary IgA antibody activity to the specific food antigen alpha-lactalbumin was also assessed using a double antibody radioimmunoassay method.

Analysis of the results revealed no statistical differences between the total immunoglobulin concentrations of the IgA, IgG, or IgM classes in the saliva of the control and the MiRAS subjects. Salivary IgE was detected in very minute quantities in only three of the 56 subjects. Although all saliva samples exhibited IgA antibody levels to alpha-lactalbumin, these levels were too low to make accurate quantitative evaluations and comparisons between the two study groups.

The results would indicate that there are no differences between salivary IgA, IgG, and IgM immunoglobulin levels in MiRAS patients and control subjects. This would not support the hypothesis that decreased levels of salivary immunoglobulins of the IgA, IgG, and IgM classes result in increased pasage of food antigens across the oral mucosa leading to local mucosal hypersensitivity tissue destruction represented clinically as MiRAS.

The lack of high levels of salivary IgE in MiRAS patients

would also suggest that saliva does not participate in an allergic reaction leading to local mucosal tissue destruction. This study also showed that IgA antibodies specific to alpha-lactalbumin, a food antigen reported to be associated with the onset of MiRAS lesions in some individuals, are in such low levels in saliva that their involvement in the pathogenesis of MiRAS is unlikely.

Future clinical studies investigating salivary immunoglobulin levels in MiRAS patients should utilize subject selection criteria, saliva collection methods, and a highly sensitive and specific assay technique similar to those used in the present study.

A registry of MiRAS patients should be initiated and maintained in order to identify subgroups of MiRAS patients that include individuals correlating common factors with the onset of lesions. A major drawback of the present study was the lack of MiRAS patients who could identify foods, specifically milk, as a precipitating factor in lesion formation.

The present investigation represented a cross-sectional study testing saliva samples of subjects during the ulcerative stage of MiRAS when the diagnosis was made on clinical features. Differences in immunoglobulin levels may contribute to the etiopathogenesis of MiRAS during the earlier premonitory and preulcerative stages rather than the later ulcerative stage. Therefore, future studies should be longtitudinal in nature, investigating total immunoglobulin concentration of various classes in the saliva of MiRAS patients who suffer onset of their lesions after exposure to the same purified food antigen. Saliva sampling should be performed prior to a challenge with the food antigen,

and during the various subsequent stages of the MiRAS lesions. Antibody activity of various immunoglobulin classes to the purified food antigen could be performed using a double antibody radioimmunoassay technique.

BIBLIOGRAPHY

Aarli, J.A. Phenytoin-induced depression of salivary IgA and gingival hyperplasia. Epilepsia, 17: 283-291, 1976.

Aarli, J.A., and O. Tonder. Effect of antiepileptic drugs on serum and salivary IgA. Scand. J. Immunol., 4: 391-396, 1975.

Adinolfi, M., Glynn, A.A., Lindsay, M., and C.M. Milne. Serological properties of γA antibodies to Escherichia coli present in human colostrum. Immunology, 10: 517-526, 1966.

Alaluusua, S., Gronblad, E.A., and H. Tolo. Quantitation of IgA in human whole saliva: a comparison of three immunoassays. Acta Odontol. Scand., 39: 155-161, 1981.

Alvarez, W.C. Canker sores. Minn. Med., 20: 602, 1937.

Antoon, J.W., and R.L. Miller. Aphthous ulcers - a review of the literature on etiology, pathogenesis, diagnosis and treatment. J. Am. Dent. Assoc., 101: 803-808, 1980.

Arnold, R.R., Cole, M.F., Prince, S., and J.R. McGhee. Secretory IgM antibodies to Streptococcus mutans in subjects with selective IgA deficiency. Clin. Immunol. Immunopathol., 8: 475-486, 1977.

Barbash, R., Kutscher, A.H., Zegarelli, E.V., and H.F. Silvers. Recurrent ulcerative (aphthous) stomatitis: intradermal food test studies. J. Allergy, 29: 442-445, 1958.

Barile, M.F., Graykowski, E.A., Driscoll, E.J., and D.B. Riggs. L-form of bacteria isolated from recurrent aphthous stomatitis lesions. Oral Surg. Oral Med. Oral Pathol., 16: 1395-1402, 1963.

Ben-Aryeh, H., Malberger, E., Gutman, D., Szargel, R., and Y.

Anavi. Salivary IgA and serum IgG and IgA in recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol., 42: 746-752, 1976.

Ben-Aryeh, H., Nahir, M., Scharf, D., Gutman, D., Laufer, D., and R. Szargel. Sialochemistry of patients with rheumatoid arthritis. Oral Surg. Oral Med. Oral Pathol., 45: 63-70, 1978.

Bennet, K.R., and P.C. Reade. Salivary immunoglobulin A levels in normal subjects, tobacco smokers, and patients with minor aphthous ulceration. Oral Surg. Oral Med. Oral Pathol., 53: 461-465, 1982.

Bennich, H., and S.G.O. Johansson. Structure and function of human immunoglobulin E. Adv. Immunol., 13: 1-55, 1971.

Bienenstock, J., and A.D. Befus. Review: mucosal immunology. Immunology, 41: 249-270, 1980.

Bishop, P.M.F., Harris, P.W.R., and J.A.P. Trafford. Oestrogen treatment of recurrent aphthous mouth ulcers. Lancet, 1: 1345-1347, 1967.

Bluestone, R., Gumpel, J.M., Goldberg, L.S., and E.J. Holborow. Salivary immunoglobulins in Sjogren's syndrome. Int. Arch. Allergy Appl. Immunol., 42: 686-692, 1972.

Bookman, R. Relief of canker sores on resumption of cigarette smoking. Calif. Med., 93: 235-236, 1960.

