

Immunoglobulins and complement in the chronic interradicular lesions of the primary teeth

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Abstract

Eleven unrestorable primary molars with interradicular radiolucencies were extracted. The granulation tissue was curretted from the sockets and examined using immunofluorescence for the immunoglobulins IgG, IgA, and IgM, and the third component of complement. The results demonstrated the presence of IgG in 100%, C3 in 80%, IgA in 54.5%, and IgM in 18.1% of the lesions.

In an attempt to describe the nature of chronic pulpal infection, several researchers successfully have cultured and identified bacterial strains from necrotic teeth.¹⁻³ Similar inquiry has been made into the periapical lesions of such infected teeth. Although the literature reports the successful culture and identification of microorganisms from acute abscesses,⁴ most research has shown that microorganisms are not found in chronic periapical granulomas and cysts.⁵⁻⁹ Because these lesions may be sterile and are not grossly infected, a nonmicrobiologic approach to the mechanisms of periapical tissue destruction has been considered by several researchers.¹⁰⁻¹⁶

One such mechanism is the humoral immune response. Various components involved in the humoral immune response can contribute to tissue destruction, including anaphylatoxins released during complement fixations, or lysosomal enzyme release from polymorphonuclear leukocytes (PMNs) following attachment by immune complexes. There are several lines of evidence suggesting the participation of immunologically mediated events in the pathogenesis of these lesions.^{17,18}

Barns and Langeland,¹⁹ for example, sealed protein antigens into deep dentinal cavity preparations in monkeys and induced the production of serum antibody. These results suggest that antigens may penetrate through deep carious lesions and contact immunocompetent cells leading to an immune response.

In later experiments, Morse and coworkers¹⁴ successfully identified immunoglobulin-producing plasma cells in human periapical granulomas and cysts. They demonstrated the absence of such cells in periapical scars.

More recently, Pulver and coworkers¹⁵ used immunofluorescence techniques to demonstrate the presence of immunologic components within periapical lesions. They demonstrated the presence of the immunoglobulins IgG, IgM, IgA, and IgE, and the complement protein C3 in such chronic lesions. In a similar study by Kuntz and coworkers,¹¹ the presence of IgG, IgM, and IgA were observed extracellularly, as well as within plasma cells. IgG-containing plasma cells were most numerous. Positive staining for C3 was found fixed to tissues, suggesting an immunological role. They also found C3 bound to circular structures (resembling blood vessels) in the absence of any detectable antibody, suggesting alternate pathway activation.

Endotoxin positively has been identified in periapical lesions. Schonfeld and coworkers²⁰ demonstrated endotoxin in 15 of 20 periapical granulomas, but in only 2 of 10 noninflamed samples. These results have significant implications in the etiology of periapical disease. It is possible that while intact bacteria are not found in significant numbers in the periapical tissues, bacterial products (such as endotoxin) are present in sufficient quantities to trigger host immune mechanisms. Endotoxin, for example, is toxic for several types of cells, including fibroblasts, and also activates the complement cascade via the alternate pathway.

The process of bone resorption in periapical pathosis is multifactorial and it is evident that the humoral immune response is one of these factors. The necessity of a thorough understanding of chronic periapical disease can be appreciated from the viewpoint of treatment. Surely, it is advantageous to understand a disease in order to treat it properly and predictably. There is little or no

literature describing the immunological status of the interradicular or the periapical lesions in the primary dentition. Since it seems reasonable for such a mechanism to exist, it is the purpose of this research to determine if immune components can be identified in the chronic interradicular granulomas of the primary dentition.

Methods and Materials

Eleven unrestorable, untreated primary molars with interradicular radiolucencies were extracted. The granulation tissue was curetted gently from the sockets and immediately fast-frozen on dry ice. All specimens were kept frozen until processing. Frozen sections four microns thick were prepared with a cryostat-microtome. Representative sections of each lesion were stained with hematoxylin and eosin and submitted for histologic evaluation.

