A STUDY OF CHANGES IN THE GINGIVAL TISSUE AND ASSOCIATED MICROFLORA IN RENAL TRANSPLANT RECIPIENTS MAINTAINED ON CYCLOSPORINE THERAPY

Submitted in Partial Fulfillment

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BY

OLVA ODLUM

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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INTRODUCTION

Drug induced tissue changes are a frequently occurring feature in people who are maintained on long term drug therapy. Many of these changes go unnoticed or are tolerated due to the location of the affected tissues. Changes in the oral tissue, however, are often unacceptable because of their visibility and disfiguring effect. Such patients will often seek remedial care.

Reports from the early clinical trials of Cyclosporin A referred to an unwanted effect on the oral tissues, gingival hyperplasia. Cyclosporine A, an immunosuppressive drug, was being tested at that time as an anti-rejection agent in renal transplantation.

Since those trials, Cyclosporine (as it is now termed) has become routinely and effectively used in transplant management. New areas for use of the drug are now being explored, particularly in relation to the wide ranging group of diseases classified as auto-immune. As a result, an increasing number of people are being maintained on long term Cyclosporine therapy.

Patients on Cyclosporine therapy may initially present for dental care because of their concern about the changes occurring in their mouths. In addition to the direct impact of Cyclosporine on the oral tissues, the management of these patients requires consideration of the general systemic effect of the immuno-suppressed state of the patient. In the case of many of these patients, the impact of the underlying

disease that lead to treatment with immuno-suppressive drug may be another factor affecting dental care.

The dental literature on Cyclosporine at the outset of this study was confined to a few case studies which described the occurrence of gingival hyperplasia. This condition is characterized by an overgrowth of the gums which can cover as much as a third of the tooth. It is not commonly seen in the general population, but has been identified as a side effect of a number of drugs, particularly phenytoin.

Phenytoin (Diphenylphenytoin) is a long established treatment for a range of neurological conditions. Unlike Cyclosporine, Phenytoin has been studied intensively. (More than 1500 articles are listed in the Index Medicus). The impact of this drug on the oral tissue bears a close resemblance to that described for Cyclosporine. Potentially, the work on phenytoin can serve as a valuable model for the study of effects of Cyclosporine.

Although the management of Cyclosporine-induced gingival hyperplasia poses a complex series of problems, accurate information on the patients or their care is sparse. A study was designed to examine these issues.

The first objective of the present study was to determine the prevalence and severity of gingival hyperplasia in patients maintained on long term Cyclosporine therapy. It was known from the case studies that this condition was associated with use of the drug, but there was

little information on how often and how severely it occurred, or what were the characteristics of the patients who did or did not develop hyperplasia. In the study, data was collected on age, sex, duration of therapy, and serum levels of Cyclosporine during the first thirty days post-transplant. It was likely that subjects would also have systemic conditions other than renal failure, e.g. diabetes. These would be noted as they might have a bearing on oral tissue response.

Clinical examination would provide data on oral hygiene status and the occurrence of any gingival hyperplastic change. Analysis would show if any one or more of these variables was related to the presence of hyperplasia.

The second and third objectives were to identify the microbial factors which might have influenced the condition and would be associated with gingival hyperplasia in patients on early or long term Cyclosporine. Black pigmented Bacteroides are the microbes most frequently associated with gingival pathology in the dental literature. Nothing was known, however, about the association of black pigmented Bacteroides with Cyclosporine induced hyperplasia. The aim in this study was to establish the relationship between black pigmented Bacteroides and gingival hyperplasia in patients on long term Cyclosporine.

It is possible, however, that black pigmented Bacteroides may be present in renal transplant patients prior to the development of hyperplasia and even prior to

long term drug therapy. Ideally, one would determine the presence of black pigmented Bacteroides in patients before they started on Cyclosporine therapy and then follow the same group of patients through to the development of hyperplasia, monitoring the presence of black pigmented Bacteroides at frequent intervals. A longitudinal study was not feasible, however, due partly to the small number of patients undergoing renal transplantation in a given time period and partly to the even smaller number of these patients who developed hyperplasia. As an alternative, the presence of black pigmented Bacteroides was investigated in two groups; the first group was comprised of patients examined within a few days of receiving a kidney transplant; the second group was made up of patients on long term Cyclosporine with marked evidence of gingival hyperplasia.

1. CYCLOSPORINE

The immunosuppressive drug Cyclosporine is a metabolite isolated from the culture broth of a 2 soil fungi Trichoderma polysporum Rifai and Cylindrocarpum Lucidum (Morris, 1981). It was first isolated in 1970 by pharmacologists at Sandoz Ltd., Switzerland, in an attempt to develop a new anti-fungal agent. In 1972 Borel observed its strong cytostatic action and tested it for immunosuppressive capability. Non-impairment of hemopoietic tissue and marked effects on lymphoid cells led to its development as a prototype for a new generation of immunosuppressants which would have selective immunoregulation (Borel et al., 1976). By 1977 the drug was tested in animal allograft transplant studies (Kostalis et al., 1977; Green and Alison, 1978; and Horman et al., 1980) and by 1978 human clinical trials were commenced in Cambridge, England (Calne, 1978). Cyclosporine is now registered as a drug and is used by thousands of transplant recipients throughout the world.

i. Chemistry, Pharmokinetic and Metabolism

Cyclosporine is a neutral, hydrophobic cyclic peptide composed of eleven amino-acids (residues) all having the S-configuration of the natural L-amino acids with the exception of the O-alanine in position 8, which has the R-configuration (Borel et al., 1976). Although the drug can be administered intramuscularly, it is poorly absorbed, and is usually taken orally as a suspension in olive oil.

Absorption levels for the individual are steady with a clearance rate of approximately 27 hours from plasma. In the blood, 50 per cent of Cyclosporine is bound to lipoproteins, 5 per cent to other plasma proteins, and the remainder is free in plasma water (Morris, 1981). Work by Ryfeel et al. (1980) on murine lymphocytes identified lymphocytes of high affinity-binding sites for Cyclosporine. Radioactive labelling techniques isolated about 12 metabolites of Cyclosporine in plasma and 9 in excreted urine. The major route of excretion in man is the biliary system (50 per cent) with about 6 per cent leaving the kidney (Beveridge et al., 1981).

To be effective, as an anti-rejection drug Cyclosporine administration must begin at the time of the transplant surgery i.e. when the host is first exposed to the antigenic allograft. The drug is relatively ineffective if administered after the rejection process has been initiated (Horman et al., 1980).

It is believed that in humans Cyclosporine acts predominantly in preventing proliferation of helper T-cells by inhibiting interleukin 2 production. This does not affect suppressor T-cell activation. According to Snyder et al. 1987 antigen dependent T-cell proliferation is affected by Cyclosporine, inhibiting antigen presentation, via the monocyte system. This theory is supported by numerous animal studies where responses of lymphotoxins to

histocompatability antigens were tested and found to be suppressed (Farrar et al., 1980, Bunjes et al., 1981).

It had been widely held that Cyclosporine did not have an effect on the B-cell system. Evidence however is mounting to suggest that certain T-independent B-lymphocyte subgroups are sensitive to Cyclosporine (Pauvonen and Hayry, 1980; Kamkl and Klaus, 1980).

The overwhelming concern with new drugs is the effect on other body tissues and systems. Clearly the impact of reducing the body's immune capacity poses grave risks, added to this most therapies produce specific effects in other tissues (Ota and Bradley, 1983). To date, because of its focussed suppression of the cell-mediated immune system, Cyclosporine comes closest to the 'ideal' rejection suppressant, as its action does not lead to a blanket lowering of resistance to invasive micro-organisms.

Early in the clinical trial period, a number of unwanted effects were reported in subjects, including nephrotoxicity, hypertension, hirsutism and gingival hyperplasia (Sweeney et al., 1981). Among reported and potential problems, gingival hyperplasia would appear of relatively minimal concern, but due to the location of these tissues at the entrance of the respiratory and digestive tracts they could create a potential nidus of infection. Further, due to the visibility of this disfiguring feature, it could become the focus of patient's concern and anxiety (Jenson, 1980). It is estimated that by 1985 approximately

1,400 transplant patients in Canada were maintained on Cyclosporine. The present study was designed to explore possible mechanisms involved in the development of oral changes in patients maintained on long term immunosuppression.

2. THE GINGIVAL TISSUE

i. Structure and Histology

The human dentition is supported in the bony sockets of the jaws by a specialized soft tissue system. A series of periodontal fibres originating in the bone is connected to the cemental layer of the roots creating a ligamentous joint which can normally withstand the forces of speech, mastication and deglutition. Covering the alveolar bone and the cervical portion of the tooth is a layer of mucous membrane known as gingival epithelium (Ainamo and Loe, 1966). This tissue is differentiated into three areas.

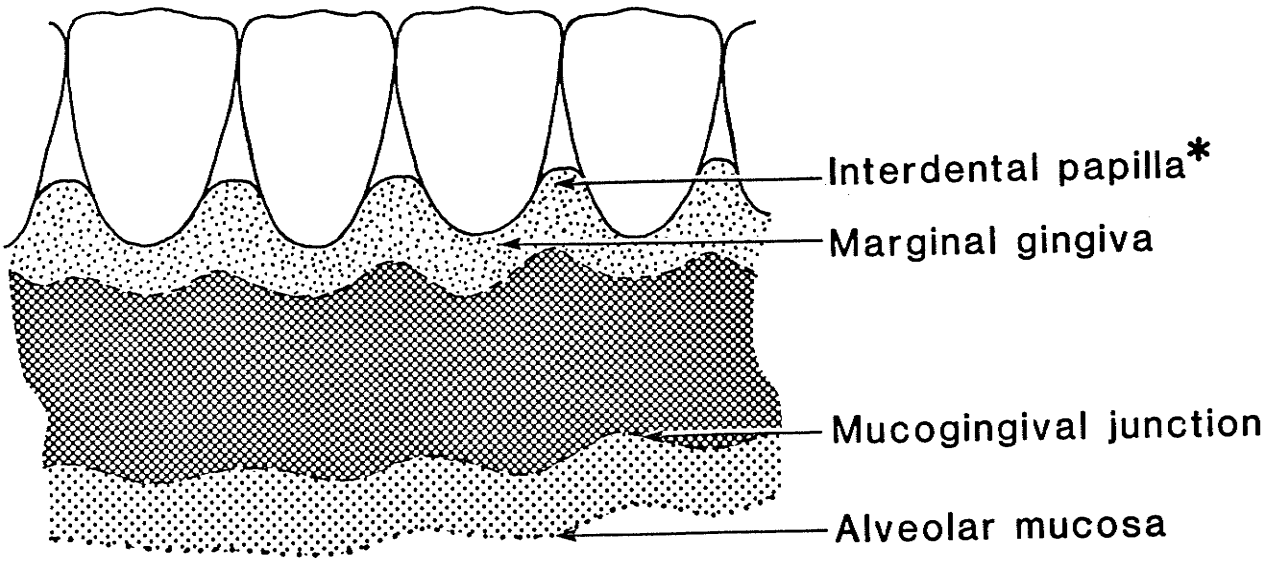
The attached gingiva is made up of stratified squamous epithelium tightly bound to the alveolar bone. The underlying stroma of connective tissue is very dense. This tissue is the demarcation between oral mucous membrane and the free gingival tissue known as the marginal gingiva.

The marginal gingiva surrounds each tooth in a collar-like fashion and a shallow linear groove, the free gingival groove, demarcates the tissue between adjacent teeth. The gingival sulcus is the indentation formed between the tooth and the free margin of the gingiva. In normal tissue this has a depth of 1-2 mm (Gurgulio et al., 1961). Histologically the marginal gingiva consists of an outer layer of keratinized squamous epithelium with obvious Rete pegs extending into the underlying connective tissue. In the healthy state the connective tissue contains a system of collagen fibre bundles, the gingival fibres. These fibres

connect the marginal to the attached gingiva with another series encircling the teeth (Arrim and Hagerman, 1953).

