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Concentrations of Immune Complexes, IgG, IgM, IgE, and C3 in Patients with Acute Apical Abscesses

Concentracion de Complejos Inmunes IgG, IgM, IgE y C3 en Pacientes con Abscesos Apicales Agudos

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The serum concentrations of circulating immune complexes, immunoglobulins G, M, and E, and the C3 complement component of 35 patients with acute apical abscesses were measured. These levels were compared with those of people without acute apical abscesses. Statistically significant differences between the levels of immune complexes, immunoglobulins G and M, and C3 complement component were found between the two groups. In addition, significant differences were also noted in eight patients in the mean levels of concentration of immune complexes, immunoglobulins G, M, and E, and C3 complement component before and after root canal therapy or extraction of involved teeth. It appeared that acute periapical lesions may lead to measurable systemic immunological reactions, but the clinical significance of these changes remains unclear.

La concentración de complejos inmunes circulando en el suero, inmunoglobulinas G, M, E y el complemento C3 fue medida en 35 pacientes con abscesos apicales agudos. Estos niveles fueron comparados con los de personas sin abscesos apicales agudos. Estadísticamente fueron significativas las diferentes entre los niveles del complejo inmune, inmunoglobulinas G y M, y el complemento C3 que se encontraron entre los dos grupos. Agregado a ésto, se notó significativa diferencia en ocho pacientes en los niveles absolutos de concentración de complejos inmunes, inmunoglobulinas G, M y E y el componente complemento C3 antes y después del tratamiento de conducto o extracción de la pieza involucrada. Aparentemente esa lesión periapical aguda podría causar una reacción inmunológica mesurada, pero el significado clínico de estos cambios aún queda poco claro.

The formation of immune complexes is the result of interaction of circulating antibodies with exogenous or endogenous antigens, usually to help the host eliminate potential pathogens. Antigen-antibody complexes can interact with the humoral and cellular components of the immune system and can enhance or suppress immune responses. In addition, immune complexes have the potential to initiate or perpetuate pathological changes in many sites in the body (1). Immune complex-associated diseases include glomerulonephritis, infective endocarditis, arthritis, vasculitis, spondylitis, ulcerative colitis, uveitis, dermatitis, liver diseases, and probably myocardial infarctions.

Several bacterial species have been identified from the root canals of pathologically involved teeth (2–7). Egress of these bacteria and their products into the periapical tissues can lead to the formation of antibodies (8). Various classes of immunoglobulins have been found in human periapical lesions (9–14). A noncovalent interaction between immunoglobulins and inciting antigens can result in the formation of immune complexes in periapical tissues. By using the anticomplement immunofluorescence technique, immune complexes were detected in human dental periapical lesions (15). The levels of circulating immune complexes (IC's), IG and IgM, and the C3 complement component have been measured in the sera of patients with chronic periapical lesions (16). When the mean levels of these components in patients with chronic periapical lesions were compared with those from control volunteers, no statistical difference was noted between the two groups. The purpose of present investigation was to compare the concentrations of circulating IC's, IgG, IgM, and IgE, and the complement component C3 in subjects with and without acute apical abscesses.

MATERIALS AND METHODS

The study population consisted of 35 patients who had presented themselves for treatment of acute apical abscesses at the emergency clinic of the School of Dentistry at Loma Linda University or either of two

private dental offices. All patients had acute symptoms of severe swelling, pain, and fever. The patients ranged from 12 to 53 yr, with a mean age of 31.6 yr. One patient had a history of rheumatoid arthritis; the others had no known systemic autoimmune or collagenous diseases. None had any history of allergic reactions to medications or other antigens. The control group consisted of 30 faculty, graduate students, and staff at the School of Dentistry. These individuals ranged from 24 to 62 yr, with a mean age of 38.6 yr. None had apparent systemic diseases or periapical dental abscesses at the time of the study.

The nature and purpose of the study as well as the risks and discomforts involved in drawing blood were explained and informed consents were obtained from all of the patients and from the guardians of patients under the age of 18. Ten milliliters of blood were drawn from each patient. The patients were informed that if their concentrations of IC's, IgG, IgM, IgE, or C3 complement component were significantly higher than those of the control group, they might be asked to donate another 10 ml of blood after treatment when symptoms had subsided. Eight of 13 patients with elevated levels of IC submitted a second blood sample for further analysis. Ten milliliters of blood were also drawn from each participant in the control group. All blood samples were clotted, and the sera separated, aliquoted, and frozen at −30°C. The samples were assigned code numbers and tested for the concentrations of IC's, IgG, IgM, and IgE, and the C3 complement component.