The technique for processing the frozen sections has been described by Kuntz and coworkers.¹¹ Sections were fixed for 30 seconds in 90% ethanol and air-dried. The sections then were washed twice in phosphate buffered saline (PBS) for 15 minutes each. Two sections of each specimen were incubated with each of the following fluorescein-conjugated goat antibodies: antihuman IgG, antihuman IgA, and antihuman IgM.^a Two sections of each specimen also were incubated with rhodamine-conjugated IgG fraction goat antibody to human C3.^a Following incubation, each specimen was washed three times in PBS for 10 minutes. (Specimens incubated with different antibodies were not washed in the same baths.) The sections then were air-dried, mounted with buffered polyvinyl alcohol and glycerine, and examined using a microscope equipped with vertical fluorescence illumination and dichroic filter combinations specific for fluorescein and rhodamine.

Negative controls consisted of human skin tissue sections which were treated in exactly the same manner as described above.

All samples were evaluated as being positive or negative. No attempt was made to quantify the amount of antibody present in the positive samples.

Results

Using the fluorescein-specific filter, tissue sections positive for IgG, IgA, or IgM showed areas of apple green fluorescence concentrated in plasma cells and regions of tissue containing immunoglobulins. Tissue sections negative for the above immunoglobulins showed only a dull field. For samples stained for IgG, 100% were positive. Six of the samples examined for the presence of IgA were positive for that immunoglobulin — five samples were negative. Only 2 of the 11 samples were positive for the immunoglobulin IgM — the remaining 9 samples were negative (Table 1).

For samples incubated with rhodamine-conjugated anti-C3, a positive reaction was characterized by bright

^a Cappel Laboratories, Cochranville, Pa.

orange fluorescence against a dull field. Ten samples were available for this study. Eight of ten were positive — 2 were negative (Table 1).

The negative controls, which consisted of human skin tissue, were negative for IgG, IgA, IgM, and C3.

The results of the histologic evaluation also are found in Table 1. No relationship between the presence or absence of immunologic components and histopathologic evaluation was observed using the Fisher Exact Test). Likewise, no relationship between the presence or absence of the various immunologic components was found when they were compared with each other.

Discussion

In this study immunofluorescence was used to demonstrate the presence of the immunoglobulins IgG, IgM, IgA, and complement C3 in chronic interradicular lesions of carious primary teeth. The presence of these immunologic components is consistent with studies of chronic periapical granulomas in the permanent dentition.¹¹ It is believed that mediators of immunologic events contribute to the pathogenesis of the supporting periodontium in endodontic disease.^{10-16, 19, 20} The formation of immune complexes from the reaction of antigens with IgG and IgM antibodies can stimulate the release of such mediators through their ability to interact with both humoral and cellular immune systems.²¹ Bacterial fragments and endotoxins are examples of such substances.²⁰ In particular, the component system can be activated either by immune complexes or by

Table 1. Histologic Evaluation* and Results of Human Granulation Tissue Incubated With Antihuman IgG, IgA, IgM and Complement C3**

Patient #	IgG	IgA	IgM	C3	Diagnosis
1	+	-	-	NE	I
2	+	+	-	+	CP
3	+	-	-	+	CP
4	+	+	-	+	CP
5	+	+	-	+	CP
6	+	+	-	+	CP
7	+	+	-	+	CAP
8	+	+	+	-	CP
9	+	-	-	+	CAP
10	+	-	+	+	CP
11	+	-	-	-	CAP

* Results of histologic evaluation of tissue samples stained with hematoxylin and eosin: CP = Chronic Periodontitis; CAP = Chronic and Acute Periodontitis; I = Inconclusive.

** IgG, IgA, and IgM identified in human granulation tissue by staining with fluorescein-conjugated goat antisera: Antihuman IgG, antihuman IgA, and antihuman IgM; complement component C3 identified by staining with rhodamine-conjugated goat antihuman complement C3.

+ indicates positive reaction; - indicates negative reaction; and NE indicates not examined.

endotoxin.²² The presence of either component in periapical or interradicular tissues would stimulate complement activation at these sites.