Brandtzaeg, P. Immunochemical comparison of proteins in human gingival pocket fluid, serum and saliva. Arch. Oral Biol., 10: 795-803, 1965.

Brandtzaeg, P. Local factors of resistance in the gingival area.

J. Periodont. Res., 1: 19-42, 1966.

Brandtzaeg, P. Human secretory immunoglobulins. VII. Concentrations of parotid IgA and other secretory proteins in relation to the rate of flow and duration of secretory stimulus. Arch. Oral Biol., 16: 1295-1310, 1971.

Brandtzaeg, P. In Oral Mucosa in Health and Disease. (A.E. Dolby, ed.) Blackwell Scientific Publications, Oxford, 1975.

Brandtzaeg, P., Fjellanger, I., and S.T. Gjeruldsen. Adsorption of immunoglobulin A onto oral bacteria in vivo. J. Bacteriol., 96: 242-249, 1968.

Brandtzaeg, P., Fjellanger, I., and S.T. Gjeruldsen. Human secretory immunoglobulins. I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. Scand. J. Haematol., 12: 1-83, 1970.

Bratthall, D., and A. Carlen. Salivary agglutinin and secretory IgA reactions with oral streptococci. Scand. J. Dent. Res., 86: 430-443, 1978.

Bratthall, D., and R.P. Ellen. Determination of immunoglobulin A in saliva by immunobead enzyme-linked immunosorbent assay: comparison with single radial immunodiffusion. J. Clin. Microbiol., 16: 766-769, 1982.

Brody, H.A., and S. Silverman. Studies on recurrent oral aphtae: 1. Clinical and laboratory comparisons. Oral Surg. Oral Med. Oral Pathol., 27: 27-34, 1969.

Burdon, D.W. The bactericidal action of immunoglobulin A. J. Med. Microbiol., 6: 131-139, 1973.

Burton-Kee, J.E., Mowbray, J.F., and T. Lehner. Different cross-

reacting circulating immune complexes in Behcet's syndrome and recurrent oral ulcers. J. Lab. Clin. Med., 97: 559-567, 1981.

Bystryn, J.C., Abel, E., and A. Weidman. Antibodies against the cytoplasm of human epidermal cells. Arch. Dermatol., 108: 241-244, 1973.

Carruthers, R. Recurrent aphthous ulcers. Lancet, 2: 259, 1967.

Ceska, M., and V. Lundkvist. A new and simple radioimmunoassay method for the determination of IgE. Immunochemistry, 9: 1021-1030, 1971.

Chajek, T., and M. Fainaru. Behcet's disease. Report of 41 cases and a review of the literature. Medicine, 54: 179-196, 1975.

Challacombe, S.J., Batchelor, J.R., Kennedy, L.A., and T. Lehner. HLA antigens in recurrent oral ulceration. Arch. Dermatol., 113: 1717-1719, 1977.

Coe, J.E., Feldman, J.D., and S. Lee. Immunologic competence of thoracic duct cells. I. Delayed hypersensitivity. J. Exp. Med., 123: 267-281, 1966.

Cole, M.F., Bowen, W.H., Sierra, L., Espinal, F., Aquirra, M., Kingman, A., Kemp, L.J., Gomez, I., Reilly, J.A., Hsu, D., Ciardi, J.E., and G. Gillespie. Immunoglobulins and antibodies in plaque fluid and saliva in two populations with contrasting levels of caries. Adv. Exp. Med., 107: 383-392, 1978.

Cooke, B.E.D. The diagnosis of bullous lesions affecting the oral mucosa. Br. Dent. J., 109: 83-95, 1960.

Cooke, B.E.D. Recurrent Mikulicz's aphthae. Dent. Pract., 12: 119-124, 1961.

Cooke, B.E.D., and P. Armitage. Recurrent Mikulicz's aphthae treated with topical hydrocortisone hemisuccinate sodium. Br. Med. J., 1: 764-766, 1960.

D'Amelio, R., Palmisano, L., Le Moli, S., Seminara, R., and F. Aiuti. Serum and salivary IgA levels in normal subjects: comparison between tonsillectomized and non-tonsillectomized subjects. Int. Arch. Allergy Appl. Immunol., 68: 256-259, 1982.

De Cree, J., Verhaegen, H., De Cock, W., and F. Verbruggen. A randomized double-blind trial of levamisole in the therapy of recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol., 45: 378-384, 1978.

Delespesse, G., Sarfati, M., Yanagihara, Y., and A.H. Sehon. In IgE Antibodies: Their Synthesis and Effector Functions. (A.H. Sehon, ed.) Excerpta Medica, Amsterdam, 1983.

Demetriou, N., Drikos, G., and A. Bambionitakis. Relation between gingival fluid and mixed and parotid salivary IgA. J. Periodontol., 49: 64-66, 1968.

De Meyer, J., Degraeve, M., Clarysse, J., De Loose, F., and W. Peremans. Levamisole in aphthous stomatitis: evaluation of three regimens. Br. Med. J., 1: 671-674, 1977.

Dolby, A.E. Recurrent Mikulicz's oral aphthae - their relationship to the menstrual cycle. Br. Dent. J., 124: 359-360, 1968a.

Dolby, A.E. A double-blind trial of an antihistamine drug in the

treatment of Miculicz's recurrent oral aphthae. Dent. Pract., 18: 347-348, 1968b.

Dolby, A.E. Recurrent aphthous stomatitis: effect of sera and peripheral blood lymphocytes upon oral epithelial tissue culture cells. Immunology, 17: 709-714, 1969.

Dolby, A.E. Mickulicz's recurrent oral aphthae: the effect of antilymphocyte serum upon the in vitro cytotoxicity of lymphocytes from patients for oral epithelial cells. Clin. Exp. Immunol., 7: 681-686, 1970a.