The final portion of the gingival tissue is the segment that fills the embrasure between adjacent teeth and is known as the papillary gingival tissue. In normal tissues this is histologically similar to the marginal gingiva but with the absence of the binding collagen fibres. In the healthy mouth the gingival tissue shows a characteristically firm, pink stippled appearance. As a consequence of its keratinized layer it is able to resist normal oral trauma. The gingival tissues are prone to a number of diseases, the most commonly occurring being gingivitis. The mechanisms involved in gingivitis, initiating factors, local and possibly systemic responses are of note and will be described in the following chapter.

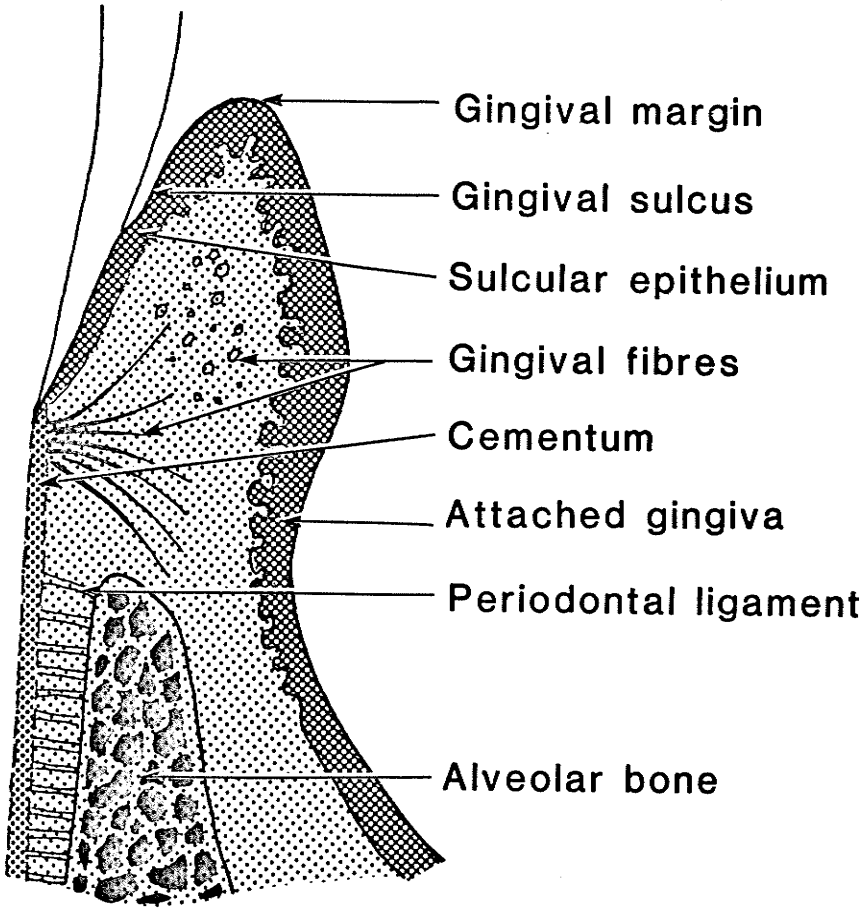
Figure 1
SURFACE TOPOGRAPHY OF NORMAL GINGIVA



*Gingival site most commonly affected by hyperplasia

Figure 2

CROSS-SECTIONAL ANATOMY OF THE NORMAL PERIODONTIUM



3. GINGIVITIS

i. Signs and Symptoms

Gingivitis is recognized clinically when a change in appearance of the firm, pink gingival tissue to a smooth red shiny surface takes place. The tissue bleeds readily under minimal pressure and in some patients is reportedly painful. Work by Lindhe and Nyman (1975) and Loe et al., (1965) showed that gingival inflammation was associated with the presence of plaque on the teeth, the degree of inflammation corresponding to the amount of plaque in otherwise healthy subjects. Classical gingivitis is confined to the margins of the gingival tissue and is not destructive to the underlying structures. Thirty per cent of the dentate adult population of North America are known to have gingivitis (Loe, 1986).

ii. Etiology

The earliest report on this topic was published in 1883 when van Leevenoek collected samples of plaque for observation in his microscope research work (Marquis, 1968). On viewing the specimen of plaque he described a large number of 'living animalcules'.

For years gingivitis has been considered a unique, microbially elicited condition due to the fact that the plaque microbes were believed to provoke the inflammatory response without penetration of the tissues (Frank and Voegel, 1978; Allenspach and Guggenhein, 1983). Similarly colonized exposed areas of e.g. skin or gut do not respond

in this way. Acute gingivitis was proved to be a plaque-related disease by a study where a group of subjects with healthy mouths developed symptoms by refraining from any oral hygiene activities. Within 3-5 days marginal bleeding was readily elicited (Loe, 1965). Resumption of oral hygiene practices reversed the condition.

The dental plaque adjacent to an area of gingivitis is well established and is comprised of bacteria with their own ecosystem and includes extra and intracellular products and cells which are being killed and lysed (Bibby, 1953; Ellison, 1970; Gibbons et al., Darwish et al., 1978). This, so called supragingival plaque is thicker and more complex than in healthy tissues. Most of the constituent bacteria are facultative or total anaerobes. Obligate aerobes constitute an insignificant portion of this plaque. It has been reported that the relative proportions of the various bacteria in a plaque change over time as the system becomes established (Hardie and Bowden, 1975).

Streptococcus	Neisseria
sanguis	flavescens
salivarius	mucosa
mutans	sicca
mitior	
mitis	
Actimomyces	Bacteroides
viscosus	gingivalis
naeslundi	melaninogenicus
israelli	ochraceus
odontolyticus	ruminicola
	oralis
Lactobacillus	
casei	
acidophilus	
salivarius	
plantarum	
fermentum	

Fig. 3 Constituent microbes of normal supra-gingival plaque (from Bowden et al., 1979).

Plaque in the gingival sulcus in gingivitis is very similar to supragingival plaque in normal tissues but with more species that are obligate anaerobes. Difficulties in accurate sampling and culturing limit absolute value determination of anaerobic bacteria at present (Slots, 1976).

It appears likely that a critical mass of plaque accumulation is a requirement for initiation of inflammation. When this occurs there is an effusion of plasma from the arterioles and neutrophils move into the intercellular areas. If the condition continues for a few days, macrophages and lymphocytes appear as would be expected in a chronic infection (Walter, 1977).

iii. Immunology

Within two weeks of cessation of oral hygiene practices, in study populations, the normally low levels of immunoglobulins in the gingival tissues increase with IgG being the predominant contributor while IgM, IgA and occasionally IgE contribute low levels (Schroeder and Lindhe, 1975). Berglund (1971) showed that immunoglobulins from inflamed tissue cross-react with antigens from bacterial plaque, strongly suggesting that a humoral response is taking place. There is also evidence that antibodies to oral micro-organisms can be shown in the general circulation (Ivanyi and Lehner, 1974; Nisengard, 1977; Ebersole et al., 1985; Tollefsen et al., 1986). More recently studies have been able to identify an antibody for specific pathogens at sites of gingival infection and to find the same antigen in circulating serum (Tew et al., 1985).

iv. Histology

The histology of gingivitis has to be described longitudinally in so much as 'clinically normal' gingivae generally exhibit degrees of inflammatory involvement with lymphocytes, neutrophils and macrophages present in the connective tissue adjacent to the functional epithelium.The onset of observable gingivitis is noted by increased cellular infiltration. The vascular tissues show increased permeability and fluid can be found in the gingival

crevices. With time the number of functional vascular units expands (Soderholm and Egelberg, 1973).

Over time a steady state is developed characterized by the presence of large numbers of plasma cells as the predominant cell in the connective tissues. At this time the dento-gingival epithelium is thinned and becomes infiltrated by lymphocytes and plasma cells. Transmigration by neutrophilic granulocytes can be observed, Muller-Glasser and Schroeder (1982).

v. Hormonal Interaction

Prostaglandins are a group of aliphatic acids found at increased levels in inflamed gingivae. They can be produced by many body cells and react at local sites. Prostaglandin can in the gingival tissues (a) modify the acute inflammatory response, (b) inhibit mitogenic and antigenic responses of lymphocytes, (c) interfere with repair of periodontal fibre damage by inhibiting fibroblast mitosis, (d) block the synthesis of collagen and non-collagenous proteins of connective tissue and, (e) resorb bone (Ralsz et al., 1981).

The continuing persistence of gingivitis has the potential for considerable tissue damage. It has been reported that removal of supra-gingival plaque and maintenance of good oral hygiene would reverse the inflammed tissue to normal within a few days (Loe, 1965). The question still remains as to why in some patients, if the plaque is not removed, the gingivitis may continue for years with no

progression, while in other patients the gingival disease will enter a destructive phase. Sixty-two percent of North American dentate adults show chronic gingivitis but only 22% have a progressively destructive disease (Loe, 1986).

Based on this outline of some of the factors inherent in or commonly affecting gingival health, it can be seen that the introduction of an immunosuppressive drug with such wide ranging effects such as Cyclosporine could have an effect on the gingivae and on its relationship with the gingival microbial system.

4. DRUG-INDUCED GINGIVAL HYPERPLASIA

i. Clinical Appearance

Hyperplasia has been defined as an increase in the size of tissue or an organ produced by an increase in the number of its component cells. Non-inflammatory gingival hyperplasia is produced by factors other than local irritants and is not common (Glickman, 1953).

Most commonly reported in the literature is gingival hyperplasia occurring in patients maintained on phenytoin (Carranza, 1984). Phenytoin is generally the drug of choice in the treatment of epileptic seizures. It is prescribed on a continuous basis for patients with this affliction. Other conditions such as puberty and pregnancy gingivitis are apparently a type of hyperplasia and appear to be related to hormonal influences (Litwack et al., 1970). A rare condition, known as idiopathic hyperplastic enlargement of the gingivae, is known to occur in certain families and is genetically determined (Setterston et al., 1980).

Recently several cases of hyperplasia have been reported to be induced by newly developed drugs. These are medications which are taken on a long term basis for management of chronic conditions. Examples are nifedipine (Procardia) which is taken for control of angina pectoris and hypertension (Lederman et al., 1984) and Cyclosporine (Wysocki et al., 1983). Due to the recent development of these two drugs, studies to date are few. Reports which included histology, indicate a situation indistinguishable

from that found in the phenytoin-induced cases of hyperplasia (Lucas et al., 1984). Literature on Cyclosporine suggests many similar effects to those produced by phenytoin. Both drugs can produce hirsutism, acne, immunosuppression, skin thickening (Vittek, 1979; Ota and Bradley, 1983) and minor central nervous system reactions. In transplant patients who are also epileptic it has been found that phenytoin acts on the liver to produce enzymes that speed up the degradation of Cyclosporine leading to greater doses being required to achieve immunosuppression. As a result these two drugs are now rarely prescribed together for a specific patient.

