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Changes in the Titer of Tooth Root Antibodies Accompanying Root Resorption Associated with Orthodontic Tooth Movement

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INTRODUCTION

Root resorption is one of the most common problems associated with orthodontic tooth movement. Especially, intrusive tooth movement has been suggested to enhance the risk of root resorption, though individual's responses are quite varied(De Shields, 1969; Sjφlien and Zachrisson, 1973; Copeland et al., 1986; Dermaut and De Munck, 1986; Levander and Malmgren, 1988; Melsen et al., 1989). According to the recent report(Brezniak and Wasserstein, 1993), loss of apical root material is unpredictable and when extended into the dentin, it is irreversible.

Bates(1856) was perhaps the first to discuss root resorption of permanent teeth. Ottolengui(1914) related root resorption directly to orthodontic treatment. Ketcham(1927) was among the first interested in root resorption as a consequence of orthodontic treatment. He demonstrated the differences between root shape before and after orthodontic treatment with radiographic evidences. Rudolph(1940) and others reported resorbed lacunae of the root surface visible on radiographs. This was followed by a wide range of histologic, clinical, and physiologic research on root resorption and orthodontic treatment(Graber and Swain, 1985; Brezniak and Wasserstein, 1993).

Root resorption related to orthodontic treatment is reported to be surface resorption (Andreasen, 1988) or transient inflammatory resorption(Tronstad, 1988). Replacement resorption is rare if ever seen after orthodontic treatment(Brezniak and Wasserstein, 1993). In a great number of cases root resorption is elicited more easily when an intruding force is applied rather than any other type of force. That is because the apical area is prone to the greatest concentration of force since it is the surface that faces toward the direction of physiologic movement during intrusion, and the bone of the apical region is fairly compact

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(Graber and Swain, 1985; McFadden et al, 1989; Profitt, 1993).

Although many root resorption studies attempted to investigate the etiologic factors and predictability of this phenomenon, its causes remain equivocal. Individual susceptibility, genetic predisposition, trauma, age of the patient, the stage of root formation at the beginning of treatment, systemic, local, and anatomic factors associated with orthodontic mechanotherapy are commonly cited components(McFadden et al, 1989; Newman, 1975). The various factors causing root resorption of permanent teeth are as follows: physiologic tooth movement, adjacent impacted tooth pressure, periapical or periodontal inflammation, tooth implantation or replantation, continuous occlusal trauma, tumors or cysts, endocrine or metabolic disorder, local functional or behavioral problems, orthodontic treatment, and idiopathic factors(Becks and Cowden, 1942 ; Phillips, 1955; Newman, 1975; Shafer et al, 1983 ; Reitan, 1985; Harris and Butler, 1992).

Root resorption is also the most common finding in traumatized teeth(Andreasen, 1985). Moreover, it has frequently been cited as an important cause for failure in dental transplantations(Robinson and Rowlands, 1973). The mechanism by which root resorption results from these stimuli is poorly understood. Evidence exists which seems to support the hypothesis that trauma may act non-specifically to disrupt the integrity of the periodontal ligament, resulting in the loss of possible "protective" capacity belived to be provided by the ligament or epithelial cells(King and Courts, 1988). This could lead to the chemotaxis and activation of resorptive cell types, possibly from sites in bone, which cause the progressive loss of root structure(Karring et al, 1980; Nyman et al., 1980; Aukhil et al., 1986).

Several clinical features of root resorption are difficult to explain with such a non-specific mechanism : first, idiopathic root resorption has no apparent traumatic etiology; second, genetics seems to play some poorly-defined role in idiopathic and orthodontic root resorption; third, considerable individual variation in all types of root resorption exists without significant variation in the trauma experiences; and fourth, in susceptible individuals, the idiopathic and orthodontic root resorption responses are site specific with certain teeth exhibiting consistently higher prevalences than others(Newman, 1975). Such observations have prompted speculation that root resorption may be mediated or modified by a specific systemic mechanism(Becks and Cowden, 1942). However, such a mechanism has yet to be convincingly demonstrated.

In spite of the clear demonstration that root resorption is the principal tooth transplant rejection response(Riviere et al, 1971), no pertinent data exists on whether there are specific immunological responses accompanying active root resorption in other contexts. However, titers of autoantibodies to a tooth root antigen preparation have recently been reported, and evidence demonstrating that immune complexes associate preferentially with resorbing roots exists(King and Courts, 1988).

A role of the specific immune response in root resorption during orthodontic tooth movement, mediated by antibody production and immune regulation, has never been explored. Therefore, the purpose of this investigation is to follow changes in levels of serum antibody to autologous tooth root antigen preparations accompanying root resorption associated with

orthodontic tooth movement in animal models.