Dolby, A.E. Mikulicz's recurrent oral aphtha: the effect of hydrocortisone succinate sodium upon the in vitro lymphocyte cytotoxicity. Br. Dent. J., 128: 579-580, 1970b.

Dolby, A.E., and R.T. Allison. Quantitative changes in the mast cell population in Mikulicz's recurrent oral aphthae. J. Dent. Res., 48: 901-903, 1969.

Dolby, A.E., and D.M. Walker. A trial of cromoglycic acid in recurrent aphthous ulceration. Br. J. Oral Surg., 12: 292-295, 1975.

Dolby, A.E., Walker, D.M., Slade, M., and C. Allan. HL-A histocompatibility antigens in recurrent aphthous ulceration. J. Dent. Res., 56: 105-107, 1977.

Donatsky, O. Epidemiologic study on recurrent aphthous ulcerations among 512 Danish dental students. Community Dent. Oral Epidemiol., 1: 37-40, 1973.

Donatsky, O. An immunofluorescence study on the cross-reaction between Strep. 2A and human oral mucosa. Scand. J. Dent. Res., 83: 111-119, 1975.

Donatsky, O. A leukocyte migration study on the cell-mediated immunity against adult human oral mucosa and streptococcal antigens in patients with recurrent aphthous stomatitis. Acta Pathol. Microbiol. Scand.[C], 84: 227-234, 1976a.

Donatsky, O. Comparison of cellular and humoral immunity against streptococcal and adult human oral mucosa antigens in relation to exacerbation of recurrent aphthous stomatitis. Acta Pathol. Microbiol. Scand.[C], 84: 270-282, 1976b.

Donatsky, O. Cell-mediated and humoral immunity against oral streptococci, neisseria, staphylococci, and adult human oral mucosa antigens in recurrent aphthous stomatitis. Scand. J. Dent. Res., 86: 25-34, 1978.

Donatsky, O. An immunoelectrophoretic analysis of the Strep. sanguis and adult human oral mucosa antigen extracts used for immunological investigations of recurrent aphthous stomatitis. Acta Pathol. Microbiol. Scand.[C], 88: 219-225, 1980.

Donatsky, O., and G. Bendixen. In vitro demonstration of cellular hypersensitivity to Strep. 2A in recurrent aphthous stomatitis by means of the leukocyte migration test. Acta Allergol., 27: 137-144, 1972.

Donatsky, O., and E. Dabelsteen. An immunofluorescence study on the humoral immunity to Strep. 2A in recurrent aphthous stomatitis. Acta Pathol. Microbiol. Scand.[B], 82: 107-112, 1974a.

Donatsky, O., and E. Dabelsteen. An immunofluorescence study on the humoral immunity to adult human oral mucosa in recurrent aphthous stomatitis. Acta Allergol., 29: 308-318, 1974b.

Donatsky, O., and E. Dabelsteen. Deposits of immunoglobulin G and complement C_3 in recurrent aphthous ulcerations. Scand. J. Dent. Res., 85: 419-425, 1977.

Dorsey, C. More observations on relief of aphthous stomatitis on resumption of cigarette smoking - a report of three cases. California Medicine, 101: 377-378, 1964.

Drinnan, A.J., and S.L. Fischman. Randomized, double-blind study of levamisole in recurrent aphthous stomatitis. J. Oral Pathol., 7: 414-417, 1978.

Eddie, D.S., Schulkind, M.L., and J.B. Robbins. The isolation and biologic activities of purified secretory IgA and IgG anti Salmonella typhimurium "O" antibodies from rabbit intestinal fluid and colostrum. J. Immunol., 106: 181-190, 1971.

Embil, J.A., Stephens, R.G., and F.R. Manuel. Prevalence of recurrent herpes labialis and aphthous ulcers among young adults on six continents. Can. Med. Assoc. J., 113: 627-630, 1975.

Everhart, D.L., Klapper, B., Carter, W.H., and S. Moss. Evaluation of dental caries experience and salivary IgA in children ages 3-7. Caries Res., 11: 211-215, 1977.

Eversole, L.R., Shopper, T.P., and D.W. Chambers. Effects of suspected foodstuff challenging agents in the etiology of recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol., 54: 33-38, 1982.

Fahey, J.L., and E. McKelvey. Quantitative determination of serum immunoglobulins in antibody agar plates. J. Immunol., 94: 84-90, 1965.

Fahmy, M.S. Recurrent aphthous ulcerations in a mixed Arab community. Community Dent. Oral Epidemiol., 4: 160-164, 1976.

Farmer, E.D. Recurrent aphthous ulcers. Dent. Pract., 8: 177-184, 1958.

Ferguson, M.M., McKay-Hart, D., Lindsay, R., and K.W. Stephen. Progesterone therapy for menstrually related aphthae. Int. J. Oral Surg., 7: 463-470, 1978.

Ferguson, M.M., Wray, D., Carmichael, H.A., Russell, R.I., and F.D. Lee. Coeliac disease associated with recurrent aphthae. Gut, 21: 223-226, 1980.

Ferguson, R., Basu, M.K., Asquith, P., and W.T. Cooke. Jejunal mucosal abnormalities in patients with recurrent aphthous ulceration. Br. Med. J., 1: 11-13, 1976.

Francis, T.C. Recurrent aphthous stomatitis and Behcet's disease: a review. Oral Surg. Oral Med. Oral Pathol., 30: 476-487, 1970.

Francis, T.C., and J.J. Oppenheim. Impaired lymphocyte stimulation by some streptococcal antigens in patients with recurrent aphthous stomatitis and rheumatic heart disease. Clin. Exp. Immunol., 6: 573-586, 1970.

Frost, M. Cromoglycate in aphthous stomatitis. Lancet, 2: 389, 1973.

