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Humoral immune response of patients with dental trauma and consequent replacement resorption

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Abstract – Replacement dental resorption may be a consequence of trauma and may cause dental transplants or reimplants to fail. Previously, we demonstrated the participation of the immunopathological response in inflammatory dental resorption. The induction mechanisms of the two types of dental resorption are well known to be different. The aim of the present study was to observe the immune response of patients who suffered dental trauma with subsequent replacement dental resorption. Four patients with replacement radicular resorption and four healthy individuals with no evidence of radicular resorption participated in the study. The results of ELISA demonstrated that serum from patients with replacement dental resorption contained larger amounts of IgG and smaller amounts of IgM anti-total human-dentin extract and anti-fractions of extract than did serum from control individuals. These results signal the hypothesis that dentin is immunogenic and the serological profile of patients with replacement dental resorption may be identified through biochemical analysis of their blood. Precise screening by this method may allow early diagnosis of dental resorption before it becomes visible radiographically.

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Dental resorption brought on by traumatic lesions causing alveolar-dental ankylosis (1) is a frequent clinical problem that often limits the use of transplants and reimplants. It is not presently possible to control resorption, because its regulatory mechanisms and mode of induction are incompletely understood. Advances in this field would have immediate clinical repercussions in all fields of odontology, by establishing protocols for early detection and treatment of dental resorption.

Previous studies have established that dentin has immunogenic potential, because antibodies are produced in rabbits immunized with dentin extract, and the sera of these animals can recognize its different fractions. In addition, increases in serum levels of IgG and IgM were observed in patients with inflammatory dental resorption, and it was possible to trace a unique serological profile and possible marker for the process, associating an immunopathological mechanism with its etiopathogeny (2, 3).

Our study was based on the hypothesis that dentin may act as a sequestered antigen (4). During dentinogenesis, the crown dentin is protected by recently formed enamel as well as the external dental epithelium, stellate reticulum, stratum intermedium, and ameloblasts. The radicular dentin is protected by Hertwig's epithelial root sheath, by the intermediate cementum, and, after fragmentation of the sheath, by the cementoblasts and cementum. Because they are isolated from the immune system during development of natural tolerance, when the

dentin proteins are exposed they may provoke a response against the organisms' own components, i.e. auto-immunity, one of the forms of immunopathological reaction. Of the dentin proteins, there are two, which have been isolated only from dentin and are considered to be specific: dentin phosphoprotein (DPP, also called phosphophoryn) and dentin sialoprotein (DSP) (5).

Since 1964, when Coburn and Henriques (6) commented in the general meeting of the IADR on the necessity of detecting the presence of antibodies or sensitization in the host in experimental dental transplants, the possible immunogenic potential of dental structures (7–14) and their action as sequestered antigens (15–17) have been much discussed. However, King, Courts and collaborators (18–20) initiated actual investigations of the relationship between antibodies and dental resorption. Their works were followed by other investigators, who also studied the humoral (21) and cellular (22) immune responses in animals.

The mechanisms of induction of the two types of dental resorption, inflammatory and replacement are clearly different. The purpose of this investigation was to elucidate, in preliminary fashion, the humoral immune response of patients who suffered dental trauma with subsequent replacement resorption.

Materials and methods

Sera were collected from a total of four patients (between 16 and 19 years old) who showed replacement radicular resorption following dental trauma and who were under orthodontic treatment at the time of this study. The patients were selected on the basis of data from their histories and clinical and radiographic examinations (Fig. 1). Four healthy individuals (ages between 15 and 25), age and gender matched to the study population and with no evidence of radicular resorption, were also selected. None of them had been treated with antibiotics during the 3 months prior to collection of the material. All of the participants (or their guardians, in the case of minors) freely gave their informed consent to undergo the procedure. The Ethics Committee of the Bauru Dental School, University of São Paulo, approved the study.

The human-dentin protein extract was obtained through a modification of the technique described by Wheeler and Stroup (21), in which the dentin of intact extracted molars was drilled out by high-speed bit, oven-dried, placed in a demineralizing solution of guanidine-5 M HCl containing 10% EDTA at pH 5.0, and then centrifuged twice, dialyzed, and lyophilized. The dentin extract was fractioned by HPLC, using a *Hydropore SEC*

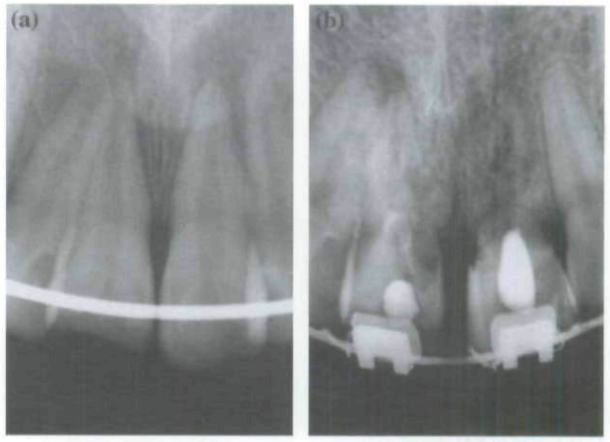

Fig. 1. Region of upper incisors in one of the patients, who suffered dental trauma: (a) soon after the accident when he sought orthodontic treatment and (b) 34 months after beginning orthodontic treatment, when replacement radicular resorption was diagnosed.

10 × 250 mm column and a *Dynamax* Fraction Collector, both from *Varian Inc.*, USA.