METHODS

Material and appliance

Five adult mongrel dogs, 2 years of age, were used in the study. They were premedicated with atropine(0.1ml/kg) and combelen(0.03ml/kg), anesthetized with Ketamine(50mg/kg) and maxillary impressions were taken. Appliances were constructed with 1.2mm stainless steel wire on the maxillary models. They consisted of labial and transpalatal parts. Closed coil springs(008X036 Unitek) were soldered in the labial parts for intrusion of anterior teeth(Chang and Park, 1992)(Fig. 1,2). Lingual buttons were bonded on six anterior teeth and closed coil springs were ligatured to generate 200-250gm intrusive force(Fig. 3). A heavy intruding force was applied in an attempt to bring an extensive apical root resorption artificially. Readjustment of force was done every week. In the 9th week, six maxillary anterior teeth were extracted. Serum samples were taken from each dog prior to intrusion and weekly for 11 weeks thereafter. These were analyzed for antibody titer to dentinal antigen preparation in succession on the same day to eliminate equipment variance that could occur if blood samples were assayed on separate days. Root resorption was monitored monthly using occlusal radiographs. And then root resorption patterns were observed with a zoom stereo microscope(Model SZH-121, Olympus optical Co. Ltd.).

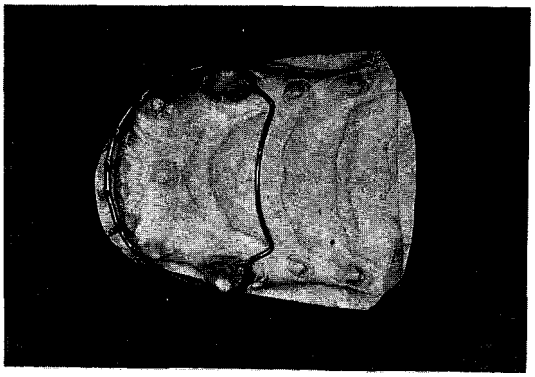

Fig. 1. Occlusal view of the appliance that was constructed on the maxillary model.

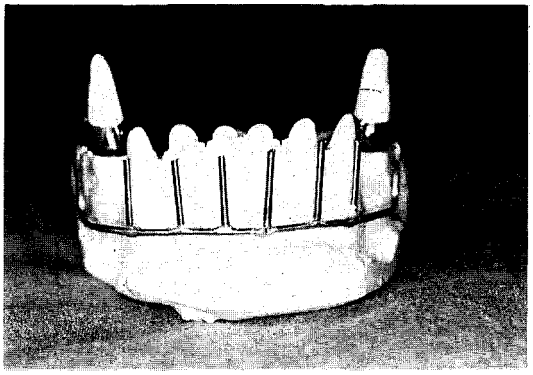

Fig. 2. Frontal view of the appliance that was constructed on the maxillary model.

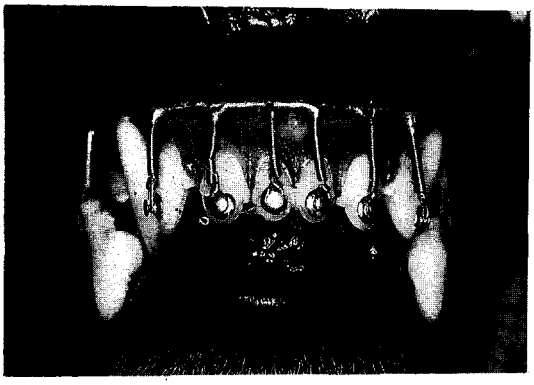

Fig. 3. View of the appliance setting in the mouth.

TOOTH ROOT ANTIGEN PREPARATION

Six lower incisors were removed as sources of homologous antigen in the dogs, respectively. Tooth root antigen preparations were made from extracted teeth by removing the crown at the cementoenamel junction, carefully scaling the periodontal ligament and cementum from the roots, bisecting them longitudinally to expose the pulp chambers and exhaustively washing in phosphate buffered saline(PBS: pH 7.4; 4) using a mechanical stirring apparatus over 24 hours. During this period the buffer solution was replaced every 4 hours. These were then pulverized in a mortar and pestle and extracted under dissociative conditions in 6M Guanidine-Hcl-10% EDTA(pH 5.0) for 48 hours at 4 on rotator. The extracted protein was measured by microassay procedure every 12 hours(Table 1), and time course of the mean of protein extracted from a pulverized dentin was recorded(Fig. 4). The extracts were then cleared by centrifugation and the supernatants were renatured by dialysis against several changes of water over a 12 hour period. The dialysis membranes were used which excluded molecules above 1000 dalton. These preparations were then lyophilized and stored for later use at -20℃. These preparations were considered to consist primarily of dentinal components and were used as an antigen source for immunizations and analyses for serum antibody concentrations.

ANTIBODY ASSAY

An enzyme-linked immunosorbent assay (ELISA) was used to quantify antibody titers in serum samples(Engvall and Perlmann,

Table 1. The amount of protein extracted hrom a pulverized dentin.