Gallina, G., Cumbo, V., Messina, P., and C. Caruso. HLA-A, B, C, DR, MT, and MB antigens in recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol., 59: 364-370, 1985.

Gausset, P., Delespesse, G., Hubert, C., and A. Govaerts. In

vitro response of subpopulations of human lymphocytes. II. DNA synthesis induced by anti-immunoglobulin antibodies. J. Immunol., 116: 446-453, 1976.

Gier, R.E., George, B., Wilson, T., Rueger, A., Hart, J.K., Quaison, F., and P.K. Hardman. Evaluation of the therapeutic effect of levamisole in treatment of recurrent aphthous stomatitis. J. Oral Pathol., 7: 405-413, 1978.

Girard, J.P., and A. de Kalbermatten. Antibody activity in human duodenal fluid. Eur. J. Clin. Invest., 1: 188-195, 1970.

Glynn, A.A. The complement lysozyme sequence in immune bacteriolysis. Immunology, 16: 463-471, 1969.

Graykowski, E.A., Barile, M.F., Lee, W.B., and H.R. Stanley. Recurrent aphthous stomatitis: clinical, therapeutic, histopathologic, and hypersensitivity aspects. J. Am. Med. Assoc., 196: 637-644, 1966.

Graykowski, E.A., Barile, M.F., and H.R. Stanley. Periadenitis aphthae: clinical and histopathologic aspects of lesions in a patient and of lesions produced in rabbit skin. J. Am. Dent. Assoc., 69: 118-126, 1964,

Greenspan, J.S., Gadol, N., Olson, J.A., and N. Talal. Antibody-dependent cellular cytotoxicity in recurrent aphthous ulceration. Clin. Exp. Immunol., 44: 603-610, 1981.

Gronblad, E.A. Concentration of immunoglobulins in human whole saliva: effect of physiological stimulation. Acta Odontol. Scand., 40: 87-95, 1982.

Gugler, E., Pallavicini, J.C., Swerdlow, H., Zipkin, I., and P.A. di Sant'Agnese. Immunological studies of submaxillary saliva

from patients with cystic fibrosis and from normal children. J. Pediatr., 73: 548-559, 1968.

Guven, O., and J.G.A.M. De Visscher. Salivary IgA in periodontal disease. Oral Surg. Oral Med. Oral Pathol., 53: 334-335, 1983.

Haim, S. Pathogenesis of Behcet's disease. Int. J. Dermatol., 22: 101-102, 1983.

Hanson, L.A., and P. Brandtzaeg. In Immunologic Disorders in Infants and Children. (E.R. Stiehm, and V.A. Fulginiti, eds.) W.B. Saunders, Philadelphia, 1973.

Heft, M., and D. Wray. Anxiety levels in recurrent aphthous stomatitis (RAS) patients. J. Dent. Res., 61: 264, 1982.

Heremans, J.F., Crabbe, P.A., and P.L. Masson. Biological significance of exocrine gamma-A-immunoglobulin. Acta Med. Scand., 179: 84-88, 1966.

Honma, T. Electron microscopic study on the pathogenesis of recurrent aphthous ulceration as compared to Behcet's syndrome. Oral Surg. Oral Med. Oral Pathol., 41: 366-377, 1976.

Hyyppa, T. Salivary immunoglobulins in children with asthma. J. Periodont. Res., 15: 227-231, 1980.

Hyyppa, T.M., Koivikko, A., and K.V. Pauanio. Studies on periodontal conditions in asthmatic children. Acta Odontol. Scand., 37: 15-20, 1979.

Ishizaka, T., Ishizaka, K., Borsos, T., and H. Rapp. C'1 fixation by human isoagglutinins: fixation of C'1 by γG and γM but not by γA antibody. J. Immunol., 97: 716-726, 1966.

Johansson, S.G.O. Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet, 2: 951-953, 1967.

Johnson, R.B., and J. Liu. The application of enzyme immunoassay to the study of salivary IgA. J. Immunoassay, 3: 73-89, 1982.

Johnstone, A., and R. Thorpe. In Immunochemistry in Practice. (A.P. Johnstone, and R. Thorpe, eds.) Blackwell Scientific Publications, Oxford, 1982.

Kakizawa, T., Noma, H., and K. Omori. The evaluation of secretory IgA in human saliva. Bull. Tokyo Dent. Coll., 14: 125-139, 1973.

Kilian, M. Degradation of immunoglobulins A_1, A_2, and G by suspected principal periodontal pathogens. Infect. Immun., 34: 757-765, 1981.

Kilian, M., Thomsen, B., Petersen, T.E., and H.S. Bleeg. Occurence and nature of bacterial IgA proteases. Ann. NY Acad. Sci., 409: 612-624, 1983.

Klinman, N.R., and R.B. Taylor. General methods for the study of cells and serum during the immuno response: the response to dinitrophenyl in mice. Clin. Exp. Immunol., 4: 473-487, 1969.

Knop, J., Breu, H., Wernet, P., and D. Rowley. The relative anti-bacterial efficiency of IgM, IgG, and IgA from pig colostrum. Aust. J. Exp. Biol. Med. Sci., 49: 405-413, 1971.

Kowolik, M.J., Muir, K.F., and I.T. MacPhee. Di-sodium cromoglycate in the treatment of recurrent aphthous ulceration. Br. Dent. J., 144: 384-386, 1978.

Kutscher, A.H., Barbash, R., Zegarelli, E.V., and J. Amphlett. Citric acid sensitivity in recurrent ulcerative (aphthous) stomatitis. J. Allergy, 29: 438-441, 1958.

Larsson, U., Attstrom, R., and A.B. Laurell. Complement factors in gingival crevice material and in saliva. I.A.D.R. Abstracts (Scandinavian Division), No. 86, 1973.