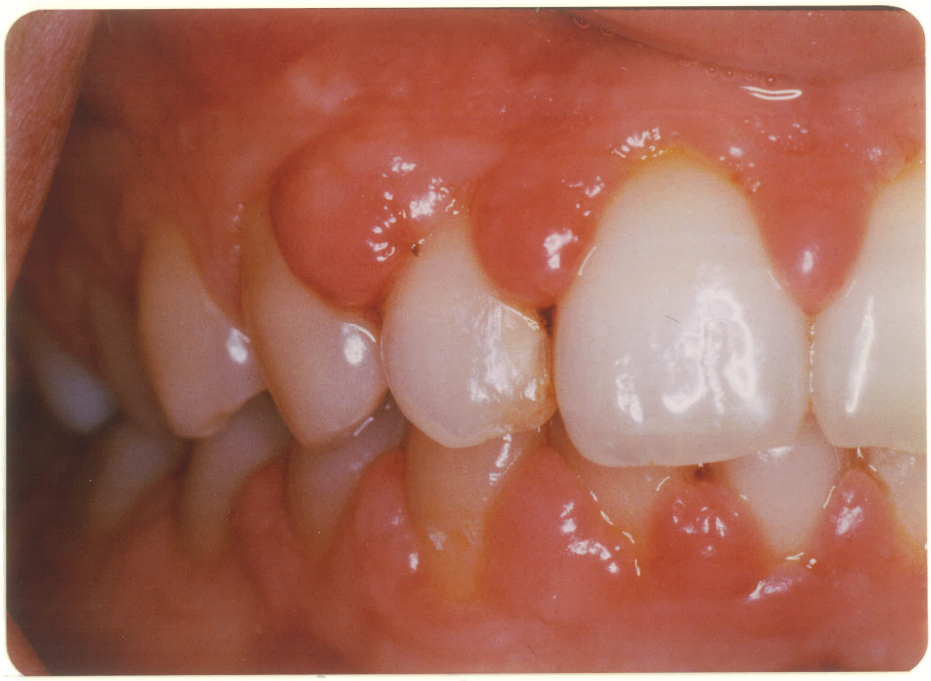


Figure 4
Photograph of Cyclosporine-induced localized hyperplasia

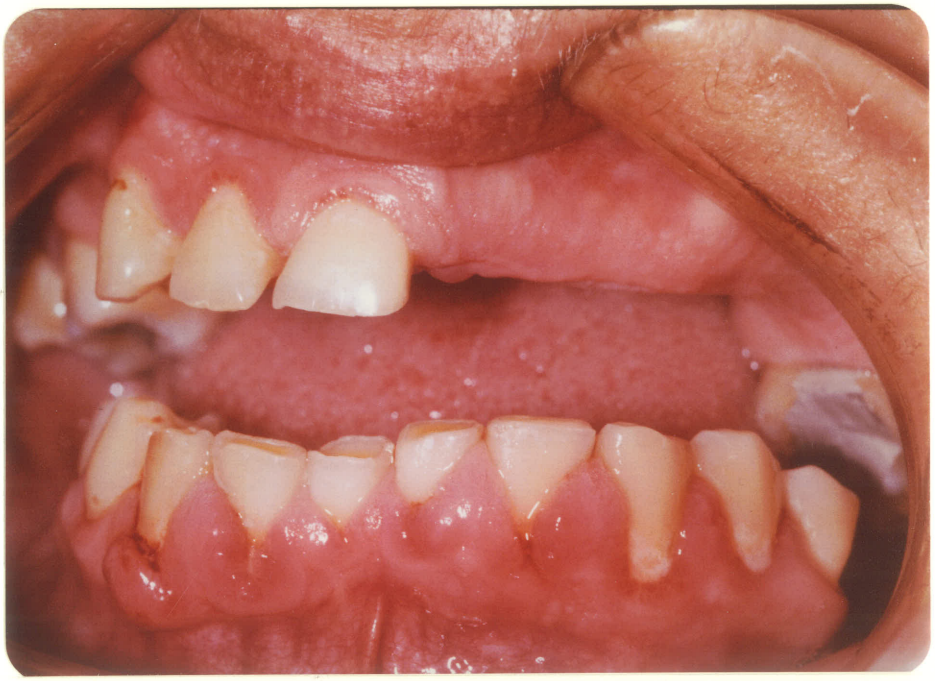


Figure 5
Photograph of Cyclosporine-induced generalized hyperplasia

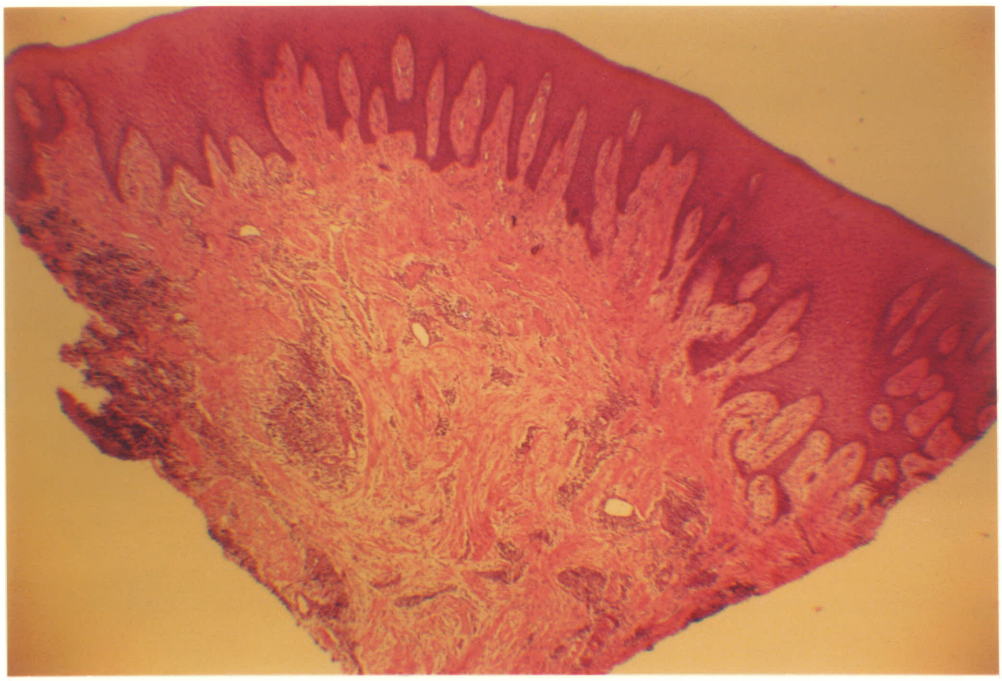


Figure 6
Section of hyperplastic gingival tissue showing elongated Rete-pegs (x31 stained Hematoxolin and eosin).

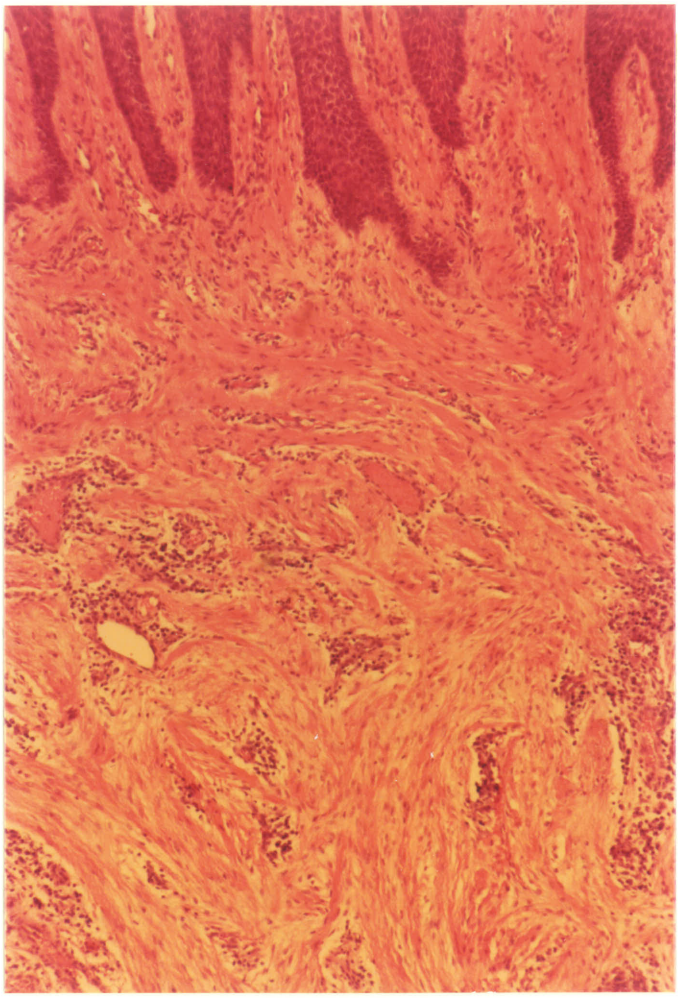


Figure 7
Section of hyperplastic tissue showing dense fibroblastic formation of connective tissue (x78 stained Hematoxolin and eosin).

The severity of drug-induced hyperplasia varies between patients. It may be localized or generalized, the latter being the more common situation in the case of phenytoin (Esterberg and Whiter, 1945). A mild case presents as an enlargement of the interdental papillae which may be accompanied by swelling of the gingival margin most commonly on the buccal surface (Steinberg, 1981). In severe cases, enlargement may be widespread and covers the crowns of all erupted teeth (Strean and Loeni, 1959). While Cyclosporine hyperplasia is very similar in appearance to the phenytoin induced condition, it is more frequently localized and can present as a single enlarged papilla. The enlargement in adults has not yet been reported as growing over the teeth but has been observed in pre-pubertal children (Daley et al., 1986). Where there is no secondary inflammation, the surface is firm and light pink in colour. When secondary infection occurs, the surface darkens to a red colour and the surface becomes friable and bleeds to the touch (Kerr, 1952). The inflammed tissue unlike 'normal' hyperplasia is painful during eating and brushing.

ii. Histology

Histologically it is very difficult to differentiate between the Cyclosporine and other drug-induced hyperplasia. Fibrous hyperplasia presents as a surface layer of parakeratinized squamous epithelium with many Rete ridges showing elongation. The connective tissue is characterized by fibroplasia with increased numbers of fibroblasts. A

cellular infiltrate of mostly plasma cells occurs in tissue spaces to varying degrees (Wysocki et al., 1984). This cellular picture is similar to that found in chronic gingivitis where the presence of plasma cells and the local increased level of immunoglobulins is thought to be a response to microbial antigens in the gingival crevice area (Tew et al., 1985).

iii. Etiology

Drug-induced fibrous hyperplasia was first described by Kimball in 1939 a year after the introduction of phenytoin for the control of Epilepsy. Considerable work has been carried out on the mechanism involved in drug-induced hyperplastic growth (Vittek et al., 1982; Hassel, 1981) etc. The search for the exact initiating factors have thus far not produced definitive results. The following are some of the factors that have been studied in relation to drug induced hyperplasia.

Cylosporine

The Cyclosporine data at this time is scant; a study of 18 subjects by Wysocki et al. (1983) found a prevalence rate of 30% but 12% only showed disfiguring hyperplasia, no specific reference was made to personal characteristics. A report from a conference by Hamilton et al. (1981) suggested that gingival hyperplasia occurred in all Cyclosporine recipients. The basis for this statement by the nephrologists however was not supported by research data. A

well documented study by Tyldesley and Rotter (1984), where 36 patients were followed for 1 year, found a prevalence rate of 25%.

Phenytoin

Most phenytoin studies have looked at mixed populations and as such the influence of age as a factor has been obscured (Steinberg, 1981). A study by Steinberg and Steinberg (1981), examined only children between ages 4 and 15 years and found a prevalence rate for gingival hyperplasia to be 94%. Aas (1963) suggested that new cases after the age of 40 are rare. In a longitudinal study, by Esterberg and White (1941), with 244 patients maintained on phenytoin were followed for ten years, the condition peaked in prevalence and severity at age 17. Nevertheless due to other associated variables that are present in medically complex and institutionalized patients a simple linear age relationship can not be accepted as the sole determinant of the condition. Sex and race appear not to be factors in development of the condition (Gardner et al., 1963).

iv. Predisposing Systemic Factors

Phenytoin has been used for close to fifty years for conditions ranging from classical epilepsy to migraine, stuttering, mood and behaviour problems, neuromuscular pathologies, cardiac problems and any other condition where cellular bioelectric activity requires regulation (Bogoch and Dreyfus, 1970). In these widely ranging systemic

conditions, no particular disease-determined trends for hyperplasia have been reported. Comparative data, from the now wide use of Cyclosporine for management of diabetes, rheumatoid arthritis, multiple sclerosis etc. have not yet been reported.