Complement factors C3a, C5a, and C567 are chemotactic for leukocytes. Lysosomal release by leukocytes causes thrombosis, hemorrhage, edema, endothelial destruction, and local necrosis of host tissue.²² C3a and C5a are also anaphylatoxins. They stimulate the release of vasoactive amines by degranulation of mast cells which causes increased vascular permeability and increased blood flow, increasing the local inflammatory response.²³ In addition, the activation of complement has been implicated in the stimulation of osteoclastic activity and bone resorption.²⁴

The results of this study demonstrate the presence of IgG in 100%, C3 in 80%, and IgA in 54.5% of samples studied. Kuntz and coworkers, in their study of 10 periapical lesions in the permanent dentition,¹¹ also found IgG present in 100% of lesions. In those samples treated by the fast-frozen method, C3 similarly was found in all of the lesions, as was IgA. In contrast to our findings of IgM in only 18.1% of the lesions, Kuntz and coworkers localized IgM in 70% of lesions studied. The low frequency with which IgM was found may reflect the chronic nature of most of these lesions.

The significance of these findings can be appreciated from the standpoint of treatment. In the permanent dentition, traditional endodontic therapy eliminates the antigenic source of the immune response through debridement and sanitation of the root canal system. In the primary dentition traditional therapy for periapically or interradicularly involved teeth also has been debridement and sanitation of the root canal system. Recently, however, some authorities have suggested formocresol pulpotomies in treating extensively involved teeth in lieu of the above.²⁵ While formocresol is a tissue fixative which binds to the proteins of microorganisms and is thus bacteriocidal,²⁶ it also has been shown to be capable of rendering autologous pulp tissue antigenic and to stimulate a specific cell-mediated lymphocyte immune response in animal studies.²⁷ Given this antigenic potential, it seems likely that the use of formocresol in teeth with total pulpitis and interradicular or periapical pathology could enhance the immunologic consequences of such pathology.

The short-term clinical effectiveness of the formocresol pulpotomy in the treatment of primary teeth with coronal pulpitis has been demonstrated. In a recent three-year clinical study of 98 primary molars with coronal pulpitis, Rolling and coworkers²⁸ found a formocresol pulpotomy success rate of 91% after three months, 83% after one year, 78% after two years, and 70% after three years. In all failures, they reported interradicular or periapical pathology. The study by Rolling and coworkers thus demonstrated a progressive increase in failure rate with time, which might have approached

unacceptable levels if it had been a more lengthy study. It is possible that the clinical success of this procedure is related to the exfoliation of primary molars.

This study suggests that while short-term success with formocresol pulpotomy can be achieved in primary teeth with coronal pulpitis, long-term effectiveness may be enhanced by attempts to remove all sources of antigenic stimulation.

Conclusions

Immunoglobulins IgG, IgA, complement component C3, and to a lesser extent, IgM have been demonstrated in the chronic interradicular lesions of primary teeth. This data supports the notion that immunologic mechanisms are at least partially responsible for the tissue destruction that is seen. Further research is needed to describe the antigenic potential of the medicaments used in the pulp therapy of primary teeth.

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Quotable Quote

For years, diabetologists and nutritionists have taught that there are two major classes of carbohydrates: simple and complex. Simple carbohydrates, which are the sugars like glucose, sucrose, and fructose, are absorbed immediately by the gut and cause a rapid rise in blood sugar and blood insulin. Complex carbohydrates, such as the starches found in rice and potatoes, take longer to be absorbed and so result in a slower and more moderate rise in blood glucose and blood insulin.

Or so the dogma goes. But it turns out that the dogma is incorrect. The problem, said Jesse Roth, a diabetes specialist at the National Institutes of Health, is that, "I believed it. Everyone believed it. But no one ever tested it."

When Phyllis Crapo of the University of Colorado Health Sciences Center in Denver thought to test the dogma, she was astonished to find just how wrong it is. Crapo and other researchers are learning, for example, that a bowl of ice cream does almost nothing to blood glucose, nor does a sweet potato. But, a white potato or a slice of whole wheat or white bread sends blood glucose soaring. To further confound the matter, the effects of carbohydrates on blood glucose are unpredictable. The only way to learn the effect of a particular food is to test it on volunteers.

These discoveries are of major consequence for diabetics who must avoid large swings in blood glucose. They also may be important for nondiabetics since, at the very least, large amounts of glucose in the blood make persons sleepy.

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