Lehner, T. Recurrent aphthous ulceration and autoimmunity. Lancet, 2: 1154-1155, 1964.

Lehner, T. Immunofluorescent investigation of Candida albicans antibodies in human saliva. Arch. Oral Biol., 10: 975-980, 1965.

Lehner, T. Stimulation of lymphocyte transformation by tissue homogenates in recurrent oral ulceration. Immunology, 13: 159-166, 1967.

Lehner, T. Autoimmunity in oral diseases, with special reference to recurrent oral ulceration. Proc. R. Soc. Med., 61: 515-524, 1968.

Lehner, T. Pathology of recurrent oral ulceration and oral ulceration in Behcet's syndrome: light, electron, and fluorescence microscopy. J. Pathol., 97: 481-494, 1969a.

Lehner, T. Characterization of mucosal antibodies in recurrent aphthous ulceration and Behcet's syndrome. Arch. Oral Biol., 14: 843-853, 1969b.

Lehner, T. Immunoglobulin estimation of blood and saliva in human recurrent oral ulceration. Arch. Oral Biol., 14: 351-364, 1969c.

Lehner, T., Batchelor, J.R., Challacombe, S.J., and L. Kennedy.

An immunogenetic basis for the tissue involvement in Behcet's syndrome. Immunology, 37: 895-900, 1979.

Lehner, T., and J.E. Cardwell. Immunoglobulins in saliva and serum in dental caries. Lancet, 1: 1294-1297, 1967.

Lehner, T., Wilton, J.M.A., and L. Ivanyi. Double-blind crossover trial of levamisole in recurrent aphthous ulceration. Lancet, 2: 926-929, 1976.

Levinsky, R.J., and T. Lehner. Circulating soluble immune complexes in recurrent oral ulceration and Behcet's syndrome. Clin. Exp. Immunol., 32: 193-198, 1978.

Lichtig, C., Haim, S., Hammel, I., and R.F. Friedman-Birnbaum. The quantification and significance of mast cells in lesions of Behcet's disease. Br. J. Dermatol., 102: 255-259, 1980.

Lindstrom, F.D., and L.E.A. Folke. Salivary IgA in periodontal disease. I.A.D.R. Abstracts, No. 410, 1968.

Loe, H. The gingival index, the plaque index and the retention index systems. J. Periodontol., 38: 610-616, 1967.

Mach, P.S., Amor, B., Messing, B., Chicault, P., Ghozlan, R., and F. Delbarre. Salivary immunoglobulin determinations: their diagnostic value in Sjogren's syndrome. Biomedicine, 25: 31-35, 1976.

Malmstrom, M., Salo, O.P., and F. Fyhrquist. Immunogenetic markers and immune response in patients with recurrent oral ulceration. Int. J. Oral Surg., 12: 23-30, 1983.

Mancini, G., Carbonara, A.O., and J.F. Heremans. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2: 235-254, 1965.

Mancini, G., Vaerman, J.P., Carbonara, A.O., and J.F. Heremans. In Protides of the Biological Fluids. (H. Peeters, ed.) Elsevier, Amsterdam, 1963.

Mandel, I.D., and H.S. Khurana. The relationship of human salivary γA globulin and albumin to flow rate. Arch. Oral Biol., 14: 1433-1435, 1969.

Martin, D.K., Nelms, D.C., Mackler, B.F., and D.L. Peavy. Lymphoproliferative responses induced by streptococcal antigens in recurrent aphthous stomatitis and Behcet's syndrome. Clin. Immunol. Immunopathol., 13: 146-155, 1979.

McClelland, D.B.L., Samson, R.R., Parkin, D.M., and D.J.C. Shearman. Bacterial agglutination studies with secretory IgA prepared from human gastrointestinal secretions and colostrum. Gut, 13: 450-458, 1972.

Merchant, H.W., Gangarosa, L.P., Glassman, A.B., and R.E. Sobel. Betamethasone-17-benzoate in the treatment of recurrent aphthous ulcers. Oral Surg. Oral Med. Oral Pathol., 45: 870-875, 1978.

Mikulicz, J. von, and W. Kummel. In Die Krankheiten des Mundes. Jena, Gustav Fischer, 1888.

Miller, M.F. Use of levamisole in recurrent aphthous stomatitis. Drugs, 19: 131-136, 1980.

Miller, M.F., Garfunkel, A.A., Ram, C.A., and I.I. Ship. Inheritance patterns in recurrent aphthous ulcers: twin and pedigree data. Oral Surg. Oral Med. Oral Pathol., 43: 886-891, 1977.

Miller, M.F., Garfunkel, A.A., Ram, C.A., and I.I. Ship. The

inheritance of recurrent aphthous stomatitis, observations, and susceptibility. Oral Surg. Oral Med. Oral Pathol., 49: 409-412, 1980.

Miller, M.F., Silvert, M.E., Laster, L.L., Green, P., and I.I. Ship. Effect of levamisole on the incidence and prevalence of recurrent aphthous stomatitis: a double-blind clinical trial. J. Oral Pathol., 7: 387-392, 1978.

Modeer, T., Tomson, G., Falk, O., and A. Rane. Phenytoin and IgA concentrations in plasma and saliva in epileptic children. Acta Paediatr. Scand., 70: 373-378, 1981.

Morris, T.J., Matthews, N., and J. Rhodes. Serum and salivary immunoglobulin A and free secretory component in ulcerative colitis. Clin. Allergy, 11: 561-564, 1981.

Nakamura, M., and J. Slots. Salivary enzymes: origin and relationship to periodontal disease. J. Periodont. Res., 18: 559-569, 1983.

Navazesh, M., and C.M. Christensen. A comparison of whole mouth resting and stimulated salivary measurement procedures. J. Dent. Res., 61: 1158-1162, 1982.