	12hr	24hr	36hr	48hr
A	18.78	20.31	32.74	33.50
B	18.61	20.47	21.00	23.20
C	18.97	21.44	22.96	24.99
D	21.04	25.12	25.35	26.47
E	22.14	22.48	24.14	27.00
F	11.77	19.02	23.20	35.85
Mean	18.55	21.47	23.40	28.50

Analytical wavelength:596nm, Concentration units:$\mu g/m\ell$

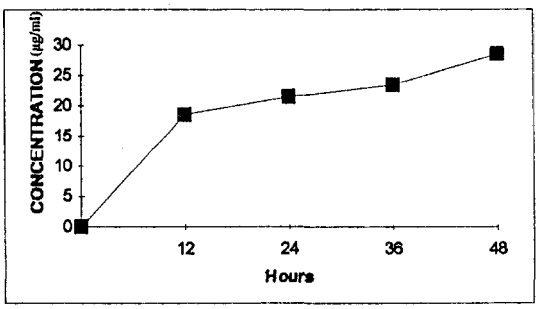

Fig. 4. Time course of the mean of protein extracted from a pulverized dentin.

1971). 100μl of tooth root antigen preparations (5μg/ml, dilution in buffer I) were attached to 96 well microtiter plates via 24 hour absorption at 4. The next day the wells were washed with buffer II. Then 350μl of fat free milk(5%) was used to block all the wells for 1 hour at 25. The wells were washed as before, and then 100μl of each dog serum sample(dilution 1:50 in buffer III) was added to antigen-coated wells and incubated for 2 hours at 37. The wells were washed again and then 100μl of goat antidog IgG (γ)(dilution 1:100 in buffer III), as a 2nd antibody, was added to the wells and left for 2 hours at room temperature on rotator. After again washing in 0.15M PBS containing 0.05% Tween-20 and then 100μl of alkaline phosphatase conjugated rabbit anti-goat

Table 2. Composition of buffers used in ELISA

Stock buffers	0.2M Sodium carbonate 0.2M Sodium bicarbonate 10% Sodium azide 10X PBS	Buffer III	for diluting sample and antisera 300ml 10X PBS 1.5ml Tween 20 6ml 10% sodium azide bring to 3L with distilled water
Buffer I	for coating plates with antigen pH 9.6 80ml sodium carbonate stock 170ml sodium bicarbonate stock 1ml sodium azide bring to 500ml with distilled water	Buffer IV	for dissolving NPP substrate pH 9.8 55ml sodium carbonate stock 70ml sodium bicarboante stock 100ml magnesium chloride bring to 500ml with distilled water
Buffer II	for washing batween steps 27g sodium chloride 1.5ml Tween 20 bring to 3L with distilled water	Buffer V	for stopping reaction 1N NaCL 20g sodium hydroxide bring to 500ml with distilled water

IgG(dilution 1:5000 in buffer III), as a 3rd antibody, was added and incubated overnight at room temperature on rotator. The wells were washed a final time, and 200 μl of a freshly made enzyme substrate solution (p-nitrophenyl phosphate, 1mg/ml in buffer IV) was added to each well and left to stand within 30 minutes at room temperature on rotator. Reaction should be stop with 100 μl of a buffer V. Optimal dilutions of antigen and antibody were determined by the checker-board method. Enzyme activity was then quantified by spectrophotometric analysis at 405nm(Automated microplate reader, Model EL311SL, Bio-Tek instrument, INC).

Specific immunoglobulin bound per serum sample was determined by subtracting background optical densities. The latter were determined from preparations containing all reagents except serum. As controls for antibody specificity, sera which were previously incubated with tooth root antigen as well as sera to an unrelated bacterial antigen(Porphyromonas gingivalis 33277) for 3 hours at 25 , were measured in all runs.

STATISTICAL ANALYSIS

The analysis of variance procedure including one-way ANOVA was used to determine the possible effect of time on the changes of the titer of tooth root antibodies. And the difference between control and each sample was analysed by Student's t-test for unpaired observations. Significance was predetermined at the level of significance α =0.05.

RESULTS

The incisors did not show clear radiographic evidence of progressive root resorption though periodontal ligament space had widened(Fig. 5). But root resorption was observed on the apical part of the maxillary incisors when examed with a zoom stereo microscope. The teeth showed shallow depressions that generally accompany deep resorption(Fig. 6, 7, 8, 9).

The mean value of autoantibody(Table 3) and changes of the individual autoantibody levels to the dentinal antigen are recorded

during intrusive tooth movement(Fig. 11). Variations between individuals were not negligible(Fig. 10).

The titers of autoantibody to the tooth root antigen preparation measured during the course of active root resorption are shown in Fig. 11. The data were reported as a ratio of treated densities divided by pretreatment baseline optical densities. In these instances a ratio greater than 1.00 signifies an increase in titer and those less than 1.00, a decrease. These demonstrate a slight tendency for an immediate decrease followed by rebound to levels above the pre-treatment baseline. A peak titer of autoantibody to dentin antigen occurred on day 28, then steadily decreased during the 9th week period as the roots resorbed and then rapidly increased when the resorbing teeth were extracted. The result of the ANOVA tests evaluating the effect of time on the changes of the tooth root antibodies revealed statistical significance(Pr > F = 0.0170). However, there were no significant differences in Student's t-test between the control and each sample, and more or less difference between the 9th and 10th week as Prob > F = 0.0810.