In terms of kidney failure, studies of transplant recipients maintained on prednisone, azathioprine drug regimens by Oshrain et al. (1979), Tollefsen et al. (1978), Schuller et al. (1973), Robertson et al. (1978) showed no tendency towards gingival hyperplasia thus ruling out kidney failure per se as a risk factor. These studies indicated that a below average rate of gingival or periodontal involvement, in particular the absence of inflammatory reaction to local irritants was a common feature.

v. Hyperplasia Inducing Drug Therapy

The Cyclosporine data varies in establishing the onset of the clinical appearance of hyperplastic change. It ranges from 1-3 months or 1-12 months (Wysocki et al., 1981, Tiddsley and Rotter, 1984).

Onset of hyperplastic growth in phenytoin studies appears to vary between subjects. Clinical observers such as Jewison (1980) suggest that hyperplasia can occur at any time up to one year after commencement of therapy. In a small study, 13 cases and 14 controls were assessed within 14 days of commencement of therapy (Philstrom et al., 1980). It was found that slight gingival enlargement occurred

within the first 6 months despite a rigorous oral hygiene program.

a. Serum Drug Levels

The dosage of phenytoin and Cyclosporine that are required to attain a specific serum level varies considerably between subjects. Dependent on the patient's levels of impairment, serum level required to produce the therapeutic effect may also vary. In the case of phenytoin used for seizure control, serum levels in the range of 10-15 ug/ml are required. It has been noted that serum levels kept below 15 ug/ml will not result in hyperplasia (Dilantin Product Information brochure, 1964). An animal model study by Staple (1954), found that after development of hyperplasia in the Macaque monkey by giving phenytoin at the human therapeutic range, subsequent lowering of the dosage resulted in a natural regression of the hyperplasia with no other therapy being used to deal with the overgrowth.

Cyclosporine levels vary between patients. A balance is sought between rejection control and a nephrotoxic effect. Considerable dose adjustment is usually required in the first 60 days post transplant. It has been suggested that the early appearance of gingival hyperplasia could be the outcome of initially high levels of Cyclosporine during this time. The continual monitoring and individual adjusting of levels could explain the variation in the interval between commencement of treatment and onset of gingival hyperplasia. Until a longitudinal study of these two factors is carried

out, a true assessment of relationship between serum levels and development of hyperplasia cannot take place.

b. Salivary Drug Levels

A study by Phillips et al., (1986) showed that Cyclosporine was present in both whole saliva and in pure parotid fluid in clinically significant amounts thus providing an added source of the drug. As patients ingest Cyclosporine in a suspension of olive oil, an additional amount of the drug would likely be retained on the oral tissues and absorbed by supragingival plaque.

In the work of Philstrom et al. (1980) with phenytoin, it was found that mean salivary levels of phenytoin were approximately 10% of that in serum. Saliva from the parotid gland appeared to have the highest levels of all salivary glands. It was postulated that plaque acted as a filter to hold salivary phenytoin around the gingival margin of teeth if there was poor oral hygiene, hence exacerbating the hyperplasia.

vi. Effects of Oral Hygiene and Local Irritants

Studies involving patients with conditions managed by potentially hyperplasia-producing medications have rarely involved a pre-treatment dental examination. Authors such as Kerr (1952), Hall (1969), Philstrom et al., (1980) claim that good oral hygiene will prevent the development of hyperplasia but as documentation of pre-existing oral

hygiene problems is generally not available, this limits the assessment of the true predisposing role of plaque.

Numerous studies have shown that progression of hyperplasia is influenced by oral hygiene status (Glickman and Lavitus, 1941). Clinical studies have noted that local irritants such as orthodontic bands or rough-edged restorations that allow plaque build up appear to encourage early onset and enhanced reaction at that site (Aas, 1963; Nukik and Cooper, 1972). Tissue change around and under prosthetic appliances have been notebly absent. A large study by Esterberg and White (1945) commented specifically on this issue as many of their subjects wore prosthetic appliances of one kind or another.

Nukik and Cooper (1972) used a cat model in an attempt to isolate the role of plaque in gingival hyperplasia development in phenytoin recipients. The cat teeth were prepared to a plaque and inflammation-free state prior to commencement of phenytoin therapy. The control group were documented for plaque and inflammation levels and had additional irritants added to their teeth to trap further plaque as part of the methodology. It was found that for the plaque-free teeth inflammation but no hyperplasia developed, where there was pre-existing inflammation, tissue overgrowth was obvious at the end of the 6 week study period.

In the Cyclosporine studies, authors report a broad range of onset times for the appearance of hyperplasia from one month to one year. This questions the role of plaque as

an initiator but most agree that progression is hastened by poor oral hygiene (Wysocki and Daley, 1984). No study to date has documented oral hygiene status and hyperplasia development longitudinally over time, neither have the studies have documented pre-transplant oral hygiene status.

In the study by Wysocki et al. (1983) no evidence was found to suggest tissue changes in edentulous ridge areas.

vii. Microbiology of Gingival Crevices

It could be argued that changes in the chemical composition of saliva and/or gingival crevice exudates could result in changes in gingiva-related flora. With the exception of an animal study by Staple et al. (1978), this area has not been explored. Using a Macaque monkey model, the microbial plaque composition was assessed before use of Phenytoin and after the development of hyperplasia. He found only a slight change in members of Leptotrichia buccalis but the number of other organisms, in particular Bacteroides melaninogenicus and Actinomyces viscosus, both frequently found associated with periodontal disease, were unchanged.

To date no published data is available on this aspect of Cyclosporine hyperplasia. Any microbial analysis will therefore reflect studies done in other areas of periodontal research as a starting point. One group of bacteria, commonly referred to as black pigmented Bacteriodes, has been widely studied for its association with periodontal disease. According to Slots (1979) this group could be present in high number in gingival environments modified by

hyperplastic change. This possibility can be studied in the Cyclosporine-induced patient group.

5. IMMUNOSUPPRESSION AND GINGIVAL HEALTH

The evidence that the development of gingivitis is closely related to the presence of bacterial plaque is well accepted (Loe et al., 1965; Lind et al., 1975). Similarly reduction of gingivitis is achieved by the control of the level of plaque around the dento-gingival tissues. The factors involved in the disease process may include antigens from bacterial sources infiltrating the gingival cells. It is likely that a change from the acute reaction type of gingivitis to chronic or hyperplastic conditions would require the involvement of other factors. These would include most factors relating to the inflammatory mechanism; immune complexes, complement sensitized lymphocytes, lymphokines, prostoglandins, histamines, etc. (Taichman, 1974).

The hypothesis that the immune system was the underlying mechanism that maintained gingivitis as a chronic condition was first tested by examination of patients with congenital defects of the immune system (Robertson et al., 1980). They found that patients with congenital abnormalities of the immune system, such as IgA deficiency and agammaglobulinemia, had less gingivitis than matched controls when plaque scores were equal. Studies of such immunologically compromised patients provided useful indicators, although the association could in part be due to other associated variables (Legler et al., 1982).

Consequently, chemically induced modification of the immune system was seen as an area for further study.

Patients undergoing anti-inflammatory therapy for the management of auto-immune diseases e.g. rheumatoid arthritis, lupus etc. were valuable cases for studying anti-inflammatory reactions. These chronic conditions are considered to be the result of an initial bacterial invasion in which the expected inflammatory response is maintained long after the original infection is controlled, resulting in tissue damage (Weigle, 1980). A variety of drugs are used to control these diseases, (Goldman, 1970; Barranco et al., 1976). Dental researchers used patients who were on long term immunosuppressive therapy to test their theories with respect to the role of the immune system in the progression of chronic gingivitis to a tissue destructive condition, periodontitis (Waite et al., 1981).

i. Anti-Inflammatory Drugs

The effect of anti-inflammatory agents on gingival tissues has been assessed by examination of patients taking drugs in this category for prolonged periods. In one such study by Waite et al., (1981), 22 rheumatoid arthritis patients who had taken acetylsalicylic acid as an anti-inflammatory drug for a minimum of one year were examined and compared with a group of age and sex matched controls to assess differences in gingival inflammatory responses. Subjects taking systemic anti-inflammatory drugs showed reduced levels of gingival inflammation, a decrease in

periodontal pocket depth and a reduction in the loss of attachment whereas controls showed no change. The authors concluded that blocking of prostaglandin synthesis in the latter stage of the acute inflammatory response by the salicylates prevented continuation of the reaction in which migration of mono-nuclear cells and stimulation of the complement system would have led to tissue destruction.

These results, which showed a reduction in chronic inflammation in patients maintained on acetyl salicylic acid, confirmed an earlier study by Nyman et al., (1979) that had tested indomethacin to control the inflammatory response in artificially induced periodontitis in beagle dogs. A further study by Weaks-Dybig et al., (1982) found similar outcomes for experimental gingivitis in dogs.

ii. Prednisone and Azathioprine

Opportunities to examine 'healthy' immuno-compromised patients became available with the success of kidney transplantation techniques. Subjects were now available who were restored to functional health but who would be required to take medication indefinitely to control the body's attempt to reject the transplanted organ. It must however be noted that these patients having suffered renal failure prior to transplant had disturbed metabolic balance and defective protein synthesis and cell division (Isaacson, 1952). Uremia, a feature of renal failure also contributes to diminished cell-mediated and humoral response (Birkland, 1976). Hemodialysis for extended periods further complicates

the picture for some patients who may have developed disturbances in calcium metabolism and hence possible bone loss (Stahbury and Lumb, 1962). Thus these patients were subject to a degree of immunosuppression prior to initiatiion of drug-induced immunosuppression.

The anti-rejection drugs first studied were the combined azathioprine/prednisone regimens (Tollefsen et al., 1978). These drugs suppress both the B and T cell components of the immune system (Bach, 1972). In an early study by Schuller et al., (1973) 33 transplant patients were examined to assess the relationship between plaque scores and gingival pathology. They found no association between plaque levels and periodontal disease. In addition there was no correlation between age and periodontal disease, despite the fact that patients were older and thus being in a high risk age group for periodontal pathology, suggesting that immuno-suppression did alter the periodontal response.

A study by Tollefsen et al., (1978) was designed to study cellular infiltration of the gingival tissues in uremic patients on hemodialysis for renal failure and patients who had received a kidney transplant and were being maintained with prednisone and azathioprine. Two control groups of healthy subjects were included, one with zero plaque scores, another with poor oral hygiene. Plaque levels were measured in the usual manner using a scale developed by Greene and Vermillion (1964). The inflammatory response was measured histologically from biopsies taken from all

subjects. Pocket depth was also measured. IgG, IgA and IgM levels from blood samples were quantified in the uremic and transplant groups. The authors concluded that the transplant group showed markedly lower scores of cellular infiltration than the uremic group. Amongst the controls the more marked infiltration was apparent in subjects with high plaque scores (i.e. poor oral hygiene) but the "plaque-free" were shown also to display significant infiltration, more in fact than the 2 renal groups who underwent no special oral care programs. The results suggest the effect of immunosuppression on the cellular infiltration response to bacterial stimulation was similar to the Schuller study, showing lowered inflammatory response in the group taking the immunosuppressive drugs (Schuller et al., 1973).