Newcomb, R.W., and B.L. de Vald. Antibody activities of human exocrine γA diphtheria antitoxin. Fed. Proc., 28: 765, 1969.

Ogra, S.S., and P.L. Ogra. Immunologic aspects of human colostrum and milk: I. Distribution characteristics and concentrations of immunoglobulins at different times after the onset of lactation. J. Pediatr., 92: 546-549, 1978.

Olson, J.A., Feinberg, I., Silverman, S., Abrams, D., and J.S.

Greenspan. Serum vitamin B_{12}, folate, and iron levels in recurrent aphthous ulceration. Oral Surg. Oral Med. Oral Pathol., 54: 517-520, 1982.

Olson, J.A., Nelms, D.C., Silverman, S., and L.E. Spitler. Levamisole: a new treatment for recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol., 41: 588-600, 1976.

Olson, J.A., and S. Silverman. Double-blind study of levamisole therapy in recurrent aphthous stomatitis. J. Oral Pathol., 7: 393-399, 1978.

Ozkaragoz, K., Smith, H.J., and M. Gokcen. IgE levels in serum, saliva, and urine of normal individuals. Acta Allergol., 27: 392-396, 1972.

Parkhouse, R.M.E., and M.D. Cooper. A model for the differentiation of B lymphocytes with implications for the biological role of IgD. Immunol. Rev., 37: 105-126, 1977.

Peavy, D.L., Nelms, D.C., and B. Mackler. Failure of autologous oral epithelia to activate RAS lymphocytes. Clin. Immunol. Immunopathol., 22: 291-295, 1982.

Peppard, J.V. Quantitative estimation of IgA in rats: a comparison of two methods. J. Immunol. Methods, 31: 129-139, 1979.

Pimlott, S.J., and D.M. Walker. A controlled clinical trial of the efficacy of topically applied fluocinonide in the treatment of recurrent aphthous ulceration. Br. Dent. J., 154: 174-177, 1983.

Platts-Mills, T.A.E., Snajdr, M.H., Ishizaka, K., and A.W. Frankland. Measurement of IgE antibody by an antigen-binding assay: correlation with PK activity and IgG and IgA antibod-

ies to allergens. J. Immunol., 120: 1201-1209, 1978.

Platz, P., Ryder, L.P., and O. Donatsky. No deviations of HLA-A, and -B antigens in patients with recurrent aphthous stomatitis. Tissue Antigens, 8: 279-280, 1976.

Plaut, A.G., Genco, R.J., and T.B. Tomasi. Isolation of an enzyme from Streptococcus sanguis which specifically cleaves IgA. J. Immunol., 113: 289-291, 1974.

Reimer, G., Luckner, L., and O.P. Hornstein. Direct immunofluorescence in recurrent aphthous ulcers and Behcet's disease. Dermatologica, 167: 293-298, 1983.

Reimer, G., Steinkohl, S., Djawari, D., and O.P. Hornstein. Lytic effect of cytotoxic lymphocytes on oral epithelial cells in Behcet's disease. Br. J. Dermatol., 107: 529-536, 1982.

Richter, J., Kral, V., Zukov, I., Subrt, P., and J. Rahm. Circadian changes of the sIgA, lysozyme, albumin and copper content of saliva. Czech. Med., 4: 249-254, 1980.

Rogers, R.S. Recurrent aphthous stomatitis: clinical characteristics and evidence for an immunopathogenesis. J. Invest. Dermatol., 69: 499-509, 1977.

Rogers, R.S., Movius, D.L., and R.E. Jordon. Serum studies in patients with recurrent aphthous stomatitis and periodontal disease. J. Dent. Res., 54: 178, 1975.

Rogers, R.S., Sams, W.M., and R.G. Shorter. Lymphocytotoxicity in recurrent aphthous stomatitis: lymphocytotoxicity for oral epithelial cells in recurrent aphthous stomatitis and Behcet syndrome. Arch. Dermatol., 109: 361-363, 1974.

Ross, R., Kutscher, A.H., Zegarelli, E.V., Silvers, H., and J.D.

Piro. Relationship of mechanical trauma to recurrent ulcerative (aphthous) stomatitis. NY State Dent. J., 24: 101-102, 1958.

Rowe, D.S., Crabbe, P.A., and M.W. Turner. Immunoglobulin D in serum, body fluids and lymphoid tissues. Clin. Exp. Immunol., 3: 477-490, 1968.

Rowley, D., and K.J. Turner. Number of molecules of antibody required to promote phagocytosis of one bacterium. Nature, 210: 496-498, 1966.

Sallay, K., and J. Banoczy. Remarks on the possibilities of the simultaneous occurrence of hyperkeratosis of the mucous membranes and recurrent aphthae. I. Clinical investigations. Oral Surg. Oral Med. Oral Pathol., 25: 171-175, 1968.

Salmon, S.E. IgE globulin in secretions. Clin. Res., 18: 135, 1970.

Salmon, S.E., Mackey, G., and H.H. Fudenberg. "Sandwich" solid phase radioimmunoassay for the quantitative determination of human immunoglobulins. J. Immunol., 103: 129-137, 1969.

Schroeder, H.E., Muller-Glauser, W., and K. Sallay. Stereologic analysis of leukocyte infiltration in oral ulcers of developing Mikulicz aphthae. Oral Surg. Oral Med. Oral Pathol., 56: 629-640, 1983.

Scully, C., Yap, P.L., and P. Boyle. IgE and IgD concentrations in patients with recurrent aphthous stomatitis. Arch. Dermatol., 119: 31-34, 1983.

Seager, J., Jamison, D.L., Wilson, J., Hayward, A.R., and J.F. Soothill. IgA deficiency, epilepsy, and phenytoin treatment.

