IL-12 receptor β2 and CD30 expression in paranasal sinus mucosa of patients with chronic sinusitis


**ABSTRACT:** The aetiology of chronic sinusitis is still poorly understood. The expression of T-helper 1 (Th1) and T-helper 2 (Th2) cell markers, interleukin (IL)-12 receptor β2 subunit (IL-12Rβ2) messenger ribonucleic acid (mRNA) and CD30, respectively, were investigated in the paranasal sinus mucosa of patients with chronic sinusitis in an attempt to elucidate the involvement of Th1 and Th2 cells in this disease.

Anterior ethmoidal mucosae were surgically obtained from two groups of patients with chronic sinusitis: those who had allergic rhinitis (allergic group, n=11) and those without allergy (nonallergic group, n=11). IL-12Rβ2 mRNA was quantified by means of the reverse transcription polymerase chain reaction, and CD30-positive cells were examined immunohistochemically.

Both IL-12Rβ2 mRNA and CD30 were expressed in the sinus mucosa of the allergic and nonallergic groups. The proportion of mononuclear cells which were CD30-positive in the sinus mucosa was significantly greater in the allergic than in the nonallergic group. The expression levels of IL-12Rβ2 mRNA were virtually equivalent in both groups.

These results suggest a T-helper 2-dominated mucosal reaction in the allergic compared to the nonallergic group, and indicate T-helper 1 activity in the sinus mucosa of both groups. The ubiquity of T-helper 1 cells suggests that they play a role in maintaining local mucosal defences against foreign antigens, which continually enter the upper respiratory tract.

superfamily, is preferentially expressed in Th2 cells. In the present study, the expression of IL-12Rγ2 mRNA and CD30 in the paranasal sinus mucosa of patients with allergic and nonallergic sinusitis was examined in an attempt to elucidate the involvement of Th1 and Th2 cells in this disease.

Materials and methods

Reagents

Protease type XIV from Streptomyces griseus, collagen type IV from human placenta, dianisobenzidine tetrahydrochloride (DAB) and bovine serum albumin (BSA) were bought from Sigma Chemical Co. (St Louis, MO, USA). Oligo-deoxynthymidine (oligo-dT) primer and reverse transcriptase (SUPER SCRIPT premplification system for first strand complementary deoxyribonucleic acid (cDNA) synthesis), Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F-12 (F-12) were obtained from Gibco/BRL Life Technologies (Rockville, MD, USA). Ultroser G serum substitute (USG) was purchased from BioSepra Inc. (Marlboro, MA, USA). Mouse antihuman CD30 mouse monoclonal antibody (clone Ber-H2) was bought from Dako (Glostrup, Denmark). Normal horse serum, biotinylated horse antioimmunoglobulin G and avidin–biotinylated peroxidase complexes (Vectastain Elite ABC reagent) were purchased from Vector Laboratory, Inc. (Burlingame, CA, USA). Hansel solution (Eosinostain) was obtained from Torii Co. (Tokyo, Japan). Thermostable Taq deoxyribonucleic acid (DNA) polymerase (TaKaRa ExTaq) and rhodamine-X-isothiocyanate (XRITC)-labelled oligonucleotide primers were provided by Takara Co. (Otsu, Japan). Normal mouse IgG1 was bought from Chemicon International, Inc. (Tecumela, CA, USA).

Patients

Samples were obtained from 22 patients with chronic sinusitis. The patients had had nasal obstruction and purulent nasal discharge for >3 months. They also showed purulence in the nasal cavity on rhinoscopic examination and soft tissue density shadow in paranasal sinuses including the ethmoidal sinus(es) on computed tomographic scanning. The patients were divided into two groups: those who had allergic rhinitis (allergic group) and those without allergy (nonallergic group). Patients in the allergic group met the following three criteria: 1) clinical symptoms of allergic rhinitis and/or anterior rhinoscopic findings characteristic of allergy, 2) eosinophils in the nasal smear, and 3) a positive radioallergosorbent test (RAST) for serum IgE directed against house dust mite. Four patients in this group also had bronchial asthma. A positive result in the nasal provocation test is important in the diagnosis of allergic rhinitis/rhinosinusitis. However, this test brings about unpleasant nasal symptoms, and was not performed in the present study. Patients in the nonallergic group showed normal levels of total serum IgE; no eosinophils in the nasal smear; negative RASTs for house dust mite and Japanese cedar, ragweed and orchard grass pollen and did not have symptoms of allergic rhinitis or bronchial asthma. The allergic group consisted of 11 patients, eight male and three female, ranging in age 16–71 yrs, with a mean age of 49.7 yrs. The nonallergic group consisted of 11 patients, five male and six female, ranging in age 18–72 yrs, with a mean age of 49.2 yrs. Normal control specimens were obtained from three patients with normal ethmoidal mucosa, two with tumours of the sphenoid sinus and the other with nasolacrimal duct obstruction.