When the sera was incubated with tooth root antigen, serum activity in the ELISA was almost absent.

Mean value of individual ELISA activity to the unrelated bacterial antigen was recorded (Table 4, Fig. 12). Serum ELISA activity to the unrelated bacterial antigen remained essentially unchanged in all animals throughout the experimental period. The result of the ANOVA tests revealed significant differences (Pr > F = 0.0005). When the time course of changes in autoantibody to homologous tooth root antigen prepatration and unrelated bacterial antigen was compared, no significant differences were found(α =0.05). In general, the pattern of changes in autoantibody was similar to the two antigens(Fig. 13).

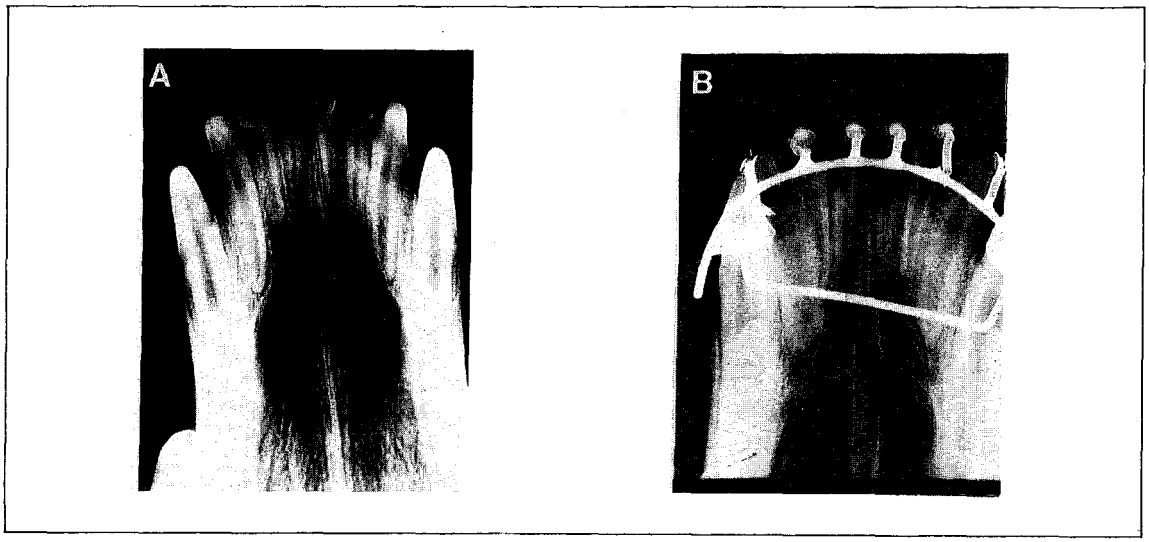

Fig. 5. Occlusal views of maxillary incisors from experimental animals.
A. before intrusive movement.
B. 11weeks after intrusive movement, there was no clear evidence of significant and progressive root resorption but the periodontal space had widened.

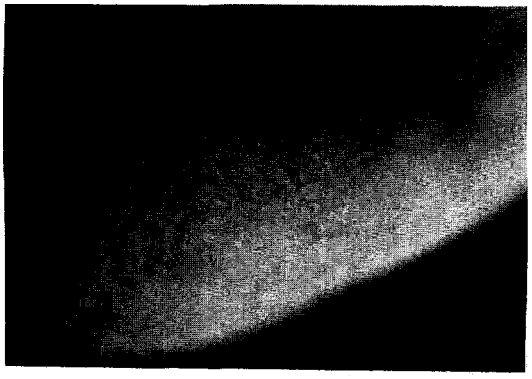

Fig. 6. Scanning zoom stereo microscope of a dog incisors at 11 weeks. Multiple small resorption lacunae were observed at the root apex.

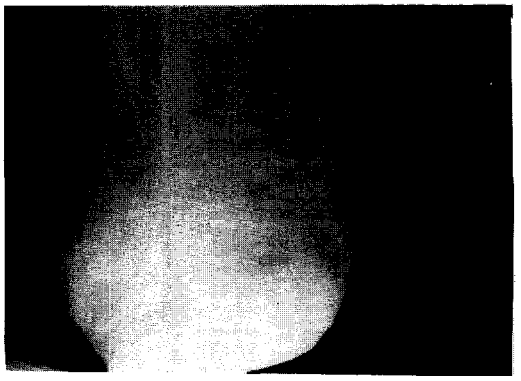

Fig. 7. Representative root resorption lesion from a dog's incisors. Small and large resorption lacunae were observed at the apical region.

Fig. 8. Representative deep root resorption lesion was observed on the apex.