A study by Oshrain et al., (1982), appears to contradict findings of their own earlier study (Oshrain et al., 1979) which had shown reduction of inflammatory response in the steroidal suppressed group (20 subjects, 20 controls). This earlier study is consistent with the findings of other researchers previously mentioned. In the more recent study, Oshrain et al. (1982) suggest that by following transplant patients over 2 1/2 - 4 years, they were able to show that gingival index scores increased whilst controls with good oral hygiene showed no change. Due to the small size of this study group and the lack of information concerning underlying systemic conditions, the

findings should be set aside until better designed studies be conducted.

iii. Cyclosporine

The advent of a new drug for use in organ transplantation did not occasion the interest in the dental literature that these earlier studies might have suggested. As described, Cyclosporine's depressant action is largely on the T-cell and T-cell dependent B-cell systems (Ryffel et al., 1980). This fact would conceivably allow studies that would differentiate between the role of the band T-cell systems in gingivitis. The only work currently available in the literature on this subject is a carefully designed study reported by Guggenheim et al., 1981. The work was carried out on rats mono-infected with Actinomyces viscosus (Nyl). The authors assessed loss of supporting bone in the periodontal structures. They found little difference between the rats treated with Cyclosporine and non-treated controls. However, both groups showed bone loss. They concluded that periodontal destruction could not be eliminated by T-cell suppression and therefore other pathomechanisms were involved. Perhaps a more positive conclusion would have suggested that tissue destruction was B-cell system dependent.

Human studies on the impact of T-cell suppression by Cyclosporine on gingivitis changed course when a report of gingival hyperplasia development appeared in the literature

when Cyclosporine was first being tested as an anti-rejection drug in organ transplantation.

This new development was first reported in the dental literature in a case report study by Rateitschak-Pluss et al. (1983). The 3 documented cases made no reference to the marked difference between the effect of Cyclosporine and prednisone on the inflammatory response of gingivae.

In another descriptive study (Wysocki et al., 1983) hyperplasia was reported to have occurred in 6 cases. In these cases hyperplasia occurred in a generalized pattern throughout the mouth and in a cyclical manner. The degree of hyperplasia was related to local gingival irritation. The possible initiating mechanism was examined in relation to serum levels of Cyclosporine. However, individual fibroblast sensitivity, similar to phenytoin hyperplasia, was the more likely explanation. The occurrence of hyperplasia in some cases whilst others showed no clinical change regardless of plaque scores suggested that suppression of the T-cells does in some way interfere with the lineal plaque to gingivitis system as occurred in the other gingivitis studies Loe, (1965). The development of hyperplastic tissue may nevertheless be due to some other process such as the involvement of a possible metabolite of Cyclosporine to which individual patients with specific cell types are sensitive.

6. KIDNEY TRANSPLANTATION

i. History and Development

The ability to explore and discover the mechanisms of bodily function in the normal state and in response to infection have made possible the development of many new medical and surgical procedures over the last twenty years, one of the most significant being organ transplantation.

The concept of replacement of diseased tissues and organs by healthy parts taken from another individual was pursued as a remote hope for centuries (Kahan, 1981). The possibility of removing an organ from an animal to compensate for a failed human organ was most favored and did ultimately become the first case for experimentation in organ transplantation. Such an experiment took place in France in 1906 when Jaboulay connected goat and pig kidneys to the arm of an uremic man. The first attempt of transplantation of a human organ took place in Boston in 1951 using cadaveric kidneys with success lasting a few days only. It was the result of work by Medawar (1946) on the subject of tissue tolerance that resulted in an understanding of the rejection mechanism and hence the failures of transplantation. The subsequent clarification of the mechanism of tissue rejection allowed a systematic approach to potential organ transplantation to begin (Bodner, 1978; Morris, 1978).

Studying tissue compatibility and matching led to focussing on identical twins as potential transplantation

subjects. In these cases tissue incompatibility factors would not be an issue (Hamburger et al., 1981). The organ system selected as most urgent for study was the renal system. Acute and chronic renal failure in the period prior to the development of dialysis as a means of treatment were lethal states for a high percentage of patients. Experimentation with transplantation in these hopeless situations was seen as an ethically acceptable action (Hamburger et al., 1981).

ii. Rejection Management

The development of tissue typing and close tissue matching resulted in limited success in non-identical twin transplant efforts. Adjunct rejection-controlling methods were needed to prolong survival of the grafts (Ting and Morris, 1980). Many approaches such as tissue irradiation, thymectomy and the trial of a range of immuno-suppressive drugs were and continue to be explored for this purpose (Roitt, 1980). The latest major break-through in rejection control was the development of the immuno-suppressive drug Cyclosporine A which was ready for clinical trial for effectiveness in 1979.

While the goal of achieving donor-specific tolerance to grafted tissue, without resorting to broadly immuno-suppressive treatments, had not been achieved in clinical circumstances, transplantation of tissues between genetically distinct donors and recipients had been possible (Baldwin et al., 1981). Richly vascularized tissue such as

kidneys, heart, liver, etc., elicit similarly severe immunological responses when transplanted to a non-identical recipient (Roitt, 1980). As the majority of patients requiring donated organs cannot receive perfectly matched tissue, clinicians must control the immunological response created when mismatched HLA tissue is transplanted. Currently, drugs such as prednisone/azathioprine combinations and Cyclosporine are used for this purpose. They have made cadaveric and live donor transplantation available to large numbers of patients (Calne, 1980).

Failure of organ transplantation is caused most frequently by organ rejection. This can occur within hours of implantation and is termed hyperacute rejection. More commonly rejection begins 7-12 days post-transplantation (Williams, 1974). In these instances, termed acute rejection, the renal allograft recipient may develop chills, fever and oliguria. Within the kidney, cellular infiltration with oedema of the tissue causes swelling of the graft. Histologically a heavy intertubular infiltration of mononuclear cells occurs. It is thought that rejection is initiated by T-cells. Donor-specific "killer" cytotoxic lymphocytes have been recovered from rejected organs. In addition lymphocytes, natural killer cells and macrophages appear as rejection proceeds. High doses of corticosteroids are required to reverse the process at this stage (Busch et al., 1975). Cyclosporine's ability to control T-cells has been an advantage in preventing acute rejection in many

patients. Nevertheless, rejection can occur and the use of high dosages of prednisone are required for varying periods of time to reverse the process. A low grade sub-clinical type of rejection, known as chronic rejection can also occur and only becomes apparent months or years after a successful transplant. This presents as a narrowing of numerous arterioles and thickening of the glomerular capillary basement membrane in renal allografts. Serial biopsies of chronically rejecting kidneys have shown that these lesions are formed by adherence of platelets and fibrin aggregates to the vessel wall (Dempster and Williams, 1963). These deposits become covered by endothelium and are incorporated into the intima which often contains IgM and complement, emphasizing the role of the humoral system in this type of rejection. Other than preventing intermittent acute episodes that aggravate the avascularization of the chronic rejection process, no therapy is at present available to offset this form of rejection (Terasaki, 1981).

iii. Current Status

The expansion of transplantation as a viable alternative to dialysis occurred through the use of broad spectrum steroidal immunosuppression with azathioprine. Due to the potential for severe post-surgical infection with the use of these drugs, advances in techniques for organs other than kidneys were not seen as realistic. It was the discovery of a more specific anti-rejection drug, Cyclosporine that made transplantation of complex organ

systems connected to major blood vessels feasible. Today, successful transplantation of liver, pancreas, heart and lung are carried out in increasing numbers.

Patients who become renal allograft recipients now can have varied medical histories that lead to renal failure (Leaf and Cotram, 1980). In the early years of transplant surgery cases of simple renal failure were selected for transplant surgery. This condition usually left the other organ systems relatively unimpaired, individuals being maintained on hemodialysis until a suitable organ became available. As the technique was refined, subjects with chronic failure or diabetes-induced renal impairment were offered the transplantation option.

The varied medical histories of current patients means that recipients of allografts require particularly complex medication regimens for their maintenance. An example would be the diabetic subjects who require daily insulin and medications to offset circulatory failure in addition to their anti-rejection therapy. Successful outcomes of renal transplantation have encouraged the extension of the technique to older patients. This results, in North America, in a wider age range of recipients from the young child to 70 year olds.

The wait for transplantation varies depending on the availability of organs and the tissue type of the individual requiring the new kidney. The majority of organs are harvested from cadaveric sources with the result that the

selected recipient is given very short notice prior to transplant surgery being planned. It is not therefore likely, given the intensity of preparation required, that it would be possible to conduct too many non-essential tests on subjects at this time despite their value in longitudinal studies such as monitoring changes in the oral tissues.

iv. The Canadian Multi-Centre Transplant Study

This twelve centre study was designed as the first prospective randomized clinical trial to compare the course and outcome of recipients of cadaveric renal transplants maintained on Cyclosporine and prednisone with those maintained on standard therapy that included azathioprine and prednisone.

The management protocol for the Cyclosporine/prednisone group was specified as follows:

> "A loading does of 20 mg of cyclosporine per kilogram of body weight was given orally within 12 hours before surgery, and thereafter the patients received 10 mg per kilogram every 12 hours. Trough cyclosporine levels (in serum obtained 11 hours after the last dose) were determined by radioimmunoassay, and the oral dose was adjusted to achieve trough levels between 100 and 400 ng per milliliter. After 30 days, the dose was reduced by 2 mg per kilogram if trough levels were above 100 ng per milliliter. It was then reduced monthly, provided that the patients were clinically and immunologically quiescent and that the trough level was not less than 100 ng per milliliter.
>
> On Day 14 the patients were started on prednisone, 1 mg per kilogram, on alternate days; if clinical conditions allowed, this dose was reduced by 5 mg every other day, to 0.3 mg per kilogram given on alternate days. This dosage was maintained for three months, and then it was lowered to 0.25 mg per kilogram if there was not evidence of graft rejection. At six months, the prednisone dose was further reduced to 12.5 mg every other day.

> Acute graft rejection was treated with oral or intravenous steroids, for a maximum of 4.5 g of methylprednisolone or its equivalent over seven days. If rejection occurred during the first 14 days after transplantation, maintenance prednisone was started after completion of therapy to prevent rejection. It was recommended that the cyclosporine dose be increased to a maximum of 20 mg per kilogram per day, provided that trough serum cyclosporine levels were less than 400 ng per milliliter. Once a quiescent state was established, the dose was reduced as indicated above. Cytotoxic agents and antilymphocyte globulin were not used in the cyclosporine group. If rejection was unremitting, the clinician could discontinue cyclosporine and use the therapy of his or her choice ("switching").

A second protocol was adapted when the outcome data of the first clinical trials were analyzed which involved the following modification:

> Prednisone was prescribed from day 1, the loading dose for Cyclosporine became 15mg/kg and trough serum levels of 100-300ng/ml were sought but levels above 400ng/ml would not be accepted. Cyclosporine levels were to be lowered to 0.3mg/kg.

Later in the study modifications were introduced such as dropping prednisone from the standard protocol for some cases and for others azathioprine was added. The organization and communication network set up for the study allowed numerous sub-studies such as the present one to be conducted. Outcome data from the study showed an overall one year graft survival of 85% and a 95% patient survival, Canadian Multicentre Transplant Report (1983).

7. BLACK-PIGMENTED BACTEROIDES

Morphological change in the oral cavity may be the result of normal age-related development or induced change, such as trauma or the impact of a drug. Whatever the cause, such changes may modify the environmental conditions at the affected site, leading to shifts in the commensal flora (Bowden et al., 1976). In the case of patients exhibiting Cyclosporine-induced gingival hyperplasia, a structural change occurs in the gingival crevices around the teeth where the hyperplasia develops. The overgrowth produces enclosed crevices, conducive to the establishment of a flora with a higher proportion of anaerobic bacteria. Studies in the periodontal literature suggest a group of bacteria that could likely colonize these 'false pockets' would be the anaerobic, gram rods known as black-pigmented Bacteroides (Slots, 1979; Sokransky, 1977, Loesche et al., 1985).

i. Taxonomy

Black-pigmented Bacteroides are so called as they produce a black pigment when cultured on appropriate media, (Oliver and Wherry, 1921). The group present as non-motile rods that stain gram negative, favour anaerobic environments but are not killed in significant numbers, if exposed to oxygen for short periods Buchanan and Gibbons (1974). A complex medium containing peptone, yeast extract, vitamin K and hemin is recommended for isolation of the human types (Bergey's Manual, 1984).

