

Serum Immunoglobulin Levels and Bacterial Flora in Subjects with Acute Oro-facial Swellings

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Blood chemistry determinations, including serum immunoglobulin (Ig) levels, were correlated with bacterial flora within root canals of subjects with endodontic flare-ups. Except for a several-fold rise in IgE, all serum immunoglobulin levels and blood chemistry results were within normal limits. Endotoxin-producing gram-negative obligate anaerobes were cultured from all but one subject.

There is a great need in endodontic therapy for a better understanding of why "flare-ups" (rapidly occurring facial swelling accompanied by pain and suppurant drainage related to a nonvital tooth) occur. In recent studies, specific anaerobic bacterial species have been cultured from nonvital teeth associated with positive symptomatology (1-3). In addition to a possible microbial etiology, endotoxin and tissue immunoglobulins (Ig), except for IgD, have been associated with periapical granulomas and cysts (4-7). However, only one serum antibody study, which evaluated only IgG and IgM, has been reported on patients without flare-ups but with endodontically involved teeth (8). The purpose of our study was to evaluate all serum immunoglobulin levels in response to endodontic flare-ups and to correlate these findings with the bacterial flora cultured from the corresponding teeth.

Three groups of patients were analyzed for the purpose of this study. Subjects in the experimental group consisted of nine individuals with endodontic flare-ups ranging from 20 to 63 yr in age and in good health. None of these subjects presented with a history of allergies or receiving radiation or chemotherapy of any type which could adversely affect serum hematology or blood chemistry results. Subjects in control group 1 consisted of four patients, 27 to 44 yr of age, in good health, with no significant medical history, and not undergoing endodontic therapy. The subjects on control group 2 consisted of two individuals not undergoing any endodontic therapy and with noncontributory medical histories except for the presence of known allergies.

Upon presentation to the clinic (time 1), all subjects in the experimental group had oral temperatures re-

corded and venous blood drawn for complete blood count and differential. Serum immunoglobulin levels including IgG, IgM, IgA, IgE, and IgD were also evaluated. All serum immunoglobulins except for IgE were quantitated using a single radial immunodiffusion technique (9). Serum IgE was quantitated through solid phase radioimmunoassay using a sandwich technique (10, 11). For IgE, the experimental readings are calibrated against the first British Standard 75-502 for human IgE. The anti-IgE is raised in rabbits and is specifically raised against human IgE. In addition, an anaerobic culture was obtained from the nonvital teeth associated with the flare-up. The tooth in question was isolated with rubber dam and access to the pulp chamber performed with a sterile bur at high speed. Purulent exudate was present in all teeth.

All experimental patients were placed on potassium penicillin, 500 mg, four doses/day for 7 days. Ten to 14 days later (time 2), venous blood samples were drawn for a second set of serum immunoglobulin titers. Endodontic therapy was continued to completion.

A modified 3-ml glass syringe, equipped with a T-shaped glass connector, was used for obtaining the bacterial specimens. This T-shaped glass connector permitted purified argon gas to continuously flow over a sterile paper point in order to maintain an anaerobic environment until the paper point could be transferred to the prerduced culture media.

The bacterial specimens were placed in prerduced brain heart infusion broth (Difco) with 0.5% cysteine added using the modified Hungate procedure of Miller and Wolin (12). Incubation was carried out anaerobically in an atmosphere of 85% nitrogen, 10% hydrogen, and 5% carbon dioxide at 37°C for 48 to 72 hr to promote the growth of all facultative and anaerobic organisms present in the samples (the ability to isolate organisms which may be present in small numbers in such a mixed population is greatly enhanced by this preincubation procedure; T. McNamara, unpublished data.) The selective media used were examined with the aid of a dissecting microscope and each different bacterial colony type on each plate was subcultured in broth and checked for purity by microscopic examination of a gram-stained smear. All cultures were

identified as to genus utilizing a modification of the procedure as outlined by Ellner, and applying the criteria of Bergey (174) to the results of carbohydrate and biochemical reactions for confirmation (13).

Each subject in each of the control groups had venous blood drawn only once to measure serum immunoglobulin titers. Blood samples were drawn at different times during the course of the experiment to test the accuracy of the laboratory's normal values.

RESULTS

Gram-negative obligate anaerobes—including *Bacteroides*, *Veillonella*, and *Fusobacterium*—were cultured from all nonvital teeth. With the exception of patient #7, gram-positive organisms were found in conjunction with gram-negative organisms. However, no one organism was found common to all subjects.

Serum immunoglobulin levels for IgG, IgM, IgA, and IgD were all within normal limits for all groups with no significant change from time 1 to time 2. Seven of the nine patients in the experimental group demonstrated abnormally high values of IgE at initial presentation to the clinic. Only one of the nine experimental patients (#6) demonstrated a normal IgE value for both time 1 and time 2 readings. With the single exception of patient #5, the time 2 readings for serum IgE titers were consistently elevated (Table 1).