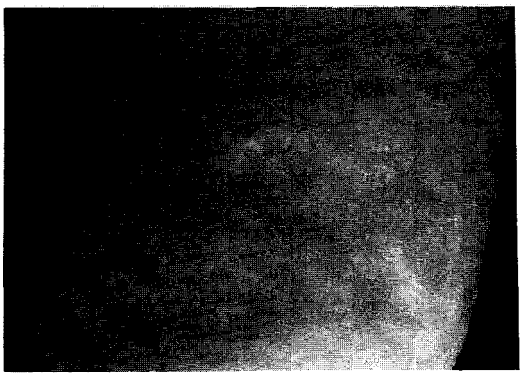

Fig. 9. A large and shallow root resorption lesion was observed on the apical surface.

Table 3. Mean value of individuai's ELISA

	A	B	C	D	E	Mean	SD	Signficance
Control	1.97	2.03	1.80	1.04	1.30	1.63	0.40	NS
1wk	0.80	1.96	1.72	1.03	1.07	1.32	0.47	NS
2wk	2.00	2.01	1.69	1.04	0.97	1.54	0.48	NS
3wk	2.00	1.93	1.73	1.03	1.10	1.56	0.43	NS
4wk	2.19	2.07	1.89	1.07	1.24	1.69	0.47	NS
5wk	1.25	1.80	1.84	1.01	1.02	1.38	0.38	NS
6wk	1.11	1.85	1.70	0.96	1.10	1.35	0.38	NS
7wk	1.03	1.80	1.79	0.95	1.13	1.34	0.39	NS
8wk	1.22	1.66	1.64	1.04	1.04	1.32	0.29	NS
9wk	1.41	1.59	1.42	1.00	1.04	1.29	0.27	NS
10wk	2.12	1.89	1.88	1.10	1.15	1.63	0.44	NS
11wk	2.08	1.73	1.93	1.18	1.33	1.65	0.36	NS

Significance determined by Student's t-test on the difference between hte contron and each week.
NS,not significant at α=0.05.

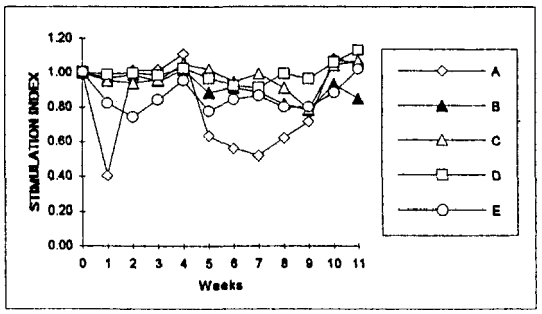

Fig. 10. Changes in the levels of autoantibody to dentin antigen from five dogs during active root resorption.

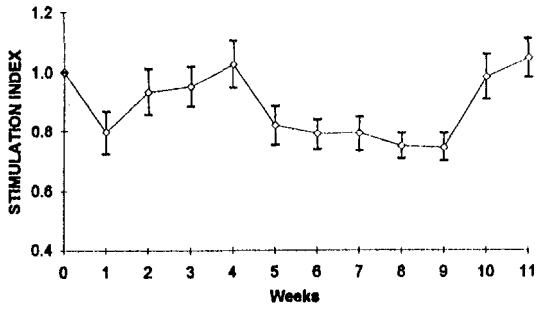

Fig. 12. Mean antibody titers to unrelated bacterial antigen during orthodontic tooth movement. The vertical bars denote two standard deviations from the sample mean.

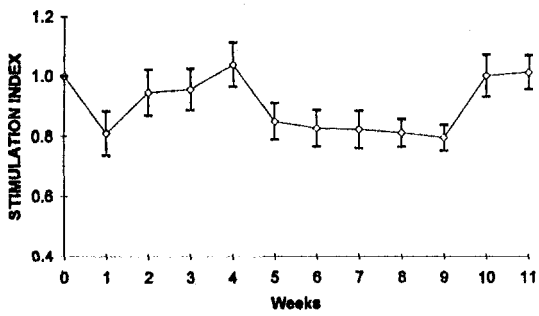

Fig. 11. Mean antibody titers to autologous dog

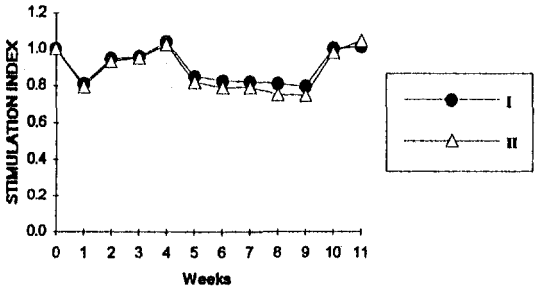

Fig. 13. Comparison of the time course of changes in autoantibody to homologous tooth root antigen preparation with unrelated bacterial antigen. There was no significant difference (α=0.05).

I : Mean antibody titers to autologous dog dentin antigen during root resorption.

II : Mean antibody titers to unrelated bacterial antigen during root resorption.