MEASURING CONCENTRATIONS OF CIRCULATING IC

The Raji cell assay described by Theofilopoulous et al. (17) was used to measure the levels of circulating IC. Raji cells (2×10^6), suspended in 50 μl of Spinner's medium, were placed in a tube. Replicate samples were run for each serum. Each patient's serum was diluted to 25% in 0.15 *M* sodium chloride, and 25 μl of the dilution were added to the Raji cells. After incubating for 45 min at 37°C, the cells were washed three times with Spinner's medium. The washed cells were then incubated for 30 min at 4°C with an optimal amount of ^{125}I-labeled rabbit anti-human IgG diluted to 50% with Spinner's medium containing 1% human serum albumin. After this incubation, the cells were again washed three times with Spinner's medium containing 1% human serum albumin. Supernatant fluids were removed, and the radioactivity of the remaining pellets was measured with a gamma counter.

Solutions containing 0.625 to 80 μg of heat-aggregated human γ-globulin (AHG) protein in 50 μl was mixed with normal serum (a source of complement) and then tested as described above. These measurements were used to create a standard curve to which experimental values were compared. The radioactivity in the experimental sera was directly proportional to the amount of IC present. Results are expressed as micrograms of AHG equivalent per milliliter of serum.

MEASURING CONCENTRATIONS OF IgG, IgM, AND THE C3 COMPLEMENT COMPONENT

The concentrations of circulating IgG, IgM, and the C3 complement component were measured by a standard nephelometric procedure described by Killingsworth and Savory (18). In this assay, antibody to human Ig, or C3 when brought into contact with the human Ig or C3 in solution, produces an antigen-antibody reaction and an increase in light scatter.

The analyzer (Beckman Immunochemistry Analyzer) was calibrated using the manufacturer's (Beckman ICS products) serum calibration solution. The peak rate signal produced by the reaction of the antibody with the Ig or C3 in the calibrator is verified, and the analyzer internally calibrates the peak rate signal so that the calibrator sample will read the proper target value indicated for the serum calibration solution. Results as microgram or milligram amounts per deciliter of serum were reported by the analyzer only if the control value was within the tolerance limits (±2 SD) for the assay.

MEASURING CONCENTRATIONS OF IgE

The concentrations of circulating IgE were measured by a radioimmune assay according to Ishizaka et al. (19) and Johansson and Bennich (20). In this assay, IgE-specific antibody binds to ^{125}I-labeled IgE, the radioactive tracer. Serum samples were mixed with a constant amount of labeled IgE and primary antiserum and incubated for 4 h at 37°C. During incubation, the unlabeled IgE competed with the radiolabeled IgE for binding sites on the primary antibody. Bound and unbound fractions separate with the addition of a fixed quantity of a second antibody. Following a second incubation period of 1 h at room temperature, the IgE-bound antibody complexes were separated from the unbound IgE in a centrifuge. Each sample was tested twice. The standard curve consisted of six points covering the range of 0 to 200 IU/ml of IgE.

In patients with acute abscesses, it was determined that 13 patients had elevated concentrations of IC's: at least 20 μg of AHG equivalent/ml of serum. This point was considered a level that would be a potential indicator of a pathological level of IC's (17). Eight of these patients returned and donated another 10 ml of blood. The levels of circulating IC's, IgG, IgM, and IgE, and the C3 complement component of those who returned for the recall were measured as described above.

RESULTS

The average serum concentration of circulating IC's, IgG, IgM, IgE, and the C3 complement component in

the patients before treatment and in the control group are shown in Table 1. Thirteen of 35 (37%) patients had circulating IC concentrations higher than 20 μg of AHG in their sera. In contrast, only 4 of 30 (13%) subjects in the control group had concentrations as high. The analysis of variance (F test) demonstrated a significant difference between all acute patients and the control population in IC ($p < 0.001$), IgG ($p = 0.038$), and IgM ($p = 0.006$). Borderline significance was found for C3 ($p = 0.053$) and no significance for IgE ($p = 0.135$). When the values of IC's, IgG, IgM, IgE, and the C3 complement component in the 13 patients with high IC concentrations were compared with those of the 4 control subjects with high IC concentrations, only the concentrations of IC ($p = 0.032$) and IgG ($p = 0.012$) differed significantly. No significant difference was found when the levels of IC's, IgG, IgM, IgE, and the C3 complement component were compared between the 22 negative IC patients and the 26 control subjects with low IC concentrations.