Collecting samples

Patients did not receive antibiotics, antihistamines, antiallergic medicine or corticosteroids for ≥1 week before sample collection, and did not have acute upper respiratory tract infections at the time of surgery. Two pieces of anterior ethmoidal mucosa were obtained from each patient during sinus surgery. One was immediately frozen and stored at -80°C for reverse transcription (RT) polymerase chain reaction (PCR). The other was fixed with Zamboni’s fixative overnight at 4°C for immunohistochemical analysis. In addition, nasal polyps were obtained from patients in the nonallergic group for the culture of nasal epithelial cells, as described below.

Immunohistochemical procedure

The Zamboni-fixed mucosal specimens were dehydrated in serial concentrations (10–20% (w/v)) of sucrose solution, embedded in Tissue-Tek optimal cutting temperature (OCT) compound and sectioned at 5 µm thickness using a cryostat. The slides were soaked in 0.3% H2O2 in methanol for 10 min to block endogenous peroxidase, treated with 1.5% normal horse serum in 10 mM phosphate-buffered saline (PBS) at pH 7.2 for 30 min and reacted with 40 µg·mL−1 antihuman CD30 in PBS containing 1% BSA overnight at 4°C. The samples were treated with 5 µg·mL−1 biotinylated antioimmunoglobulin G in PBS containing 1.5% normal horse serum for 30 min, and incubated with avidin–biotinylated peroxidase complexes in PBS for 30 min. They were then developed in 0.03% DAB, 0.006% H2O2, 50 mM tris(hydroxymethyl)aminomethane (Tris)–HCl (pH 7.6) for 1 min, and counterstained with methyl green for 15 min. As a control, normal mouse IgG1 was substituted for antihuman CD30. Cryostat sections were stained with Hansel solution for 1 min in order to count infiltrated cells.

Cell counting

CD30-positive cells, neutrophils, eosinophils, mast cells and mononuclear cells in the epithelium, lamina propria and submucosal layer were counted using a light microscope at high magnification. Six to 10 fields were randomly chosen from each slide, and the results expressed in cells·mm−2.

Culture of nasal epithelial cells

Human nasal epithelial cells were cultured to serve as a negative control tissue in the RT-PCR for IL-12Rγ2 mRNA. Nasal epithelial cells were harvested from the nasal polyps and cultured according to YAMAYA et al. [15]
with minor modifications [16]. In brief, the polyps were incubated with 0.1% protease type XIV in PBS overnight at 4°C, and then vigorously agitated to dislodge epithelial cells. The epithelial cells were sedimented and resuspended in DMEM-F-12 (1:1) containing 2% USG supplemented with penicillin G, streptomycin, gentamicin and amphotericin B. The cells were then used for seeding a 24-well tissue culture plate coated with collagen type IV, and incubated in humidified 5% CO2/95% air for 5–10 days at 37°C until the cells grew confluently. The cultured cells were removed from the plate by treatment with 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid in PBS, and then subjected to RT-PCR.

Reverse transcription polymerase chain reaction for interleukin-12 receptor β2 subunit messenger ribonucleic acid