Bacteria with these characteristics were first described by Oliver and Wherry (1921) as a single group, Bacteroides melaninogenicus. Later work suggested that there were three types within the group, characterized by their fermentation capacity as weak, strong and non-fermentors (Courrant and Gibbons, 1967). Names were assigned to the three types by Holdeman and Moore, (1970): Bacteroides melaninogenicus subs. asaccharolyticus, Bacteroides melaninogenicus subs. intermedius and Bacteroides melaninogenicus subs. melaninogenicus. These sub-species were subsequently identified as B. Intermedius, B. melanininogenicus and B. asaccharolyticus. In addition the heterogenus group, B. asaccharolyticus was separated into the species B. gingivalis and B. asaccharolyticus, (Coykendale et al., 1980). B. gingivalis is an oral species while B. asaccharolyticus is found normally in other parts of the alimentary canal (Finegold and Barnes, 1977).

Black-pigmented Bacteroides have been recovered from a wide range of animals such as hamsters, guinea pigs, rabbits, dogs, sheep, cattle and horses (Burdon, 1928). They exhibit varying characteristics specific to the commensal animal. These bacterial groups found in animals differ from those found in humans.

It should be noted that due to the evolving nature of the taxonomic classification, many studies will have in the past, reported on the group in single or overly large species groupings. This classification system, particularly

those aspects that refer to specificity in disease production, may be erroneus. It is likely that with increasing sophistication of analytic methods further species sub-division may occur in the future. Nevertheless the presence of representative colonies of the black pigmented Bacteroides group remains a useful indicator of health status in the oral cavity (Burdon, 1928; Slots and Dahlen, 1985; Loesche et al., 1985).

BIOCHEMICAL, SEROLOGICAL, AND GENETIC CHARACTERISTICS OF BLACK-PIGMENTED BACTEROIDES*

Species/Subspecies	Glucose fermentation	Metabolic acid end products	Indole production	Catalase production	Esculin hydrolysis	Lactose fermentation	Sucrose fermentation	Cellobiose fermentation	Hemagglutination of sheep erythrocytes	Trypsin-like activity	Serology	% G+C	Site of isolation
B. gingivalis	-	APIbBIvPh	+	-	-	-	-	-	+	+	1 serogroup	48	Oral cavity of humans and *M. arctoides.*
B. asaccharolyticus	-	APIbBIv	+	-	-	-	-	-	-	-	2 serogroups	52	Nonoral sites of humans.
B. intermedius	+	AIbIvS	+	-	-	-	v	-	-	-	2 serogroups	43	Oral and nonoral sites of humans and animals.
B. melaninogenicus	+	AIbIvS	-	-	-	+	+	-	-	-	1 serogroup	41	Oral and nonoral sites of humans and animals.
B. levii	+	APIbBIvS	-	-					-	v	1 serogroup	49	Cattle, oral and nonoral sites of humans?
B. macacae	+	APIbBIv	+	+	-	+	-	-	-	v	1 serogroup	43	Oral cavity of *M. arctoides.*
Canine *Bacteroides*	(-)	APIbBIv	+	+	ND	ND	ND	ND	ND	v	1 serogroup	42	Oral cavity of beagle dog.

*Sign: A, acetic acid; P, propionic acid, Ib, isobutyric acid; B, buytric acid; Iv, isovaleric acid; S, succinic acid; Ph, phenylactic acid; +, 90% or more positive reactions; -, 90% or more negative reactions; v, variable (11 to 89% positive reactions); ND, no data available.

ii. Black Pigmented Bacteria Associated with Normal Human Tissues

Anaerobic bacteria can be found in many parts of the healthy human body. In particular the black pigmented Bacteroides group has been found in many areas of the alimentary and genito-urinary tracts (Coykendall et al., 1980).

The mucous tissue of the mouth begins to be colonized by bacteria at birth but the character of the flora changes over time as growth-associated local conditions and environmental factors progress. Initially there appear to be no Bacteroides (McCarthy et al., 1965) (p.17) but gradually as teeth begin to erupt at approximately 6 months the numbers of anaerobes increase (McCarthy et al., 1965, Hurst and Fendeson, 1969). By the time that tooth eruption is fully underway the black-pigmented Bacteroides mass may account for 14.9% of the microflora of the mucous membranes (Hurst and Fenderson, 1969; Osana et al., 1977). With increasing age the percentage increases particularly in areas closely associated with the teeth. Children after the age of puberty show higher levels of these flora in the gingival crevice.

The surface of the tongue was studied by Gordon and Gibbons (1966) who found sparse colonization by black pigmented Bacteroides. In contrast, using more sophisticated methods, van der Velden et al., (1986) found higher numbers. Saliva bathing the oral tissues was found to contain Bacteroides by Gibbons et al., 1964.

Tonsils were long known to harbour Bacteroides strains, (Oliver and Wherry, 1921; Brook and Grober (1983); Brook and Yocum 1984). Similar studies controlling for mouths with periodontal breakdown by van der Velden et al., (1986) and van Winkelhoff et al., (1986) confirmed colonization of normal tonsils by black pigmented Bacteroides in adult subjects.

Other parts of the digestive tract with large numbers of anaerobes are the colon and to a lesser extent the terminal illeum (Finegold, 1977).

iii. Black-Pigmented Bacteria Associated with Diseased Tissue

Indigenous anaerobic black-pigmented Bacteroides are frequently found in superficial infections of structures protected by mucous membranes e.g. vaginitis, the oral diseases, gingivitis and periodontitis (Finegold, 1977; Moore, 1987). Human bite wounds (Goldstein et al., 1984) were amongst many of the wide ranging disease states shown to harbour these bacteria in significant numbers in the infective flora (Brook, 1983). The black pigmented Bacteroides have also been found in deeper lying tissues where life-threatening conditions are created, such as aspiration pneumonia, lung abscesses, perforated appendices and post-surgical infections (Brook, 1983). Their presence in brain abscesses were reported by Mathiesen, et al., (1984).

iv Virulence Factors

In order to be pathogenic a microbe has to possess virulence factors that allow it to freely colonize an area and to withstand or offset the animal's defence system (Bowden et al., 1976). Further, the potential to destroy the host tissue must exist.

The initial criterion for pathogenicity is the ability to establish a nidus. This is achieved by the black pigmented Bacteroides by the possession of pili and fimbriae on their walls which allow them to attach to epithelial cell surfaces (Slots, 1982). In order to grow and establish colonies, bacteria have to protect themselves from the host's defences. Bacteroides although they activate both the classical and alternative complement pathway in humans appear to be able to produce substances which compete with chemotactic products and block chemotactic receptors on polymorphoneuclear leucocytes (Okuda et al., 1978; Tofte et al., 1980). This results in a marked modification of the host response (van Dyke et al., 1982). The presence of a capsule on some strains, B. gingivalis in particular, is protective and prevents phagocytosis by monocytes (Sundqvist et al., 1982) and hence promotes virulence. In addition some black-pigmented Bacteroides produce a low molecular weight fatty acid which is leukotoxic (Botta et al., 1985, Rothstein et al., 1985).

v. Disease Progression

Progression of disease caused totally or in part by these bacteria is brought about through the activity of various enzymes including collagenases, proteases, hyaluronidase, ribonuclease, desoxyribonuclease and plasma clotting and fibrinolytic activities (Gibbons and MacDonald 1961; Robertson et al., 1982; Sundqvist et al., 1985; Carlsson et al., 1984).

Work by Bulkas et al., (1979), Bulhoe et al., (1985) showed that the species, synthesized enzymes such as phospholipase, which may act as a prostaglandin mediator and initiate bone resorption (Goodson et al., 1974). Other strains produce volatile sulfur products that are cytotoxic (Ing and Tonzetich, 1981). Continuation of bone resorption leads to the production of interleukin 1 in macrophages and peripheral monocytes, which in turn can result in an auto-immune type of bone destruction (Gowen et al., 1983).

In spite of these virulence factors, the black pigmented Bacteroides may not be pathogenic in pure cultures (Takazoe and Nakamura, 1971). However, their virulence can be evoked in mixed culture when the other bacteria present provide the essential nutrient requirements such as menadione, vitamin K or succinate (Mayrand and McBride, 1980).

As stated earlier, it has not been possible to associate one particular species of black pigmented Bacteroides with a specific periodontal condition.

Nevertheless, changes in ratios of the bacteria within microbial populations associated with periodontal disease have been well documented, (Sokransky, 1970; Slots 1979; Tanner et al., 1979; Williams, et al., 1976; Loesche et al., 1985). In the case of subgingival plaque in periodontitis, the majority of bacteria are anaerobes with the black pigmented strains being prominent. Tanner et al., (1979); White and Maynard (1981) demonstrated that there was a relationship between the levels of B. gingivalis and inflammation in periodontitis.

vi. Black Pigmented Bacteroides in Pregnancy

A study of gingival bacteria during pregnancy by Kornman and Loesche (1980), found that changes in bacterial populations correlated with changes in hormone states of the women at various points during pregnancy. They found that at the beginning of pregnancy gram negative anaerobic rods represented 10% of the cultured gingival sulcus population but by 24 weeks the percentage had risen to 39%. This was associated with an increase in black pigmented Bacteroides levels, corresponding to changes in systemic hormone levels. By the third trimester, again accompanied by changes in estrogen/progesterone levels, the ratio of aerobe to anaerobe had returned to earlier levels. The researchers concluded that changes in hormone levels in the tissues would influence the flora of the gingival crevice.

vii. Summary

The studies in the years since Burdon's work in 1928 strongly suggest that bacterial populations, particularly those of anaerobic rods differ between healthy and diseased gingival tissues (Moore et al., 1982a; Moore et al., 1982b; Moore et al., 1983, Moore et al., 1985; Mandell and Sokransky 1981; Slots et al., 1980). In the case of Cyclosporine-induced hyperplasia the deep pseudo-pockets and unusual hormone levels would suggest that the anaerobic black pigmented bacteria could be associated with the condition and show increased prominence in the total bacterial colony counts in samples taken from the pockets.

8. THE STUDY

Objectives

1. TO DETERMINE THE PREVALENCE AND SEVERITY OF GINGIVAL HYPERPLASIA IN A GROUP OF PATIENTS MAINTAINED ON LONG TERM CYCLOSPORINE THERAPY. Since reports on Cyclosporine recipients noted the development of gingival hyperplasia, it was important to determine the extent of the problem and know the nature of this development i.e. whether Cyclosporine therapy meant automatic development of gingival hyperplasia. A study to document prevalence of the condition would answer this question as well as if hyperplasia assumes the same form and distribution in all cases. A systematic documentation of the morphology would give a picture of any variations between cases in this regard.

2. TO ESTIMATE THE RELATIVE POPULATION LEVELS OF BLACK PIGMENTED BACTEROIDES IN THE GINGIVAL CREVICE FLUID OF NEWLY TRANSPLANTED RENAL ALLOGRAPHT RECIPIENTS. The main concern regarding microbial populations is with the ecological changes related to the development of gingival hyperplasia. In consideration of the medical complexity of patients available for such a study it was possible that prior to commencement of Cyclosporine therapy they could as a group present unique microbial characteristics. A pre-therapy study of the gingival crevice microflora was designed to answer this question.