The complete and differential blood counts were all found to be within normal range. The oral temperatures were all slightly elevated with the exception of patient #4, who gave a reading of 103°F.

The four subjects in control group 1 exhibited normal values for all serum immunoglobulins, including IgE (Table 1). The two subjects in control group 2 demonstrated elevated IgE levels (Table 1).

TABLE 1. Serum immunoglobulin levels

Experimental Patient No.	IgG Normal 564-1715 (mg/dl)		IgM Normal 53-375 (mg/dl)		IgA Normal 85-385 (mg/dl)		IgD Normal 0-25 (mg/dl)		IgE Normal 0-41 (units/ml)		Microorganisms
	1	2	1	2	1	2	1	2	1	2	
	1		1050		280		205		1		
2	1,200		120		105		1		673		<i>Bacteroides</i> Hemolytic <i>Streptococcus</i> <i>Treponema</i>
3	760	800	255	250	150	165	1	1	111		<i>Bacteroides</i> <i>Veillonella</i> <i>Actinomyces</i> Hemolytic <i>Streptococcus</i> <i>Diphtheroides</i>
4	1,000	960	200	210	105	100	8	5	130	146	Hemolytic <i>Streptococcus</i> <i>Veillonella</i>
5	1,500	1,500	88	210	170	180	6	7	266	29	<i>Veillonella</i> <i>Actinomyces</i> Hemolytic <i>Streptococcus</i>
6	1,100	1,100	145	135	200	200	14	3	32	26	Hemolytic <i>Streptococcus</i> <i>Veillonella</i> <i>Actinomyces</i>
7	1,200	1,200	170	170	100	100	5	3	918	559	<i>Staphylococcus</i> <i>Lactobacillus</i> <i>Actinomyces</i>
8	830	1,000	235	200	200	180	1	1	86	75	
9	840	940	160	120	150	160	3	1	204	184	
Control group											
1 patient no.											
1	1,050	N/A	110	N/A	120	N/A	1	N/A	21	N/A	} Not applicable
2	1,300	N/A	150	N/A	110	N/A	1	N/A	16	N/A	
3	1,100	N/A	115	N/A	150	N/A	1	N/A	9	N/A	
4	1,050	N/A	280	N/A	115	N/A	1	N/A	26	N/A	
Control group											
2 patient no.											
1	1,100	N/A	80	N/A	390	N/A	1	N/A	313	N/A	} Not applicable
2	790	N/A	85	N/A	285	N/A	8	N/A	89	N/A	

DISCUSSION AND CONCLUSIONS

The microbiological results of our study closely correspond with Keudell et al. (8) and Sundquist (2) in that gram-negative obligate anaerobes are often associated with nonvital teeth. These anaerobes also correspond closely to the types of organisms found in both early and late stages of periodontitis (14, 15). Our findings also corroborate the nonelevated humoral antibody levels of IgG and IgM found by Keudell et al. (8) in endodontic lesions. The consistently elevated serum IgE values in our study indicate a possible mechanism for an immediate hypersensitivity reaction. Several studies in clinical medicine have indicated that elevated IgE levels are often present in allergic diseases (16). The highest IgE levels (aside from IgE myeloma) are found in parasitic diseases such as allergic bronchopulmonary aspergilliosis and active eczematous diseases (17).

IgE is cross-linked by appropriate antigens on its Fab portion which stimulates mast cells and basophils to release biologically active substances including histamine, eosinophil chemotactic factor, slow reacting substance, and platelet-activating factor. It may be these mediators which are responsible for the increased vascular permeability and edema associated with a flare-up.

One likely available antigen which could cross-link IgE may be the lipopolysaccharide endotoxin, from the cell wall of gram-negative bacteria (17). Endotoxin has been reported by several authors to be present in the root canal and in immunologically active periapical granulomas and cysts of nonvital teeth (4, 7, 18-21).

The administration of endotoxin in vivo results in rapid degranulation of mast cells, as found by Hook et al. (22). In a sensitized host, IgE is already bound to the mast cell. Sensitization could occur through prior exposure of the host to the endotoxins of gram-negative organisms found in periodontal disease. The passage of whole bacteria from the gingival sulcus into remote pulp tissue has been demonstrated by Grossman (23). Therefore, it is tempting to speculate that the rapid onset of symptoms associated with the endodontic flare-up might be an immediate hypersensitivity reaction mediated through IgE and caused by endotoxins produced by the gram-negative bacteria found in the periapical lesion.

Ongoing research presently includes monitoring serum IgE levels in patients with chronic quiescent peri-

apical lesions and treatment of flare-ups with systemic antihistamine.

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