

Case report

Immunologic evaluation of dental patient with history of hypersensitivity reaction to sodium hypochlorite

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Abstract – A 12-year-old girl, with a previous history of bronchial reaction and contact dermatitis to sodium hypochlorite, was referred for root canal treatment. Complete immunologic evaluation revealed a mild hypersensitivity condition, as it was assessed by the RAST investigation to different allergens and the DTH reactivity expressed through migration inhibition test. The absence of a serious immunologic dysregulation in the patient's immunologic profile justified the term 'non-allergic hypersensitivity' to sodium hypochlorite to describe the condition.

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Sodium hypochlorite is by far the most widely used irrigant in endodontic therapy (1, 2). The potential of this solution to cause immune mediated reactions or to immerge on the basis of a disturbed immune status has not been studied extensively. It has been postulated to cause hypersensitivity reactions based on clinical observations only (3, 4).

The present case report presents a patient with a history of sodium hypochlorite reaction and asks for immunologic background information in order to go a step further in interpreting such reactions from the immunological point of view.

The authors present a patient with a history of sodium hypochlorite reaction who underwent an extensive immunologic investigation on humoral and cellular immune responses to elucidate whether her medical and dental history is a result of an autoimmune, allergic or hypersensitivity disorder.

Case Report

Patient history

A 12-year-old girl presented with pain caused by a cariously exposed mandibular right first molar. Pulpitis was diagnosed and root canal treatment was planned. The medical history revealed bronchial hyperactivity and contact dermatitis reactions to household bleach and to the environment of indoor swimming pools which are known to contain sodium hypochlorite. The past dental history revealed that the patient had been receiving routine dental treatment but never endodontic treatment.

Pulp extirpation and instrumentation was performed. Isotonic saline was used as an irrigant and RC-Prep as a chelating agent. Root canals were temporarily dressed with calcium hydroxide and the patient was referred to the Immunology Department

for further evaluation of the alleged reactions to sodium hypochlorite. Endodontic therapy with lateral condensation of gutta-percha and Grossman's sealer was completed two weeks later when all laboratory results were available.

Immunological study

Serum Immunoglobulins levels

IgA 1.23 g/L (normal values 0.85–4.50 g/L), IgG 14.95 g/L (normal values 8.00–17.00 g/L), IgM 2.78 g/L (normal values 0.60–3.70 g/L), IgE 28.3 IU/mL (normal values 0.00–100 IU/mL).

Complement components

C3c 1.129 g/L (normal values 0.50–0.90 g/L), C4 0.371 g/L (0.10–0.40 g/L).

Total Immunocomplex levels

1.145 mg/mL (normal values 0.00–1.50 mg/mL).

Autoantibody presence

Antinuclear antibodies (Hep-2 substrate, indirect immunofluorescence technique): positive homogenous pattern (positivity was detected up to serum dilution 1/640), plus positive speckled pattern (positivity was detected up to serum dilution 1/320). Homogenous pattern of immunofluorescence implies anti-DNA autoantibodies. In order to investigate the possibility of anti-dsDNA antibody presence in the patient's serum, a test for anti-dsDNA antibodies was performed on Crithidia Luciliae substrate which proved to be negative. Additionally, since the speckled pattern of immunofluorescence reflects antibodies against small nuclear RNPs (Ro/SS A, La/SS B, Sm, Jo, U₁-RNP), a more detailed screening test for the identification of specific anti-RNP antibodies was performed using an immunoblotting technique and proved to be negative as well.

Antiphospholipid antibodies (ACA, ELISA technique): IgG ACA were 6.1 IU where as IgM ACA were found increased at 17.8 IU (normal values ranging below 12 IU and 6 IU respectively).

Antinuclearcytoplasmic antibodies (ANCA, ELISA technique): Both types of ANCA studied were found to be normal. PR-3 ANCA were 1.0 IU and MPO ANCA 6.3 IU (normal values ranging below 7 respectively).

Although total *IgE levels* were normal, an investigation for the presence of specific anti-IgE antibodies against a panel of plant, animal and fungal allergens was performed and revealed a mild to medium scale reactivity to olive, fungi and acari dermatophagoides, pteronissinus/farinae, using a RAST immunosorbent assay.

Peripheral blood T-cell cultures

Peripheral blood was collected in heparin. Lymphocytes were separated in a lymphocyte separation medium (Gibco, Pasley, Scotland). Cells were washed in culture medium and resuspended in RPMI 1640 MEDIUM (Gibco) plus 10% fetal calf serum (FCS, Gibco) plus 10 µg/mL gentamycin at a final concentration of 1×10^6 viable cells per mL of culture medium (viability of cells was assessed by means of ethidium bromosulfate-acridine orange staining, Sigma, St. Louis, MO, USA). Cells were cultured in triplicate in the presence of PHA (Sigma), a T-lymphocyte mitogen, at a concentration of 5 µg/mL of culture medium as well as different dilutions of the reference solution (1% NaOCl) for 72 h, at 37°C in a humidified atmosphere with 5% CO₂. At the end of the culture time, the cultures were collected, centrifuged and the supernatants were immediately frozen at -70°C.