The serum concentrations of IC's, IgG, IgM, IgE, and C3 complement component in eight patients with initial IC concentrations greater than 20 μg of AGH/μl before and after treatment are shown in Table 2. A paired *t* test between dependent variables indicated a significant difference between the mean levels of IC's ($p =$

Table 1. Average serum concentrations of IC's, immunoglobulins G, M, E, and complement component C3 in patients with acute abscesses (by high and low IC values)

Group	No. (%)	IC (μg AHG/ml)	IgG (mg/dl)	IgM (mg/dl)	IgE (IU)	C3 (mg/dl)
Patients with IC > 20 μg AHG/ml	13 (37)	51.6 ± 32.5*	1209 ± 279	203 ± 93	74.7 ± 71.2	176 ± 33
Patients with IC < 20 μg AHG/ml	22 (63)	8.9 ± 5.2	1022 ± 228	131 ± 53	78.8 ± 72.6	154 ± 28
Controls with IC > 20 μg AHG/ml	4 (13)	30.3 ± 9.8	952 ± 149	190 ± 34	59.3 ± 46.8	159 ± 40
Controls with IC < 20 μg AHG/ml	26 (87)	5.6 ± 5.3	882 ± 250	168 ± 65	68.8 ± 59.4	151 ± 29
Normal Range		<20†	564–1715 (mg/dl)§	53–375 (mg/dl)	0–40 (units/ml)§	160‡

* ±SD.
† A. Theofilopolous, personal communication.
‡ Muller-Eberhard (26).
§ Svetcov et al. (21).

Table 2. Serum concentrations of IC, IgG, IgM, IgE, and C3 in eight patients with elevated* levels of immune complexes before and after treatment

Patient†		IC (μg AHG/ml)	IgG (mg/dl)	IgM (mg/dl)	IgE (IU)	C3 (mg/dl)
1	Before‡	195	1430	161	34.20	195
	After§	192	1160	156	18.77	192
2	Before	192	1350	171	105.64	192
	After	116	1360	173	33.65	116
3	Before	239	1090	112	25.10	239
	After	207	1080	101	59.05	207
4	Before	134	1160	197	57.79	134
	After	108	1150	210	38.80	108
5	Before	168	770	415	84.77	168
	After	154	1210	197	51.19	154
6	Before	146	998	269	29.61	146
	After	134	894	265	13.25	134
7	Before	168	1370	163	171.35	168
	After	112	1320	152	132.92	112
8	Before	173	1180	344	190.56	173
	After	139	1050	231	142.38	139
Statistical ‖						
Mean difference (d)		34.4	78.0	47.1	34.6	31.6
SD		27.9	89.7	78.4	19.1	24.2
t		3.49+	2.46¶	2.79¶	5.0+	3.69+

* >20 μg of AHG/ml.
‡ Patient values prior to endodontic treatment.
§ Patient values after treatment; no acute symptoms.
‖ Paired *t* test between dependent variables ($n = 8$).
\+ $p = 0.01$.
¶ $p = 0.05$.

0.01), IgG ($p = 0.05$), IgM ($p = 0.05$), IgE ($p = 0.01$), and C3 ($p = 0.01$) before and after dental treatment.

DISCUSSION

By comparing data from eight patients with acute abscesses before and after treatment, significant differences in the mean concentrations of circulating IC's, IgG, IgM, IgE, and C3 were noted. Although a statistically significant difference was noted between the mean values of the various serum components, most individual's concentrations remained essentially unchanged for IgG and IgM. When comparing levels of IgE concentrations, however, seven of eight patients showed a substantial decrease in this immunoglobulin after treatment. Five of the eight patients had IgE levels higher than normal, which were lowered after treatment. Two of these patients changed from higher levels to within normal limits after treatment. The remaining three had higher than normal levels remaining after treatment, but did experience a substantial drop. Svetcov et al. (21) noted a several-fold rise only in IgE levels in subjects with endodontic flare-ups. Our IgE data compare closely with those obtained by these investigators before and after treatment. While being aware of the potential role IgE may mediate in a disease process, the data, at this time, appear to be too small to draw any conclusions as to actual mechanism.