Lancet, 2: 632-635, 1975.

Segal, A.L., Katcher, A.H., Brightman, V.J., and M.F. Miller. Recurrent herpes labialis, recurrent aphthous ulcers, and the menstrual cycle. J. Dent. Res., 53: 797-803, 1974.

Sewell, H.F., Matthews, J.B., Flack, V., and R. Jefferis. Human immunoglobulin D in colostrum, saliva, and amniotic fluid. Clin. Exp. Immunol., 36: 183-188, 1979.

Shapiro, S., Olson, D.L., and S.J. Chellemi. The association between smoking and aphthous ulcers. Oral Surg. Oral Med. Oral Pathol., 30: 624-630, 1970.

Shillitoe, E.J., and T. Lehner. Immunoglobulins and complement in crevicular fluid, serum and saliva in man. Arch. Oral Biol., 17: 241-247, 1972.

Ship, I.I. The etiology of recurrent aphthous stomatitis: the effect of a non-allergic regime in hospitalized patients. J. Dent. Res., 39: 748, 1960.

Ship, I.I. Inheritance of aphthous ulcers of the mouth. J. Dent. Res., 44: 837-844, 1965.

Ship, I.I., Brightman, V.J., and L.L. Laster. The patient with recurrent aphthous ulcers and the patient with recurrent herpes labialis: a study of two population samples. J. Am. Dent. Assoc., 75: 645-654, 1967.

Ship, I.I., Morris, A.L., Durocher, R.T., and L.W. Burket. Recurrent aphthous ulcerations and recurrent herpes labialis in a professional school student population. I. Experience. Oral Surg. Oral Med. Oral Pathol., 13: 1191-1202, 1960a.

Ship, I.I., Morris, A.W., Durocher, R.T., and L.W. Burket.

Recurrent aphthous ulcerations and recurrent herpes labialis in a professional school student population. II. Medical history. Oral Surg. Oral Med. Oral Pathol., 13: 1317-1329, 1960b.

Ship, I.I., Morris, A.L., Durocher, R.T., and L.W. Burket. Recurrent aphthous ulcerations in a professional school student population. IV. Twelve-month study of natural disease patterns. Oral Surg. Oral Med. Oral Pathol., 14: 30-39, 1961.

Shklair, I.L., Rovelstad, G.H., and B.L. Lamberts. A study of some factors influencing phagocytosis of cariogenic streptococci by caries-free and caries-active individuals. J. Dent. Res., 48: 842-845, 1969.

Sims, W. The concept of immunity in dental caries. Oral Surg. Oral Med. Oral Pathol., 34: 69-86, 1972.

Sircus, W., Church, R., and J. Kelleher. Recurrent aphthous ulceration of the mouth: a study of the natural history, aetiology, and treatment. Q. J. Med., 26: 235-249, 1957.

Smith, Q.T., Hamilton, M.J., Biros, M.H., and B.L. Pihlstrom. Salivary and plasma IgA of seizure subjects receiving phenytoin. Epilepsia, 20: 17-23, 1979.

Sorensen, C.H. Secretory IgA enzyme immunoassay application of a model for computation of the standard curve. Scand. J. Clin. Lab. Invest., 42: 577-583, 1982.

Spouge, J.D., and H.F. Diamond. Hypersensitivity reactions in mucous membranes: I. The statistical relationship between hypersensitivity disease and recurrent oral ulcerations.

Oral Surg. Oral Med. Oral Pathol., 16: 412-421, 1963.

Staley, H.R. Aphthous lesions. Oral Surg. Oral Med. Oral Pathol., 33: 407-416, 1972.

Stanley, H.R., Graykowski, E.A., and M.F. Barile. The occurrence of microorganisms in microscopic sections of aphthous and non aphthous lesions and other oral tissue. Oral Surg. Oral Med. Oral Pathol., 18: 335-341, 1964.

Strauss, K. Vitamin B_1 therapy in cyclic habitual aphthous stomatitis in women. Br. Dent. J., 83: 77-80, 1947.

Sutton, R.L. Periadenitis mucosa necrotica recurrens. J. Cutan. Dis., 29: 65-71, 1911.

Symoens, J., and J. Brugmans. Treatment of recurrent aphthous stomatitis and herpes with levamisole. Br. Med. J., 4: 592, 1974.

Taylor, K.B., Truelove, S.C., and R. Wright. Serologic reactions to gluten and cow's milk proteins in gastrointestinal disease. Gastroenterology, 46: 99-108, 1964.

Thomas, H.C., Ferguson, A., McLennan, J.G., and D.K. Mason. Food antibodies in oral disease: a study of serum antibodies to food proteins in aphthous ulceration and other oral diseases. J. Clin. Pathol., 26: 371-374, 1973.

Tomasi, T.B. Human gamma globulin. Blood, 25: 382-403, 1965.

Tomasi, T.B. Secretory immunoglobulins. N. Engl. J. Med., 287: 500-506, 1972.

Tomasi, T.B. In Basic and Clinical Immunology. (D.P. Stites, J.D. Stobo, H.H. Fudenberg, and J.V. Wells, eds.) Lange Medical Publications, Los Altos, California, 1982.

Truelove, S.C., and R. Morris-Owen. Treatment of aphthous ulceration of the mouth. Br. Med. J. Clin. Res., 1: 603-607, 1958.

Tuft, L., and L.N. Ettelson. Canker sores from allergy to weak organic acids (citric and acetic). Case report and clinical study. J. Allergy, 27: 536-543, 1956.

Tuft, L., and L.S. Girsh. Buccal mucosal tests in patients with canker sores (aphthous stomatitis). J. Allergy, 29: 502-510, 1958.

Twetman, S., Lindner, A., and T. Modeer. Lysozyme and salivary immunoglobulin A in caries-free and caries-susceptible pre-school children. Swed. Dent. J., 5: 9-14, 1981.