3. TO COMPARE THE POPULATION OF BLACK PIGMENTED BACTEROIDES TAKEN FROM GINGIVAL CREVICE FLUIDS IN AREAS AFFECTED AND NON-AFFECTED BY GINGIVAL HYPERPLASIA IN PATIENTS MAINTAINED ON LONG TERM CYCLOSPORINE THERAPY. Gingival hyperplasia is frequently highly localized in an individual's mouth. Such distribution allows for comparisons of microbial populations between affected and non-affected areas within a constant systemic environment. A difference in black pigmented Bacteroides colonization between affected and non-affected tissues would suggest that local factors such as bacteria could be associated with the hyperplastic development. On the other hand, it could also suggest that hyperplastic changes in the oral environment has an influence on microbial growth.

9. DESIGN

The study was conducted with the co-operation of two centres in the Canadian Multi-Centre Transplant Study, Winnipeg and Vancouver. Patients were selected by a set of criteria designed to meet the requirements inherent to each study objective. Variables that could have an influence on outcome were identified for documentation.

All patients were interviewed regarding participation and asked to sign a consent form prior to involvement in the study.

10. SUBJECT SELECTION

Group I

In order to determine the prevalence and severity of gingival hyperplasia in patients on long term Cyclosporine therapy, subjects had to have been successfully maintained on Cyclosporine therapy for a minimum of three months after having renal transplant surgery. They also had to have a minimum of ten functioning natural teeth.

Names of Cyclosporine-maintained patients were sequentially drawn from the transplant registries of both centres. Patients were telephoned or interviewed at clinics to assess willingness to participate and to ascertain their dental status relative to the eligibility criteria. Forty such patients were identified.

Appointments were set up for each patient to attend the hospital dental clinic where personal data was collected and they were given an oral examination to document the presence of gingival hyperplasia and its severity. Oral hygiene status was also calibrated. Medical data were collected from the patient's medical records.

Group II

In order to determine the population of black pigmented Bacteroides in the gingival crevice of newly transplanted subjects, a group was selected on the basis that they had teeth and no hyperplasia of the gingivae. They would also not have been subject to Cyclosporine therapy for more than 5 days.

It was decided that (10-15) subjects would suffice for this group. The investigator was informed by the Renal Transplant Program personnel within 24 hours that a successful transplant surgery had taken place and that permission to interview the patient had been obtained. The investigator would then visit the patient within 5 days of surgery at the Surgical Nephrology Unit to describe study details and to determine willingness to participate in the study. Dental eligibility was determined by examination of the oral tissues. Eleven subjects were included. At the next appointment one microbial sample was taken from a selected gingival crevice between two natural teeth. Gingival tissues would be examined visually for any unusual or pathological features.

Group III

In order to determine the relative populations of black pigmented Bacteroides in affected and non-affected areas of gingival hyperplasia in a subject's mouth, it was necessary to identify subjects who showed areas of obvious gingival hyperplasia when maintained on long term Cyclosporine. Patients with marked gingival hyperplastic changes were selected if they satisfied the following additional criteria: They were not undergoing supplemental anti-rejection therapy for an acute rejection phase at the time of sampling nor were they recipients of any anti-microbial therapy at the time of examination or during the four weeks preceding planned microbial sampling.

Some of the patients in Group 1 also satisfied criteria for Group 3 and were included. The patients were contacted and the consenting individuals were given an appointment for microbial sampling.

Patients and their transplant physicians were interviewed to confirm whether they met the medication-selection criteria at this point in time. Two microbial samples were taken one from the gingival crevice of an interdental papilla showing marked gingival hyperplasia, the other from the area showing the least or no hyperplastic change.

11. METHODS

i. Personal Data

Personal data relating to age and sex were collected by patient interviews.

ii. Medical History

A review of the patients' medical charts provided information on systemic conditions that could impact on the findings of the study such as: Phenytoin for the control of Epilepsy, were noted.

iii. Cyclosporine Treatment History

a. Duration of Cyclosporine Therapy

The subjects who were treated with Cyclosporine were examined at a random point in the treatment of each subject. The duration of therapy for each subject was calculated from patient medical records and recorded in months.

b. Serum Levels of Cyclosporine

A mean reading based on daily serum measurements of Cyclosporine over the first thirty days was calculated for each patient from medical records. This value was calibrated as ng/ml of serum.

iv. Oral Examination

All patients were examined with the use of a dental mirror and periodontal probe. The following were determined:

- The number of natural teeth present.
- The distribution of any gingival hyperplasia was mapped on to a periodonal chart.

- Severity of the hyperplasia was noted per quadrant on the basis of visual observation as follows.

a. Extent of Hyperplasia

- The distribution data was analyzed to categorize any hyperplasia as follows:

Localized	>50% papillae involved
Generalized	<50% papillae involved

b. Severity of Hyperplasia

No Hyperplasia
Mild -slight, bubbly changes on the surface of the papillae
Moderate -obvious bulging of the papillae
Severe -overgrowth of the papillae extending over a third of the crown of the adjacent tooth

c. Oral Hygiene Status

In order to determine oral hygiene status the simplified method for calibration which was developed by Greene and Vermillion (1964) was used. Due to the variation in the number and location of the teeth present in several subjects a further modification was introduced, 3 instead of 6 teeth were taken in the assessment. Where possible, teeth from opposing arches and from contralateral sites would be selected but where distribution presented problems, the investigator chose as wide a distribution of teeth as possible for calibration.

Oral Hygiene Scoring System

0 = No debris or stain
1 = Soft debris covering not more than one third of the tooth being examined
2 = Soft debris covering more than one third but not more than two-thirds of the exposed tooth surface
3 = Soft debris covering more than two-thirds of exposed tooth surface

12. BLACK PIGMENTED BACTEROIDES ENUMERATION

i. Microbial Sampling

Microbial sampling was carried out by isolating the selected gingival tissue by the use of cotton rolls. The area was dried by applying sterile gauze squares to the surface. A Johnson and Johnson sterile paper point #60 was then held by a pair of college pliers and inserted into the gingival crevice. The point was held in place for ten seconds, then transferred to a vial containing 250 ml of Reduced Transfer Fluid enriched with 50 ml of bovine laked blood (SR48 Oxoid Cda). The vial was sealed and taken to the laboratory for processing.

ii. Laboratory Methods

The sample was taken to the laboratory where the fluid was sonicated by placing the tip of a Kontes ultrasonic cell disruptor in the fluid for 15 seconds to disperse the bacteria. The sample was then serially diluted 1:10, 1:100, 1:1000 with 1 ul being inoculated on to a petri dish of supplemented blood agar at each diluation (5% sheep's blood, Atlas Laboratories, Winnipeg in blood agar base No. 2, Oxoid, England; supplemented with hemin and menadione). The work was carried under aerobic conditions. The agar plates were incubated anarobically (CO_2 - 10%, N_2 - 80.1%, H_2 - 10.4%).

The plates were examined after 7 days incubation. A steriomicroscope (x10) was used to identify colonies that

resembled those characteristic of black pigmented Bacteroides. Total colony counts were made for each plate as was a separate count of black pigmented Bacteroides colonies. Representative examples of colonies were selected and subcultured on to supplemented agar plates for further culturing prior to gram staining and final identification.

Identification was based on the API 20 system of biochemical reaction (Biomerieux). Bacterial counts were tabulated for each subject along with information of the age of subject, duration of Dialysis for Group I and duration of Cyclosporine therapy for Group II. The minimal detectable level for black pigmented Bacteroides based on the dilution was 2.5×10^3 per sample is recorded as 0 in the Table.

13. RESULTS

Objective 1 Prevalence and Severity of Gingival Hyperplasia (Group I).

The age and sex distribution of Group I is shown in Table 1. While the group ranged in age from 12-56, the majority (60%) were between 20-40 years of age. Slightly over half the patients were male. The group included 11 patients with complicating systemic conditions. All were currently on Cyclosporine therapy with the duration of use ranging from 2-24 months. Serum levels ranged from 99-756 microgram/litre. Six subjects had used the first study protocol and the remaining 85% were on the modified second protocol.

Table 1: Age and sex distribution of subjects in Group I.

Age [years]	Males		Females		Totals	
	Numbers	%	Numbers	%	Numbers	%
10 - 19	2	10	1	5	3	8
20 - 29	8	38	8	42	16	40
30 - 39	5	24	3	16	8	20
40 - 49	3	14	3	16	6	15
50 - 59	3	14	4	21	7	17
Totals	21	100	19	100	40	100

Oral hygiene status ranged from good to very poor. Using the Greene and Vermillion Index, 6 (15%) had a score of '0' (no plaque) and 5 had a score of '3'. If scores of 0-1 are taken as indicative of an adequate level of oral

hygiene, then only a bare majority of these patients (55%) met this standard. Only women have scores of '0', but when '0' and '1' are combined, the difference between male and female patients disappears.

Table 2: Oral hygiene scores relative to age for males and females.

	Males				Females			
	Oral hygiene scores							
Age [years]	0	1	2	3	0	1	2	3
10 - 19	0	1	0	1	0	0	1	0
20 - 29	0	5	3	0	1	3	3	1
30 - 39	0	3	1	1	1	1	1	0
40 - 49	0	1	0	1	2	0	1	0
50 - 59	1	1	2	0	2	1	0	1
Totals	1	11	6	3	6	5	6	2

Evidence of hyperplasia was found in 14 patients (35%). The condition was localized in 4 of these patients and generalized in the remaining 10. The relationship between age, sex, the severity and distribution of hyperplasia are shown in detail in Tables 3, 4. There was no significant association between the presence of hyperplasia and gender. Below the age of 50, only 27% of the patients had developed hyperplasia relative to 71% of those aged 50-59. This result suggests there may be a positive association between the presence of hyperplasia and age, but the numbers are too small for statistical test.

Table 3: Distribution and severity of gingival hyperplasia in relation to age and oral hygiene scores for females.

Age [years]	Oral Hygiene Scores				No Hyperplasia	Localized Hyperplasia			Generalized Hyperplasia		
	0	1	2	3		Mild/Mod./Sev.			Mild/Mod./Sev.		
10-19	0	0	1	0	1	0	0	0	0	0	0
20-29	1	3	3	1	6	1	0	0	0	1	0
30-39	1	1	1	0	1	0	0	0	2	0	0
40-49	2	0	1	0	3	0	0	0	0	0	0
50-59	2	1	0	1	0	1	0	0	2	1	0
Totals	6	5	6	2	11	2	0	0	4	2	0

Table 4: Distribution and severity of gingival hyperplasia in relation to age and oral hygiene scores for males.

Age [years]	Oral Hygiene Scores				No Hyperplasia	Localized Hyperplasia			Generalized Hyperplasia		
	0	1	2	3		Mild/Mod./Sev.			Mild/Mod./Sev.		
10-19	0	1	0	1	1	0	0	0	0	1	0
20-29	0	5	3	0	6	0	0	2	0	0	0
30-39	0	3	1	1	3	0	0	0	1	1	0
40-49	0	1	0	1	3	0	0	0	0	0	0
50-59	1	1	2	0	2	0	0	0	1	0	0
Totals	1	11	6	3	15	0	0	2	2	2	0

The relationship between the severity and distribution of hyperplasia and the duration of Cyclosporine therapy is shown in detail in Table 5. Among the 7 patients with less than 6 months of therapy, only 1 (14%) showed evidence of hyperplasia compared with 13 (41%) of the 32 who had been receiving therapy for more than 6 months. This result suggests that there may be an association between length of therapy and hyperplasia, but again the numbers are too small for statistical test. There appears to be little difference between patients who have been on therapy for 6-11 months compared to 12 or more months. It may be that the risk of developing hyperplasia is greatest within 6-12 months of beginning therapy rather than the first 3 months as suggested by Wysocki 1983. Although the numbers are again too small for statistical test, the data also suggest a trend towards patients who have been on therapy for longer than 12 months (see Table 5) being more likely to have generalized rather than localized gingival hyperplasia.

Table 5: The relationship between duration of Cyclosporine therapy (in months) and the presence and severity of gingival hyperplasia.