In the ELISA assays, the dentin extract or its fractions were attached to 96-well-microtiter plates via 24-hour absorption at 4 °C. The sera were diluted 1:50 and the peroxidase conjugated human anti-IgG or anti-IgM was diluted 1:15 000. The substrate consisting of *ortho*-phenylenediamine was added to each well. Enzyme activity was then quantified by spectrophotometric analysis at 492 nm. All assays were performed in duplicate, and were repeated.

Results

Sera IgG and IgM anti-extract and anti-fractions of dentin proteins from patients with replacement resorption

Serum IgG specific for the human-dentin extract and its fractions was present. The arithmetic mean for the reading at 492 nm, expressed in OD, was higher for the groups of patients with replacement radicular resorption, compared to the controls, except for the approximately 44 kDa MW fraction, which gave a similar result (Fig. 2a). The differences were statistically significant for the fractions with MW approximately 150, 78, 55 and 33 kDa (Fig. 2a*). For IgM, patients with replacement resorption showed lower or equal levels (MW ~33 kDa) than the patients of the control group (Fig. 2b), although the difference was statistically significant only for the MW ~62 kDa fraction (Fig. 2b*). In general, the readings from the IgG assays were lower than for IgM assays, especially for the controls with no resorption (Fig. 2a and b).

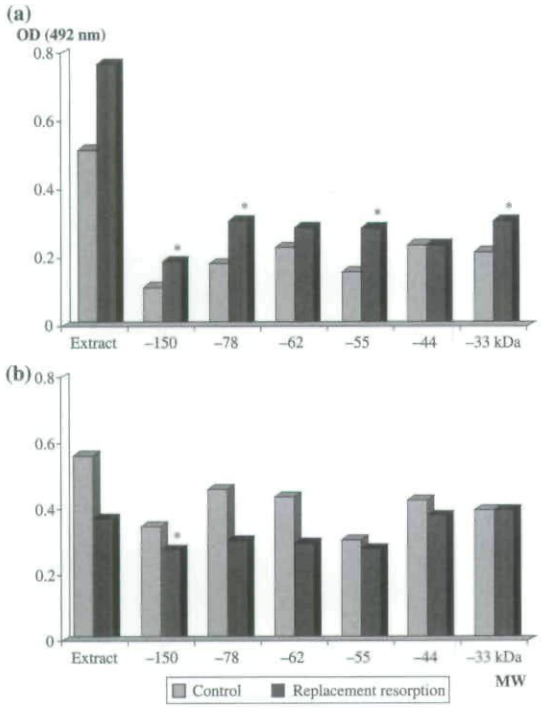

Fig. 2. Arithmetic means of sera antibodies specific for human-dentin extract and its fractions, detected by ELISA from patients with replacement resorption and from controls. In (a) IgG antibodies and in (b) IgM antibodies. *Statistically significant difference from the control group ($P < 0.05$; Student's *t*-test). Standard deviations (SDs) were at most 20% of the value of the mean.

Discussion

Previous studies have identified a unique serological profile, which is a possible identifier of the process of inflammatory dental resorption (2, 3). Since the biological model of the mechanism of replacement dental resorption differs from the model for inflammatory resorption, we investigated, in a preliminary manner, how replacement resorption behaves.

Levels of serum IgG specific for total human-dentin extract and its fractions were higher in the patients with resorption than in the controls (Fig. 2a). Replacement radicular resorption occurred in tortuously irregular areas, which multiplied the contact interface of the dentin tissue with the cells associated with the immune response and with osteoclasis. The reactions were presumably soon exacerbated. The fractions were selected for the possibility that they might correspond to intact human DPP and its three subunits (23). Among the responses to the fractions analyzed, the amount of IgG specific for the MW ~150 kDa fraction in the patients with resorption was almost half the amount of IgG specific for the MW ~78 kDa fraction, showing that the immune response was heterogenous.

The IgM levels in patients with replacement resorption were not statistically different from levels in the controls (Fig. 2b). Furthermore, in general the assays for IgG showed lower values than for IgM, especially in the controls not undergoing resorption (Fig. 2a and b). This apparent presence of dentin-reactive antibodies in the controls can be explained by the indexes of dental resorption described in the general population, which, although variable, can reach extremes of up to 100% of cases (24). Therefore, periods of immunogenic induction, although brief, may be occurring constantly.

Because these preliminary data included only a small number of samples, the results are certainly open to question. However, the data do tend to indicate that dentin is not recognized as self by the immune system (2, 3). In order to interpret the immunopathological events in dental resorption, it must be emphasized that none of the subject individuals in the study showed any caries or other factors that would cause them to produce anti-dentin antibodies. Moreover, sequestered antigens, besides being 'hidden' in the dentin, are incorporated in a mineralized matrix, which reduces their exposure to the components of the immune response and thus limits their direct inductive capacity (4). Through concomitant analysis of the IgG and IgM levels it was possible to trace a serological profile and also a potential identifier for the process of replacement dental resorption. This profile and identifier differentiated replacement resorption from inflammatory resorption, which showed larger quantities of IgG, except for MW ~44 and 33 kDa, with a corresponding sligh increase in IgM production (3).

Precise delineation of this process may lead to a biochemical diagnosis for dental resorption and its identification at early stages, before it can be diagnosed by visual imaging techniques such as radiography. Early diagnosis will allow prompt intervention and therapy, making possible more successful transplants and reimplants, allowing intentional reimplants, and treatment of traumas with no loss of teeth, preventing greater damage and making dentistry more efficient and beneficial for the patient.

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