Cytokine levels were measured in culture supernatants: Interleukine-2 (IL-2) and Interleukine-10 (IL-10) levels were measured with a microplate ELISA immunoassay. Dynamic range for IL-2: 31.2–2000 pg/mL and for IL-10: 7.8–500 pg/mL. Results are shown in Table 1.

A migration inhibition test was performed in the presence of the above mentioned dilutions of sodium hypochlorite. The method of Bendixen & Soborg (5) was used. The method is based on the principle that when monocytes sensitized by an antigen come into a secondary contact with the same antigen, they are inhibited from migration. The migration inhibition index has found application in delayed hypersensitivity reactions and/or in autoimmune conditions.

Peripheral blood was collected in heparin and left to sediment for one and a half hours, at 37°C in a humidified incubator. Then, the leukocyte rich plasma was removed, suspended on culture medium Tc-199 (Flow Laboratories, London, UK) and centrifuged to separate mononuclear cells. Afterwards, mononuclear cells were collected, washed, adjusted to a concentration of 10^7 /mL and placed into capillary tubes, sealed from one end. Then, capillary tubes were centrifuged, cut 1 mm below cell surface and placed in chambers (1 capillary/chamber), which were then filled up with culture medium in the presence of NaOCl (test chambers) or not (control

Table 1. Cytokine levels in culture supernatants

Cytokine	1/10	1/20	1/100
Interleukine -2	935	819	764
Interleukine -10	51	30	41

IL-2=31.2–2.000 pg/mL.

IL-10=7.8–500 pg/mL.

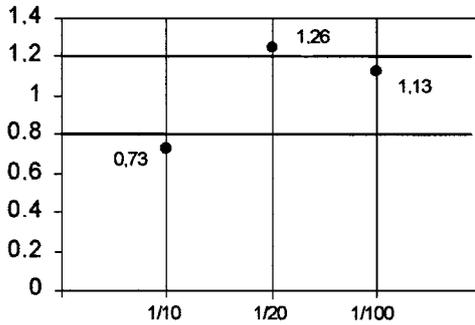


Fig. 1. Values of migration inhibition test.

chambers). Chambers were left to be incubated for 24 h at humidified atmosphere, at room temperature. The next day the image of the capillary tubes and migrated cells was projected on a viewing screen and the area of migration was traced on paper and calculated from the paper utilizing a planimeter. The amount of migration, with or without the effect of antigen (NaOCl) was measured as follows: leukocyte migration inhibition = mean migration with antigen / mean migration without antigen. Each test was performed in triplicate. Three control chambers without antigen and three test chambers containing the relevant antigen were used. The final value was taken to be the mean value from the three test chambers compared with the three control chambers. Results are shown in Figure 1.

Discussion

The patient serum investigation showed no abnormal findings concerning the conventional laboratory tests (Igs, Ics, C3c, C4, RFs). Serum autoantibody study revealed positive ANA presence, but detailed research for the detection of any autoimmune disease specific antibody was negative. It should be noted that anti-dsDNA and anti-Sm antibodies characterize systemic lupus erythematosus patients, anti-Ro and anti-La antibodies predict Sjögren syndrome, anti-Jo antibodies are found in dermatomyositis-polymyositis and U₁RNP belong to the diagnostic criteria of mixed connective tissue disease (5). The patient did not present the clinical picture of systemic autoimmune disease. Nor did she reveal serum autoantibody specificity for suspected autoimmune diseases. Relevant diagnoses should therefore be excluded at the moment.

On the other hand, in the allergiological study, despite the normal total IgE levels found in the patient's serum, a mild to medium serum reactivity was noticed against the different allergens tested. This finding, in combination with the delayed-type hypersens-

itivity reaction revealed by the migration inhibition test in the presence of sodium hypochlorite, expresses in some degree the sensitization of the patient's lymphocytes because of her exposure to sodium hypochlorite. A possible connection to the patient's hypersensitivity condition, as it was assessed by the RAST investigation and the DTH reactivity expressed through the migration inhibition test, could exist, but cannot be established on clinical grounds at the moment. For hypersensitivity reactions to external substances without involvement of an obvious immunologic mechanism, the term 'non-allergic hypersensitivity' has been used (6), and we think, applies to the patient described here.

Besides, cytokine measurements of IL-2 and IL-10 in the supernatants from PHA plus NaOCl cultured peripheral lymphocytes showed no cytokine dysregulation, since IL-2 is the fundamental cytokine regulating Th₁ pattern of immune responses and IL-10 is crucial for the Th₂ pattern of response (7). These findings also justified the term 'non-allergic hypersensitivity'. It should be emphasized that if a serious allergic immune reactivity were part of the patient's immunologic profile, IL-10 should have increased and been followed by a decrease in IL-2 levels which indicates a Th₂ pattern of immune response (8). These normal cytokine findings also explain why the patient, though presenting ANA positivity, did not develop a specific autoimmune disease profile, since in systemic autoimmune disease, cytokine dysregulation should be expected.

In conclusion, the history and the immunological study of the patient discussed here emphasize the following points in the clinical approach of similar cases. First, patients with a history of atypical allergic reactions and probable hypersensitivity should undergo a basic immunologic evaluation before sodium hypochlorite is used in endodontic treatment. In case of findings that give implications for autoimmune and/or allergic disease by entities closely related to each other and both affecting mouth pathology, they should be followed up regularly and alternative substances should be used in endodontic treatment.

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