Distribution of Dipeptidyl Peptidase IV in Patients with Chronic Tonsillitis

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In the pathogenesis of recurrent tonsillitis (RT) and tonsillar hypertrophy (TH), different immunological mechanisms are involved. Dipeptidyl peptidase IV (DPP IV) and aminopeptidase N (APN) participate in the regulation of the immune response during inflammation. In this study, the localization of DPP IV and the enzymatic activities of DPP IV and APN in 32 patients, 13 with RT and 19 with TH, who underwent tonsillectomy were investigated. The localization of DPP IV in tonsils was studied using histochemical and immunohistochemical methods. The enzymatic activities of DPP IV and APN in tonsillar lymphocytes and the patients’ sera were determined kinetically at 37°C using Gly-Pro-p-nitroanilide (for DPP IV) and Ala-p-nitroanilide (for APN) as chromogenic substrates. In samples from both RT and TH patients, DPP IV was found to localize mainly in extrafollicular areas of tonsillar tissue in a pattern corresponding to the T-cell distribution. Significantly higher (P < 0.001) levels of DPP IV and APN activities in sera from patients with TH than in sera from patients with RT were found. A correlation of DPP IV activities in sera and tonsillar lymphocytes from patients with TH was also found (r = 0.518; P < 0.05). Moreover, the results show that DPP IV and APN activities in sera decreased significantly with age. Tonsillar lymphocytes demonstrated a wide range of DPP IV and APN activities, without significant differences between the investigated groups. The results of this study show that the localization of DPP IV does not depend on the type of tonsillitis, whereas the variety in levels of DPP IV and APN activities in sera of patients with TH and RT suggests different patterns of participation of antigen-stimulated tonsils in the immune system.

Palatine tonsils are a primary site of bacterium- or virus-induced immune system activation due to their exposure to both alimentary and airborne pathogens. Previous studies of recurrent tonsillitis (RT) and tonsillar hypertrophy (TH) showed differences between these conditions in relation to the number of immunoglobulin-producing cells (26), the lymphocyte cell cycle (25), the expression of some cytokines (1), morpho-

DPP IV is a membrane-bound serine protease present in a variety of mammalian cell types, primarily on the apical surfaces of epithelia and acinar cells and also on capillary endothelial cells of different organs, including the liver, spleen, lung, and brain (11). It is a type II glycoprotein that releases X-Pro and X-Ala dipeptides from the N termini of different polypeptides, including hormones, cytokines, chemokines, and neu-

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MATERIALS AND METHODS

Tissue specimens. The study was performed with 19 children (aged 5 to 12 years) with the clinical diagnosis of TH and 13 persons (aged 10 to 58 years) with the clinical diagnosis of RT who underwent tonsillectomy at the ear, nose, and throat clinic at the Clinical Center Nis, Nis, Serbia. Tonsils were classified on the basis of clinical diagnoses of TH and RT according to the method of Surjan et al. (26), and classifications were confirmed by pathohistological examination of paraffin sections stained by the hematoxylin-eosin method.
Immediately after tonsillectomy, each tonsil was washed in saline solution to eliminate any possible blood contamination. One part of the tonsillar tissue was embedded in paraffin and used for morphological examinations, a second part was used to isolate tonsillar lymphocytes, and the rest of the tissue was frozen in liquid nitrogen and cut into 5-μm-thick sections by using a cryostat (Minotome; IEC).

Blood samples were taken from patients before tonsillectomy. After clotting, the sera were separated and kept at −20°C until the determination of enzymatic activity. Control blood samples were obtained from 15 clinically healthy persons aged 37 to 62 years.

Histochemistry of DPP IV. The enzymohistochemical determination of DPP IV activity in tonsillar tissue was performed according to the method of Lojda (17). Briefly, 4 mg of Gly-Pro-4-methoxy-b-naphthylamide (Sigma, Germany) was dissolved in 500 μl of N,N-dimethylformamide (ICN, OH). Fast blue B salt (10 mg; Gurr’s, London, England) was dissolved in 9.5 ml of Dulbecco phosphate-buffered saline (PBS; pH 7.2) mixed with Gly-Pro-4-methoxy-b-naphthylamide solution, and the mixture was filtered through a 0.22-μm-pore-size membrane filter. The sections were incubated in this mixture for 10 min at room temperature, and the reaction was stopped by extensive washing with PBS. Serial sections were counterstained with Mayer’s hematoxylin. For the negative control, the mixture was used without a substrate.