Ullman, S., and R.J. Gorlin. Recurrent aphthous stomatitis: an immunofluorescence study. Arch. Dermatol., 114: 955-956, 1978.

Vaerman, J.P., and J.F. Heremans. Effect of neuraminidase and acidification on complement-fixing properties of human IgA and IgG. Int. Arch. Allergy Appl. Immunol., 34: 49-52, 1968.

Van de Heynig, J. Levamisole in the treatment of recurrent aphthous stomatitis. Laryngoscope, 88: 522-527, 1978.

Van Hale, H.M., Rogers, R.S., Doyle, J.A., and A.L. Schroeter. Immunofluorescence microscopic studies of recurrent aphthous stomatitis. Arch. Dermatol., 117: 779-781, 1981.

Verhaegen, H., De Cree, J., and J. Brugmans. Treatment of aphthous stomatitis. Lancet, 2: 842, 1973.

Virella, G., Boudswaard, J., and R.J. Boackly. Preparation of

saliva samples for immunochemical determination of immunoglobulins and for assay of lysozyme. Clin. Chem., 24: 1421-1422, 1978.

Waksman, B.H. Auto-immunization and the lesions of auto-immunity. Medicine, 41: 93-141, 1962.

Waldman, R.H., Mann, J.J., and J.A. Kasel. Influenza virus neutralizing antibody in human respiratory secretions. J. Immunol., 100: 80:85, 1968.

Waldman, R.H., Virchow, C., and D.S. Rowe. IgE levels in external secretions. Int. Arch. Allergy Appl. Immunol., 44: 242-248, 1973.

Walker, D.M., and A.E. Dolby. Aphthous ulceration, cromoglycic acid, and cellular immune response. Lancet, 1: 1390, 1975.

Walker, D.M., Dolby, A.E., Mead, J., Llewellyn, J., and J. Rhodes. Effect of gluten-free diet on recurrent aphthous ulceration. Br. J. Dermatol., 103: 111, 1980.

Weathers, D.R., and J.W. Griffin. Intraoral ulcerations of recurrent herpes simplex and recurrent aphthae: two distinct clinical entities. J. Am. Dent. Assoc., 81: 81-88, 1970.

Widerstrom, L., and D. Bratthall. Increased IgA levels in saliva during pregnancy. Scand. J. Dent. Res., 92: 33-37, 1984.

Williams, B.D., and T. Lehner. Immune complexes in recurrent oral ulceration and Behcet's syndrome. Br. Med. J., 1: 1387-1389, 1977.

Williams, R.C., and R.J. Gibbons. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science, 177: 697-699, 1972.

Wilson, C.W.M. Food sensitivities, taste changes, aphthous ulcers and atopic symptoms in allergic disease. Ann. Allergy, 44: 302-307, 1980.

Wilson, I.D. Studies on the opsonic activity of human secretory IgA using an in vitro phagocytosis system. J. Immunol., 108: 726-730, 1972.

Wilton, J.M.A., and T. Lehner. An investigation into the antigenic relationship between oral bacteria and oral mucosa. J. Dent. Res., 47: 1001, 1968.

Wray, D. Gluten-sensitive recurrent aphthous stomatitis. Dig. Dis. Sci., 26: 737-740, 1981.

Wray, D. In Viruses in Oral Medicine. (J.J. Hooks, and G.W. Jordan, eds.) Elsevier North-Holland, New York, 1982.

Wray, D., Ferguson, M.M., Hutcheon, A.W., and J.H. Dagg. Nutritional deficiencies in recurrent aphthae. J. Oral Pathol., 7: 418-423, 1978.

Wray, D., Ferguson, M.M., Mason, D.K., Hutcheon, A.W., and J.H. Dagg. Recurrent aphthae: treatment with vitamin B_{12}, folic acid, and iron. Br. Med. J., 2: 490-493, 1975.

Wray, D., Graykowski, E.A., and A.L. Notkins. Role of mucosal injury in initiating recurrent aphthous stomatitis. Br. Med. J., 283: 1569-1570, 1981a.

Wray, D., Rubinstein, P., Walker, M., and A. Notkins. Inheritance and HLA markers in recurrent aphthous stomatitis (RAS). J. Dent. Res., 60: 378, 1981b.

Wray, D., Vlagopoulos, T.P., and R.P. Siraganian. Food allergies and basophil histamine release in recurrent aphthous stomati-

tis. Oral Surg. Oral Med. Oral Pathol., 54: 388-395, 1982.

Yeoman, C.M., Greenspan, J.S., and S.M. Harding. Recurrent oral ulceration: a double-blind comparison of treatment with betamethasone valerate aerosol and placebo. Br. Dent. J., 144: 114-116, 1978.

Zegarelli, E.V., Kutscher, A.H., Silvers, H.F., Beube, F.E., Stern, I.B., Berman, C.L., and R.E. Herlands. Triamcinolone acetonide in the treatment of acute and chronic lesions of the oral mucous membranes. Oral Surg. Oral Med. Oral Pathol., 13: 170-175, 1960.

Zegarelli, E.V., Silvers, H.F., and A.H. Kutscher. Antihistaminic agents in the treatment of recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol., 6: 302-304, 1953.

Zipursky, A., Brown, E.J., and J. Bienenstock. Lack of opsonization potential of 11S human secretory γA. Proc. Soc. Exp. Biol. Med., 142: 181-184, 1973.

Zissis, N.P., Hatziotis, A.J., Antoniadis, D., Ninika, A., and J.C. Hatziotis. Therapeutic evaluation of levamisole in recurrent aphthous stomatitis: double-blind comparison of two dosage schedules of levamisole and placebo. J. Oral Med., 38: 161-163, 1983.