Total ribonucleic acid (RNA) was isolated from the tissue samples by guanidinium isothiocyanate extraction, using the Quick Prep total RNA extraction kit (Pharmacia, Uppsala, Sweden) according to the manufacturer’s protocol, and was quantified by measuring absorbance at 260 nm. One μg RNA was converted to first strand cDNA using an oligo-dT primer and reverse transcriptase for 50 min at 42°C according to the suppliers’ instructions. Oligonucleotide primers were synthesized based on the human cDNA sequences. The nucleotide sequences of the IL-12Rβ2 primers were designed as follows: forward primer, 5'-GCAGCTAGAGGACAGCAAGCCA-3' (bases 3,345–3,367) and reverse primer, 5'-GGTGTTGTA-CAGGCTGCTGTGAGT-3' (bases 3,614–3,636) with an amplified cDNA size of 292 bp. The nucleotide sequences for β-actin primers were designed as follows [17, 18]: forward primer, 5'-ATGGTCAAGAAGATGCTCTAT-3' (bases 180–200) and reverse primer, 5'-GTTAAGGCCAATCTGAAG-3' (bases 1,178–1,197) with an amplified cDNA size of 1018 bp. The primers were 5'-labelled using XRITC. One fiftieth of the cDNA synthesis reaction product was used as a template for the PCR at a final volume of 40 μL. PCR was performed using a thermo-stable Taq DNA polymerase in a programmable thermal cycler, Model 2400 (Perkin Elmer, Norwalk, CT, USA). For the semiquantitative analysis of IL-12Rβ2 mRNA, PCR was conducted for 28 cycles: 30 s at 94°C, 30 s at 50°C and 1 min at 72°C. For the amplification of β-actin, PCR was conducted for 25 cycles: 30 s at 94°C, 30 s at 62°C and 1 min at 72°C. An incubation for 30 s at 94°C preceded the first PCR cycle, and the final cycle was followed by an extension of 7 min at 72°C. PCR products were separated by electrophoresis on a 6% polyacrylamide gel, and the density of each band measured using a fluorescent image analyser (FMBIO II; Hitachi, Tokyo, Japan). Cultured human nasal epithelial cells were used as a negative control tissue. Results were expressed as the fluorescence ratio of IL-12Rβ2/β-actin for normalization.

Statistics

Values are expressed as mean±SEM, and statistical significance of differences was tested using Student’s t-test unless otherwise indicated. Differences were regarded significant at the level of p<0.05.

Results

Profiles of infiltrated inflammatory cells

The profiles of the infiltrated inflammatory cells in the mucosa are summarized in figure 1. There were no significant differences in numbers of eosinophils, neutrophils, mast cells and mononuclear cells between the allergic and nonallergic groups. Eosinophils were more numerous than neutrophils in both groups, consistent with previous observations [11, 19, 20]. Very few eosinophils and neutrophils were seen in the normal control mucosa.

Immunohistochemical localization of CD30

Immunoreactivity of CD30 was mainly localized in infiltrated mononuclear cells in the lamina propria (fig. 2). The average number of CD30-positive cells was 2.5-times greater in the allergic than in the nonallergic group (16.0±6.4 versus 6.2±1.8 cells.mm⁻²), but this difference was not statistically significant (fig. 3a). The proportion of mononuclear cells which were CD30-positive was significantly greater in the allergic than in the nonallergic group (1.20±0.36 versus 0.49±0.15%, p<0.05; fig. 3b).

Expression of interleukin-12 receptor β2 subunit messenger ribonucleic acid

Figure 4 shows the RT-PCR analysis of IL-12Rβ2 and β-actin. IL-12Rβ2 mRNA was detected in the sinus mucosa of all the subjects examined, but not in cultured human nasal epithelial cells. The fluorescence ratios of IL-12Rβ2/β-actin, which represent the expression levels of IL-12Rβ2 mRNA, were 0.53±0.09 and 0.47±0.12 in the allergic and nonallergic groups, respectively (fig. 5). There was no significant difference between them. The fluorescence ratio of IL-12Rβ2/β-actin correlated significantly with the number of mononuclear cells, as shown in figure 6 (r=0.564, p<0.01). Results from the analysis of

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**Fig. 1.** Profile of infiltrated inflammatory cells in the paranasal sinus mucosa. Values are presented as mean±SEM. #: allergic group; #: nonallergic group; □: control. E: eosinophils; N: neutrophils; MC: mast cells; MNC: mononuclear cells, *p<0.05.
normal ethmoidal mucosae were not reproducible, probably because of the limited size of the samples and the trace amount of total mRNA isolated, and are not presented here.

**Discussion**

The present study is the first report demonstrating the existence of IL-12Rβ2 mRNA in vivo and CD30 in the upper respiratory tract.

CD30 was originally defined as a tumour-specific antigen that was recognized by the Ki-1 antibody raised against the Hodgkin and Reed–Sternberg cells of Hodgkin’s disease [21]. CD30 is also expressed in the tumour cells of lymphomas [22] and embryonal carcinomas [23]. Thereafter, it was discovered that CD30 is preferentially expressed in Th2 cells and is involved in signalling promoting the development of Th2 cells [14]. Although its expression is not exclusively in Th2 cells, high CD30 expression has been demonstrated in patients with Th2-dominant disorders such as chronic graft-versus-host disease, systemic sclerosis, Omenni’s syndrome, atopic dermatitis and bronchial asthma [24–26]. These observations suggest that the expression level of CD30 is an index of the Th2-dominant state. The present study revealed that the proportion of mononuclear cells that were CD30-positive in the sinus mucosa was greater in the allergic than in the nonallergic group (fig. 3). This suggests the presence of a Th2-dominated mucosal reaction in allergic group patients, which is in agreement with a previous report [11].