Table 4. Mean value of individuai's ELISA activity to unrelated bacterial antigen.

	A	B	C	D	E	Mean	SD	Signficance
Control	1.93	2.12	1.72	1.12	1.51	1.68	0.39	NS
1wk	0.79	2.01	1.69	1.08	1.11	1.34	0.46	NS
2wk	1.96	2.08	1.78	1.18	0.85	1.57	0.50	NS
3wk	1.96	2.09	1.74	1.03	1.17	1.60	0.44	NS
4wk	2.19	2.19	1.97	1.11	1.15	1.72	0.51	NS
5wk	1.28	1.85	1.85	0.98	0.93	1.38	0.42	NS
6wk	1.15	1.77	1.60	0.99	1.12	1.33	0.32	NS
7wk	1.03	1.79	1.74	0.97	1.12	1.33	0.37	NS
8wk	1.13	1.39	1.66	0.90	1.22	1.26	0.27	NS
9wk	1.42	1.54	1.41	0.97	0.93	1.25	0.31	NS
10wk	1.98	2.12	1.98	1.09	1.08	1.65	0.48	NS
11wk	2.16	2.08	2.01	1.36	1.17	1.76	0.42	NS

Significance determined by Student's t-test on the difference between hte contron and each week.
NS,not significant at α=0.05.

DISCUSSION

Loss of root length has been observed after intrusion of the teeth within 14days(Ku and Park, 1988) and within 35 days in a scanning electron microscope study(Harry and Sims, 1982), but in a clinical situation, the root resorption can be observed at any time during the orthodontic treatment with marked time variation(Linge and Linge, 1980). Dermaut and De Munck(1986) follow up to the 29th week. In comparison with occlusal radiographs before and after intrusive tooth movement, no significant differences were present. This designates that root resorption and deformation identifed by radiographs are already in the state of severe root resorption. Others have described the pattern of root resorption lesions in human beings(Reitan, 1974; Barber and Sims, 1981; Ku and Park, 1988), dogs(Chang and Park, 1992) and rats (King and Fishlschweiger, 1982) in response to orthodontic treatment. The features of resorption lesions reported by these investigators are also similar to those described here.

When sera is incubated with tooth root antigen, serum activity in the ELISA was almost absent. This is because serum activity in the ELISA could be removed by absorption of the serum with dog dentin antigen.

An immunological response to orthodontic tooth movement resulting in root resorption could be either non-specific or specific. The former is similar to a foreign body reaction occured against a damaged tissue designed to remove or wall-off that structure. Such a response is usually characterized by the infiltration of macrophages and occasionally foreign body giant cells(Hirsh and Johnson, 1984), but a specific immune response does not occur. Tissues undergoing this type of response also have increased levels of proteolytic enzymes, lysozyme, complement, and prostaglandins(Riviere et al, 1971). In addition to the appropriate cellular infiltration being present during root resorption, other specialized hard tissue resorptive cells such as cementoclasts, dentinoclasts, and osteoclasts are also present(Andreasen, 1966). Prostaglandins have been implicated in the root resorption associated with orthodontic tooth movement, which can be considered to be a microform of root trauma also associated with limited root resorption(Shanfeld et al, 1986).

In addition to these histological and biochemical changes, a specific type of immunological response would require the synthesis of antibodies and regulation by various leukocytes, including T and B lymphocytes and macrophages. The demonstrations that root extracts can be autoantigenic and that levels of these antibodies follow a predictable kinetics during orthodontic root resorption in the dog suggest that serious consideration should be given to the hypothesis that this type of response may be of the specific kind. However, prior to accepting this evidence as proof of a specific immunological response, two notes of caution should be considered. First, there is good evidence that autoantibodies are quite common in sera apparently playing no pathological role, probably because they are suppressed by immune regulatory mechanisms(Dighiero et al, 1986). Second, these investigations offer no evidence that changes of an autoantibody levels to root components or even the presence of immune complexes on resorbing roots play any direct role in the process of orthodontic root resorption. The existence of circulating auto-

antibodies to tooth root antigens in dogs suggests that immunological mechanisms may play a role in root resorption but does not indicate whether it is an active or passive one(King and Courts, 1988). Demonstrating that levels of an autoantibody which correlate with the severity of the disease is a necessary prerequisite for establishing a role for autoimmunity. Once this is established, a role for the autoantibody in the precise mechanism of the disease may be defined. The latter has proven to be a difficult task in most known autoimmune diseases.

Autoimmune disease is thought to result from alterations in the individual's ability to regulate autoantibody production. This could result from disease, mutation or inheritance. Depending on the type of antigen and the mode of regulation, autoimmune diseases can manifest as either organ-specific(e.g. Hashimoto's thyroiditis, Primary myxoedema) or non organ-specific(e.g. systemic lupus erythematosus, dermatomyositis). Other autoimmune disease are thought to have a multifactorial pathogenesis resulting from a combination of genetic, hormonal and environmental factors. Root resorption is likely to be an organ-specific disease with root resorption as a feature and, obviously other factors including trauma, do seem to play roles.