Duration of Cyclosporine Therapy [months]	No Hyperplasia	Localized Hyperplasia			Generalized Hyperplasia		
		Mild	Mod.	Sev.	Mild	Mod.	Sev.
0 - 5	6	0	0	0	0	1	0
6 - 10	9	1	0	2	3	1	0
11 - 15	8	1	0	0	3	1	0
16 - 20	2	0	0	0	0	0	0
21 - 25	1	0	0	0	0	1	0
Totals	26	2	0	2	6	4	0

The relationship between the severity and distribution of hyperplasia and serum levels is shown in Table 6. The range of serum levels is relatively wide (99 - 756 microgram/litre) but with the majority (65%) falling below 300 micrograms/litre.

Table 6: Relationship between serum level of Cyclosporine (mean value for first 30 days of therapy) and presence and severity of gingival hyperplasia.

Serum level of Cyclosporine [microgram/ litre]	Number of Subjects	No Hyper-plasia	Localized Hyper-plasia	Generalized Hyperplasia	Total
100 - 200	14	12(86%)	1(7%)	1(7%)	2(14%)
201 - 300	12	7(58%)	3(25%)	2(17%)	5(42%)
301 -	14	7(50%)	0(0%)	7(50%)	7(50%)
Total	40	26(65%)	4(10%)	10(25%)	14(35%)

When subjects were divided into 3 approximately equal size groups, the group with the Cyclosporine level less than 200 had the highest percentage of subjects with no hyperplasia (86%). In the group with Cyclosporine levels above 300, only 50% did not show signs of hyperplasia. The proportion of subjects with generalized hyperplasia increased with the average levels of Cyclosporine from 7% in the lowest to 50% in the highest Cyclosporine levels. This relationship was significant (Mantel-Haenszel trend test, chi square = 6.68, 1 degree of freedom, p=.01).

The relationship between the severity and distribution of hyperplasia and oral hygiene status is shown in Tables 3, 4. Poor oral hygiene status (a score of 2 or 3 on the Index) is not associated with the presence of hyperplasia. However, majority of patients with generalized hyperplasia have oral hygiene scores of 2 or 3 (6 out of the 9 patients).

Group I included 11 patients with conditions likely to have an impact on the oral tissue; 8 with diabetes, 2 with epilepsy and 1 who was pregnant. Both the epileptic patients and the woman who was pregnant showed evidence of moderate generalized hyperplasia, but gingival hyperplasia was found in only 1 out of the 8 patients with diabetes. Both the epileptic patients were maintained on Tegretol to control their seizures.

ii. Microbiological Study

Objective 2 Estimation of relative population levels of black pigmented Bacteroides in gingival fluid of early transplanted allograft recipients (Group II).

The subjects were 5 males and 6 females, all been maintained on dialysis, ranging from 6 to 192 months prior to transplant.

Nine patients exhibited unusually pale, gingival tissues, possibly due to an anaemic state, a feature common in this group of patients. Two cases, 3 and 9 however showed

marked marginal gingivitis and the tissues bled during the insertion of the paper points.

Table 7 shows the occurrence of viable counts of black pigmented Bacteroides in study subjects. Subjects 3 and 9 show significantly elevated colony counts of the bacteria as proportions of the total counts. Subjects 5, 7, 2 show low colony counts that would be within the normal range for healthy gingivae. The remaining six samples grew no black pigmented colonies. All strains of black pigmented Bacteroides were identified or B. intermedius, no B. melaninogenicus or B. gingivalis were isolated.

Table 7: Percentage of contribution of black pigmented Bacteroides to total viable count in new transplant patients.

Presence of Marginal Gingivitis

Subject	Age	Sex	Duration of Dialysis [months]	Black Pigmented Bacteroides of total count
1	52	F	192	0
2	37	F	24	0
3	38	M	24	54
4	47	M	24	0
5	27	M	4	7
6	29	M	6	0
7	21	F	2	1
8	17	F	5	0
9	30	F	5	47
10	41	M	1	0
11	29	F	2	2

Objective 3 Comparison of the population levels of black pigmented Bacteroides in samples taken from gingival crevice fluid in areas affected and non-affected by Cyclosporine induced hyperplasia (Group III).

Fourteen cases, 8 male and 6 female were studied. All cases showed localized, moderate to severe gingival hyperplasia.

Viable colony counts of black pigment Bacteroides were found in samples taken from four subjects (28.6%). Three of these subjects showed the bacteria in the affected areas whilst the fourth showed colonization of both affected and non-affected sites.

The remaining samples (71.4%) did not grow any of the test flora on culture.

Table 8: Percentage contribution of black pigmented Bacteroides to total viable count in areas affected and non-affected by gingival hyperplasia.

Subject	Age at Transplant	Sex	Duration of Cyclosporine Therapy	% Black Pigmented Bacteroides Hyperplasia Site	% Black Pigmented Bacteroides No Hyperplasia Site
1	18	M	9	15	0
2	40	F	23	0	0
3	35	M	6	0	0
4	30	F	12	0	0
5	21	M	6	26	0
6	33	F	6	16	0
7	39	M	12	0	0
8	28	M	15	0	0
9	24	F	4	0	0
10	56	M	11	0	0
11	25	M	6	0	0
12	56	F	6	0	0
13	38	M	24	4	14
14	25	F	8	0	0

14. DISCUSSION

Given the relatively small numbers and the cross-sectional rather than longitudinal character of the design, the results of this study must be interpreted with some caution. The finding that 33% showed evidence of hyperplasia suggests a relationship between the development of the condition and Cyclosporine use, but not in all patients. This lack of uniformity of reaction raises many questions, particularly those relating to the initiating factors and what causal mechanisms are involved.

There is a trend towards women being more likely than men to develop gingival hyperplasia (42% relative to 29%), although this relationship is not statistically significant. Neither was age a significant variable in this regard, but the age range of the patients was relatively narrow; all were between ten and forty years. This age range was representative of the standard age distribution of renal transplant recipients during the study period. Since that time, however, there has been a trend towards extending the age boundary as transplantation has been increasingly adopted as a management option for renal failure in older patients. A marked increase in the number of younger transplant patients is unlikely in the forseeable future, but the use of Cyclosporine therapy by younger patients will increase as Cyclosporine is adopted for the management of other conditions, such as the management of type I Diabetes.

The onset of this condition tends to cluster around the ages of 10-14 years. A study of juvenile diabetics by Wysocki et al. (1986) reported a very high prevalence of gingival hyperplasia. When reviewing long term Cyclosporine use, the age at onset of the therapy should be noted when designing a dental management program.

Previous research on the relationship between the initiation of therapy and the timing of onset has been inconclusive. On the one hand, Tyldesley and Rotter (1984) claim that change will occur within the first three months of therapy. On the other hand, Wysocki et al., (1981) claim that the time of onset is variable. Neither study looked at variations in serum levels. As the present study was not longitudinal, we could not establish a definitive time of onset; however, we could examine the relationship between the development of hyperplasia and serum levels. The Canadian Multi-Centre Transplant Study used a protocol in which the initial dosage of Cyclosporine post-surgery was determined by the weight of the patient and was subsequently individually adjusted according to achieved serum levels and calibrated kidney function. After thirty days, "provided that the patient was clinically and immunologically quiescent" dosage levels were gradually reduced. The individualized responses observed in the present study were examined in relationship to these early serum levels of Cyclosporine. The majority (86%) of the patients with a Cyclosporine level below 200 during this period did not

develop hyperplasia, whereas 50% of those with serum levels above 300 did so. The results of this study demonstrate the importance of taking into account variations in the dosage of Cyclosporine in the first month. Similar findings were reported in phenytoin studies (Staple 1954). The extreme hyperplasia reported in early Cyclosporine case studies may have reflected higher dose levels used in the initial clinical trials.

When patients on the drug for more than a year are compared with those using Cyclosporine for less than a year, a higher proportion of long term users showed evidence of hyperplasia with the majority showing generalized as opposed to localized hyperplasia. This may reflect an increased probability of developing hyperplasia with longer use of Cyclosporine, but there are other possible explanations. There were, for example, changes in the management of these patients, or the prevalence of unfavourable oral hygiene standards gradually influenced development. These and similar questions are now being raised in work done at the cellular level (Sciubba, 1988).

Oral hygiene scores did correlate with the severity of hyperplasia in affected subjects; those with poor oral hygiene showing greater enlargement of the gingival tissues. The actual development of gingival hyperplasia, however, did not appear to have any relationship with oral hygiene, although longitudinal data on plaque scores are lacking. It is a reasonable assumption that the hygiene status of these

patients was well-established and did not improve subsequent to transplant. No special program of preventive dental care existed for transplant patients and most had not been seen for dental care for a number of years prior to transplant surgery. Interestingly, few affected patients showed signs of gingivitis, such as bleeding, a fact of significance for the microbial study.

A number of the subjects were receiving medical care for conditions other than renal failure, including diabetes (8). Many researchers have stated that diabetes, both type I and type II, is a predisposing factor for periodontal disease (Manoucher-Pour, M. and Bissada, N.K., 1983). In this study, however, none of the diabetics all of whom had reached end-stage renal failure showed any significant change in their gingival tissues.

The one patient who was pregnant during the study developed marked generalized hyperplasia with a superimposed gingivitis. This latter condition is not an uncommon feature during pregnancy. It was, therefore, of interest to follow this case to notice any changes in the hyperplasia post partum when the condition regressed to a 'mild generalized' level. At present dental management of pregnant recipients of Cyclosporine is not a common event. Patients on these protocols are generally counselled not to become pregnant due to the possible effects of Cyclosporine on the unborn child as well as the increased risk to the mother.

The immediate post transplant microbiological study was designed to answer the question whether or not subjects suffering from renal failure were in some way or another predisposed to changes in the oral microflora in particular the black pigmented Bacteroides population of the gingival crevice area.

The findings from this study showed that in 8 out of 10 samples taken from new transplant subjects it was not possible to culture black pigmented Bacteroides. The two cases that produced high colony counts were noted to have had symptoms of long established gingival pathology that was exhibited as bleeding while samples were being collected which would explain the higher counts (Loesche et al., 1985) Any elevation of microbial counts in patients maintained on long-term Cyclosporine could therefore be seen as an associated occurance. Based on these data it can be stated that elevated counts of black pigmented Bacteroides from gingival crevice samples are not a regular feature of renal failure.

In the sampling for black pigmented Bacteroides in Group III, subjects the long-term Cyclosporine group, the identification of black pigmented Bacteroides, in 1 out of 14 non-affected sites suggests that no direct mechanism inherent in Cyclosporine derived either from saliva or serum promotes the growth of these bacteria.

The identification of the test bacteria in only 4 out of 14 of the samples from affected sites was perhaps more unexpected. The environment of the gingival crevices at these sites, where pseudo-pocketing conditions existed would promote anaerobic conditions and hence be more likely to encourage the development of an anaerobic flora (Moore, 1987). Similarly accumulation of debris and irritants would occur due to the difficulty in cleaning of the pseudo-pockets and likely lead to inflammation. The accompanying bleeding would provide hemin and other blood products required by black pigmented Bacteroides for proliferation Takazoe and Nakamura, (1971). It can be stated that despite numerous predisposing factors produced by Cyclosporine therapy the development of a significant population of black pigmented Bacteroides in the human gingival tissues does not result.

15. CONCLUSIONS

1. The prevalence of gingival hyperplasia in the present study was 33% and is influenced by serum levels of Cyclosporine in the first 30 days post transplant. Severity was found to be variable and influenced by oral hygiene status.

2. Black-pigmented Bacteroides are not present at increased levels in the gingival crevice areas of patients in renal failure.

3. Black-pigmented Bacteroides are not present at increased levels in gingival crevice areas of patients maintained on long term Cyclosporine therapy and exhibiting gingival hyperplasia.

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