Immunohistochemistry analysis of DPP IV. Samples for immunohistochemistry analysis were prepared in the same way as those for histochemistry analysis. The sections were then fixed in cold acetone at −20°C and incubated in PBS (pH 7.2) with 5% fetal calf serum for 20 min. The slides were washed three times with PBS and incubated with anti-DPP IV monoclonal antibody (clone MA261; 0.1 mg/ml [Bender MedSystems, Austria]). After 1 h of incubation, the slides were washed again with PBS and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Becton Dickinson, San Jose, CA) for 60 min at room temperature. The slides were washed extensively with PBS and mounted for examination under a fluorescence microscope (23).

Serum DPP IV and APN activities. DPP IV activity was determined by using 96-well plates with 0.8 mM Gly-Pro-p-nitroanilide as the chromogenic substrate in 20 mM TRIS-HCl buffer, pH 8.3 (9). APN activity was determined with buffer containing 3 mM KCl, 140 mM NaCl, 1.5 mM Na2HPO4, 9 mM NaH2PO4, and 1 mM MgCl2, pH 7.8, by using 1.5 mM alanine-p-nitroanilide (Merck, Germany) as the chromogenic substrate. After incubation (15 min at 37°C), the activity of the enzyme relative to the optical density at 405 nm was read kinetically by using Multiskan Ascent (Thermo LabSystem, Finland). One unit of activity was defined as the amount of enzyme which cleaved 1 nM substrate per min under the conditions applied.

**RESULTS**

The clinical diagnoses of RT and TH were confirmed by pathohistological analyses for both investigated groups.

Localization of DPP IV in tonsillar tissue. By using histochemical and immunohistochemical methods, DPP IV activity was demonstrated mainly in extrafollicular areas, corresponding to the distribution of T lymphocytes (Fig. 1). No difference in the localization of DPP IV activity between samples from RT and TH patients was observed. Immunofluorescence analysis detected low levels of DPP IV activity in the germinal centers of a few lymphoid follicles in samples from RT patients (Fig. 1F). Strong expression of DPP IV in blood vessels underlying tonsillar surface epithelia was found (Fig. 1B and F).
We also found strong DPP IV activity in the tonsillar crypts of some tonsils with TH (Fig. 1H).

**DPP IV and APN enzymatic activities in sera and on tonsillar lymphocytes.** DPP IV and APN activities in TH patient sera were significantly increased ($P < 0.001$) compared to those in RT patient sera (Table 1).

There were no significant differences in the DPP IV and APN activities on lymphocytes isolated from tonsils, although the median value for samples from RT patients was higher than that for samples from TH patients (Table 2).

A significant correlation ($r = 0.518; P < 0.05$) between levels of DPP IV activity in sera and on tonsillar lymphocytes from patients with TH was found (Fig. 2), whereas there was no correlation for patients with RT. A significant negative correlation between DPP IV activities in sera ($r = -0.700; P < 0.001$), as well as APN activities in sera ($r = -0.550; P < 0.01$), and ages of TH and RT patients was found (Fig. 3 and 4), whereas no correlation for the control group was observed.

**DISCUSSION**

There are very few papers dealing with the distribution of DPP IV in tonsillar tissue, particularly in patients with RT and TH. The histochemical demonstration of this enzyme, using Gly-Pro-4-methoxy-β-naphthylamide as the substrate, in lymphoid tissue and human tonsils was documented in previous studies during the 1980s. DPP IV activity was observed in extrafollicular areas (T-cell-rich zones) in normal and reactive tonsils and lymph nodes, while in lymphoid follicles (B-cell-rich zones), no positive reaction was found (21).

In accordance with previous findings, our study confirmed that DPP IV is located predominantly in the extrafollicular areas in both RT and TH patients. DPP IV cell surface expression on tonsillar lymphocytes in extrafollicular areas indicates the involvement of these lymphocytes in the immune response through the modulation of B-cell functions, as well as the synthesis of different cytokines (1). Using the classical histochemical method, we could not find DPP IV expression in lymphoid follicles, although immunofluorescence analysis with CD26 monoclonal antibody demonstrated moderate fluorescence in the germinal centers of some follicles in samples from RT patients. Considering that CD26$^+$ T cells have a potential role in T-helper-cell functions (20), we proposed that this DPP IV activity may correspond to the T helper lymphocytes inside lymphoid follicles as well. The migration of CD4$^+$ cells to the germinal center allows direct contact, providing B cells with a signal for differentiation into immunoglobulin-producing cells.