The reversible reductions in serum tooth root autoantibody demonstrated in the dogs during active root resorption experiences suggest two possible interpretations. First, the resorbing tooth could be exposing significant amounts of previously sequestered antigen to the circulation, thereby providing a "sink" into which circulating autoantibody can bind. And then most circulating autoantibody and even that produced as part of a specific immunological response could be bound. This could result in a reduced serum autoantibody titers, if the rate of antibody production were less than the rate at which it binds to the "antigen sink". Resorbing roots have been shown to contain significantly higher levels of immune-complexes suggesting that autoantibodies may bind preferentially to these altered surfaces(King and Courts, 1988). Such an interpretation is further supported by the observation that serum tooth root autoantibody levels rapidly increase after removal of the source of antigen excess as in the extraction of a resorbing root. If an antigen sink did exist, one would predict that its rapid removal might cause a short period of antibody overproduction. This is in agreement with the finding of King and Courts(1988) and Ng, et al.(1990).

A second interpretation for the reduced levels of circulating antibody to tooth root antigen during active root resorption may be the activation of immune regulatory mechanisms, probably by T-cell suppressors.

T-helper function is usually detected early after exposure to antigen, but T-cell suppression usually becomes evident only when antibody production is most active or remains for extended periods(Green, et al., 1983).

Although root resorption occurs over extended periods, there is no data directly demonstrating such immune regulation exists in this context. The linkage between immune cells and bone resorption has become partially understood in the laboratory, but the full spectrum of clinical disorders of this relationship remains to be explored(Ng, et al., 1990).

The question of how tooth root autoantibody develops also bears further consideration. At least two possibilities exist: sequestered or altered antigens. The sequestered antigen mechanism proposes that autoanti-

bodies develop when certain macromolecules are unavailable to the immune system during development of tolerance, and if they become available in large enough doses in later life, they are unrecognized and therefore become autoantigenic. This concept is appealing for several reasons. There is morphological data that dentin is lined by the lamina limitans which is a sheet-like structure, containing glycosaminoglycans, and may act as a barrier that regulates the exchange of substances between dentinal fluid and peritubular dentin (Thomas, 1984), and that the pulpal side of the root can be considered to be covered by a "membrane" of odontoblasts which appear to have the ability to regulate the chemical nature of the dentinal fluid(Haljamäe and Rö ckert, 1970; Nagai and Frank, 1974). Enamel proteins are immunogenic in the same species(Schonfeld, 1979). Certainly, removal of cementum and exposing it to the circulation could make such an antigen available. Both of these lines of evidence suggest that dentin could be sequestered until deciduous root resorption. There is evidence that the latter may be stimulated by some physiological mechanism involving the follicles of the successional teeth(Cahill and Marks, 1980).

Such resorption could then act as a sensitizing event resulting in the production of autoantibodies. If one assumes that autoantibodies can play an active role in root resorption, acute exposure of the immune system to a previously sequestered antigen could explain reports that trauma resulting in the loss of the periodontal ligament and cementum results in root resorption or the observation that root planed surfaces adjacent to gingival pockets where re-epithelializations delayed are particularly prone to resorption (Karring et al., 1980 ; Nyman et al., 1980 ; Aukhil et al., 1986). Such a mechanism might also explain the lack of an inflammatory response during deciduous root resorption (Ten Cate and Anderson, 1986) because the latter represents a sensitizing event. Longitudinal tooth root autoantibody measurements covering the periods of the deciduous, mixed and permanent dentitions would be helpful in further elucidating such a mechanism.

The altered antigen mechanism suggests that some event, either physical or chemical, leads to the alteration of previously recognized molecules so that they are no longer recognized as self. Certainly numerous different types of events, including trauma and orthodontic treatment, could lead to protein denaturation, hapten-binding or other alterations of root idiotopes. Although such a mechanism could explain tooth root autoantibody production and the surface binding seen after trauma, it fails to explain the presence of autoantibodies in dogs with no history of trauma(King and Courts, 1988).

Newman(1975) suggested that those persons with idiopathic root resorption are unusually prone to orthodontic root resorption. It is possible that genetic factors as well as immunological mechanism influence this potential. The precise nature of the immunogenic components in tooth root antigen preparations also needs further study.

This study confirms the previous observation that titers of autoantibody to tooth root antigens decrease significantly during active root resorption only to return when the resorbing teeth are removed from contact with the circulation(King and Courts, 1988). It also demonstrates the possibility that these immunologic changes precede significant development of root resorption lesions rather than merely reflecting their presence. This

suggests that these humoral changes may have some predictive value for root resorption. However, it remains to be demonstrated whether the reported changes in serum titers represent a causative antecedent event in root resorption or merely reflect the ongoing process.

SUMMARY

This study was designed to measure the changes in the titer of tooth root antibodies accompanying root resorption associated with orthodontic tooth movement in dogs to explore a role of the specific immune response in root resorption during orthodontic tooth movement.