Elevated concentrations of IgE are characteristic of immediate hypersensitivity reactions and may indicate that systemic IgE-mediated reactions could have been initiated by antigens egressing from pathologically involved root canals. Antigen-antibody concentrations are capable of interacting with the complement system and can change the concentrations of these components by forming immune complexes.

In our study, the eight treated patients all showed a decrease in levels of C3 after treatment. Even though the mean concentration of this component after treatment was significantly lower, most levels appeared to still be within the generally accepted normal limits. Present data regarding C3 levels do not essentially contrast with our previous findings in patients with large chronic periapical lesions (16).

The Raji cell assay is based on the interaction of complement-fixing immune complexes with complement receptors on the Raji cell surface. Raji cells, a lymphoblastoid cell with B cell characteristics, uncommonly lack surface immunoglobulins. Any IgG bound to the cell surface is due to interaction of immune complexes. The technique is reproducible, is easy to perform, and requires only small amounts of serum. It detects complexes of various sizes but, preferentially, larger ones. Theofilopoulos et al. (17) have shown by this technique that only 3% of normal individuals have values exceeding 12 μg of AHG equivalent/ml serum; patients with malignancies have values of 50 to 100 μg of AHG/ml and patients with autoimmune diseases range from 100 to 300 μg of AGH/ml of serum.

Our results (Table 1) showed that high concentrations of circulating immune complexes in patients with acute abscesses were almost three times as common as in the control group. Formation of immune complexes depends on the nature and ratio of the antibodies and antigens.

An increase in the concentration of immunoglobulins during periapical abscesses and a significant decrease after dental treatment could indicate that the entrance of antigens into the periapical tissues could induce an elevated, localized concentration of immunoglobulins. Interaction between immunoglobulins and root canal antigens could form circulating immune complexes. What determines the fate and pathogenicity of IC's is not completely understood. One important factor appears to be the duration of exposure to the antigens. If the exposure is short, clinical manifestation and tissue injuries are usually transient. If the exposure is prolonged, the potential for immune complex-associated diseases increases. Another factor that may be involved is the pathogenicity of immune complexes in the genetic make-up of the host (22–24).

In most cases, human exposure to circulating immune complexes, as a result of dental abscesses, is of limited duration. Because the presence of circulating immune complexes does not necessarily lead into diseases, establishing cause and effect between dental abscesses and systemic immune complexes-associated diseases is difficult. However, because these immune complexes have the potential in certain individuals to cause systemic diseases, the source of antigens should be eliminated either by root canal therapy or by extraction. Removal of the source of the antigens is one of the steps recommended by the World Health Organization (25) to attenuate the deleterious effects of immune complexes. Removal of root canal antigen by complete cleaning and debridment or by extracting teeth that cannot be saved seems to be the most practical approach to prevent the formation of IC's and the elevation of immunoglobulins.

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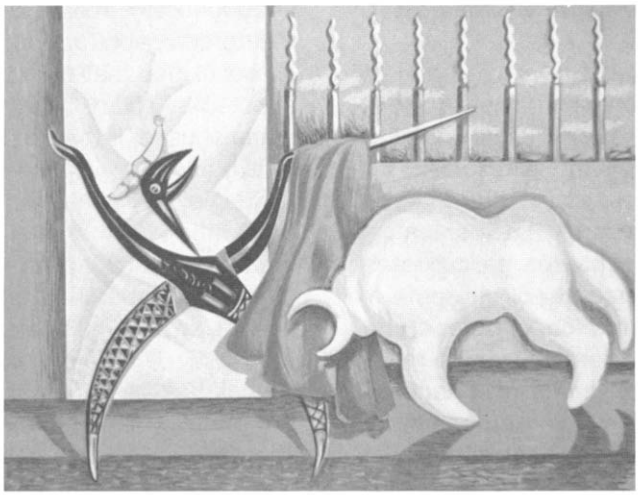

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