In this study, we found significantly elevated levels of DPP IV activity in TH patient sera compared to those in RT patient sera. Two possible explanations for these results could be proposed. Firstly, the increase of DPP IV activity in the TH group may be associated with the fact that the patients with TH were younger than the patients with RT. This explanation is in accordance with the observation of Durinx et al. (9), who demonstrated decreased DPP IV activity in serum with ageing. The second explanation may be the fact that the tonsils and peripheral blood of children have a greater number of activated lymphocytes than those of adults, especially CD4$^+$ and

**TABLE 1. Activities of DPP IV and APN in sera**

| Group | DPP IV activity (U/liter) | APN activity (U/liter) |
|-------|--------------------------|------------------------|
|       | Mean ± SD                | Median                 | Range      | Mean ± SD                | Median                 | Range      |
| Control| 22.4 ± 5.8               | 16.2–35.7              |            | 26.5 ± 6.9               | 13.9–41.6              |            |
| TH patients | 39.8 ± 6.5$^a$          | 29.9–50.9              | 28.5 ± 3.8$^b$    | 20.6–35.6              | 14.3–24.2              |            |
| RT patients | 19.2 ± 2.7               | 14.3–24.2              |            | 22.3 ± 5.0               | 16.7–34.7              |            |

$^a P < 0.001$ versus control.

$^b P < 0.001$ versus RT group.

**FIG. 2. Correlation between values for DPP IV activity in sera and values for DPP IV activity on tonsillar lymphocytes from the TH group.**

**TABLE 2. Enzyme activities of DPP IV and APN on tonsillar lymphocytes**

| Enzyme | Group | Activity (nM/h/10^6 cells) |
|--------|-------|---------------------------|
|        |       | Mean ± SD | Median | Range                |
|        |       | DPP IV    |       |                      | APN                       |                      |
|        | TH patients | 10.7 ± 5.9 | 7.8    | 3.9–26.1             | 24.7 ± 7.1               | 23.8                | 11.6–42.3 |
| RT patients | 12.3 ± 3.7 | 14.3    | 5.3–15.8            | 22.1 ± 6.0               | 21.3                | 14.9–33.0 |

**FIG. 3. Correlation between DPP IV activities in sera and patients’ ages ($r = -0.700; P < 0.001$).**

**FIG. 4. Correlation between DPP IV activities in sera and patients’ ages ($r = -0.550; P < 0.01$).**
CD8+ T lymphocytes (16, 18), which may be a source of the serum form of DPP IV. Indeed, Lojda (17) demonstrated that DPP IV activity occurs in 39% of peripheral blood lymphocytes and exclusively in T lymphocytes.

The correlation of the DPP IV activities in sera with tonsillar lymphocytes from TH patients suggests that the immune response in hypertrophied tonsils with an increased number of activated lymphocytes affects the immune system during the evolution of the disease (5).

There is strong evidence that DPP IV is involved in the metabolism of neuropeptide Y and peptide YY, important for the control of feeding and energy homeostasis (19). Considering that children with chronic tonsillitis have a loss of appetite (which is corrected after tonsillectomy), it may be speculated that high levels of DPP IV in the sera of patients with TH may be involved in the regulation of this process.

Since APN (CD13) is often localized with DPP IV, we measured APN activities on tonsillar lymphocytes as well as in patients' sera. It was reported previously that APN activity is present in tonsils and also on the surfaces of resting and stimulated lymphocytes (2, 14). The expression of APN, associated primarily with major histocompatibility complex class II expression, suggests that there is a role for APN in antigen processing (12).

In the sera from our patients with TH, the levels of APN activity were elevated, but in the sera from the other groups, the correlation of the DPP IV activities in sera with the distribution of T cells and the increased number of activated circulating lymphocytes which express DPP IV strongly correlates with the distribution of T cells and does not depend on the type of tonsillitis. However, the variety in the activities of DPP IV and APN in sera and tonsillar lymphocytes from TH and RT patients suggests different participation of the tonsils in the immune system during the course of chronic tonsillitis.

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In conclusion, the similar levels of expression of DPP IV activity clearly demonstrated in samples from RT and TH patients indicate that, in inflamed tonsils, the localization of DPP IV strongly correlates with the distribution of T cells and does not depend on the type of tonsillitis. However, the variety in the activities of DPP IV and APN in sera and tonsillar lymphocytes from TH and RT patients suggests different participation of the tonsils in the immune system during the course of chronic tonsillitis.
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