Five adult mongrel dogs, 2 years of age, were used in the study. Six lower incisors were extracted as sources of homologous antigen in the dogs. Tooth root antigen preparations were made from a 6M Guanidine-HCl-10% EDTA(pH5.0) extract of these root dentins. Root resorption was elicited by intrusion of six maxillary incisors with 200-250gm intrusive force. In 9th week, resorbing six maxillary anterior teeth were extracted. Serum samples were taken from each dog prior to intrusion and weekly for 11 consecutive weeks. Serum autoantibody titers were determined with an enzyme-linked immunosorbent assay. As controls for antibody specificity, sera which were previously incubated with tooth root antigen as well as sera to an unrelated bacterial antigen(Porphyromonas gingivalis 33277) for 3 hours at 25 were measured in all runs. Root resorption was monitored monthly using occlusal radiographs. And then root resorption patterns were observed with a zoom stereo microscope (Model SZH-121, Olympus optical Co. Ltd.).

Incisors did not show clear radiographic evidence of significant and progressive root resorption, but periodontal ligament space had widened. But root resorption was observed on the apical regions of the maxillary incisors with a zoom stereo microscope. Teeth showed the shallow depression generally accompanying deep resorption.

These demonstrate a slight tendency for an immediate decrease followed by rebound to levels above the pre-treatment baseline. A peak titer of autoantibody to dentin antigen occurred on day 28, then steadily decreased during the 9th week period as the roots resorbed and then rapidly spiked in animals when the resorbing teeth were extracted.

When sera is incubated with tooth root antigen, serum activity in the ELISA was almost absent. This is because serum activity in the ELISA could be removed by absorption of the serum with dog dentin antigen.

Serum ELISA activity to the unrelated bacterial antigen remained essentially unchanged in all animals throughout the experimental period. When the time course of changes in autoantibody to homologous tooth root antigen prepatration and unrelated bacterial antigen was compared, no significant differences were found(α =0.05). In general, the overall pattern of changes in autoantibody was similar to the two antigens.

These findings suggest the possibility that these immunologic changes precede a significant development of root resorption lesions rather than merely reflecting their presence. Therefore, this suggests that the changes of antibody levels may have some predictive value for root resorption.

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- 국문초록 -

치아이동시 치근 흡수에 따른 치근항체의 역가 변화

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치아이동시 발생되는 치근 흡수에 관련된 특이면역반응의 역할을 연구하기 위해 치근 흡수에 따른 치근항체의 역가 변화를 관찰하였다.

생후 2년된 성견 다섯마리를 실험동물로 사용하였다. closed coil spring을 사용하여 각 실험동물의 상악 6전치에 200-250gm의 함입력을 가하였으며, 하악 6전치를 발거하여 동종항원으로 사용하였다. 발거된 하악 전치의 상아질 부위를 분리하여 6M Guanidine-HCl-10% EDTA(pH 5.0)에서 해리시킨 다음 부유물 만을 투석하여 항원을 준비하였다. 치아를 함입시키기 전 1회 혈청을 채취하고 함입시키면서 매주 간격으로 11회 혈청을 채취하였다. 실험 9주째 치근 흡수가 일어나고 있는 상악 6전치를 모두 발거하여 항원의 근원을 제거하였다. 혈청내 치근항체의 역가는 ELISA(enzyme-linked immunosorbent assay)로 측정하였다. 항체 특이성에 대한 대조군으로 혈청과 치근 항원 그리고 혈청과 관련없는 박테리아 항원(Porphyromonas gingivalis 33277)을 섭씨 25도에서 3시간 반응시켜 모든 과정에서 항원-항체 반응을 측정하였다. 치근 흡수를 관찰하기 위하여 실험 전과 실험 후 한 달 간격으로 방사선 교합사진을 촬영하였고 9주째 발거한 치아의 치근단 부위를 입체현미경으로 관찰하였다. 치근항원에 대한 자가항원의 역가 변화를 측정하여 다음과 같은 결과를 얻었다.

자가항체의 역가는 치아가 함입되면서 즉시 감소하였다가 1주후 다시 증가하였으며 4주째 최고수준에 도달한 다음 다시 지속적으로 감소하였다. 흡수 중인 치근을 발거한 직후 급격히 증가하는 양상을 보였다. 치근 항원과 반응시킨 혈청내에서는 항원-항체 반응이 거의 나타나지 않았으며 관련없는 박테리아 항원과 반응을 시킨 혈청에서는 활성도가 동종 치근 항원에 대한 자가항체의 활성도와 비슷하게 나타났다. 방사선 교합사진 상에서는 육안으로 구별할 수 있는 차이가 거의 없었으나 입체현미경하에서는 치근단 부위의 다양한 흡수 양상을 관찰할 수 있었다.

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Key words : *Tooth Root Antibody, Root Resorption, Orthodontic Tooth Movement, ELISA*