The human IL-12 receptor is composed of two β-type cytokine receptor subunits, IL-12Rβ1 and IL-12Rβ2. Although either alone has a low affinity for IL-12, the binding affinity is augmented >100-fold by the combination of the two subunits [27]. IL-12Rβ2 mRNA is selectively expressed in Th1 cells, and thereby participates in the differentiation of naive cells into Th1 cells [12]. The present study demonstrated that IL-12Rβ2 mRNA was present in the paranasal sinus mucosa of all the subjects examined, whereas it was not detected in cultured human nasal epithelial cells. Normal ethmoidal mucosa would have been an ideal control specimen in the present study. However, it was technically difficult to cleanly harvest filmy mucosa from the normal sinus. The size of the normal sinus mucosa obtained was, therefore, very limited, and results from the RT-PCR analysis of the trace amount of mRNA isolated were not reproducible. For this reason,
cultured human nasal epithelial cells were used instead, serving as a complete negative control.

The expression level of IL-12Rβ2 mRNA was virtually equivalent between the allergic and nonallergic groups (fig. 5). This implies that Th1 is activated in the sinus mucosa in the allergic group to the same degree as in the nonallergic group. The expression level of IL-12Rβ2 mRNA correlated significantly with the number of mononuclear cells (fig. 6). Considering that the majority of infiltrated inflammatory cells are mononuclear cells (fig. 1), it is likely that Th1 cells are ubiquitously distributed in the sinus mucosa. Because the nasal cavity and paranasal sinuses are continuously exposed to foreign antigens, it is reasonable that Th1-type cell-mediated immunity is activated for the purpose of local mucosal defence.

IL-12 is a multifunctional cytokine which induces commitment of naive T cells to the Th1 phenotype [28], and is known to play a key role in the promotion of Th1-type cell-mediated immunity. This cytokine is mainly produced by macrophages, B cells and dendritic cells [29]. DAVIDSSON et al. [30] have demonstrated the presence of IL-12 mRNA in the paranasal sinus mucosa of patients with chronic sinusitis. Interestingly, they found that IL-12 mRNA was expressed in allergic subjects as well as in nonallergic subjects [30], which is in accord with the present result concerning IL-12Rβ2 mRNA and supports the interpretation that Th1 is activated in the sinus mucosa in the allergic as well as in the nonallergic group.

The expression level of interleukin-12 receptor β2 subunit (IL-12Rβ2) messenger ribonucleic acid, expressed as the fluorescence ratio of IL-12Rβ2/β-actin for normalization, was measured using a fluorescent image analyser. Results are expressed as fluorescence ratio of IL-12Rβ2/β-actin for normalization. There was no significant difference between the two groups.

Fig. 4. – Reverse transcription (RT) polymerase chain reaction (PCR) analysis of interleukin-12 receptor β2 subunit (IL-12Rβ2) and β-actin messenger ribonucleic acid (mRNA). Total ribonucleic acid was isolated from samples by means of guanidinium isothiocyanate extraction, and subjected to RT-PCR as described in Materials and methods. PCR products were then separated by electrophoresis on a 6% polyacrylamide gel. IL-12Rβ2 mRNA was expressed in the sinus mucosa of patients 1–4 (patients 1 and 2 are allergic and 3 and 4 nonallergic subjects) (a), but not in cultured human nasal epithelial cells 5 and 6 (b). M: molecular weight markers.

Fig. 5. – Expression level of interleukin-12 receptor β2 subunit (IL-12Rβ2) messenger ribonucleic acid in the sinus mucosa. The fluorescence intensity of the RT-PCR products on a polyacrylamide gel were measured using a fluorescent image analyser. Results are expressed as fluorescence ratio of IL-12Rβ2/β-actin for normalization. There was no significant difference between the two groups.

Fig. 6. – Correlation between the expression level of interleukin-12 receptor β2 subunit (IL-12Rβ2) messenger ribonucleic acid, expressed as the fluorescence ratio of IL-12Rβ2/β-actin for normalization, and the number of mononuclear cells. ○: allergic group; ●: nonallergic group. The correlation is statistically significant, with a Pearson correlation coefficient of 0.564 (n=22, p<0.01).
In conclusion, interleukin-12 receptor β2 subunit messenger ribonucleic acid and CD30, T-helper 1 and T-helper 2 cell markers, respectively, were expressed in the paranasal sinus mucosa of patients with chronic sinusitis. The proportion of CD30-positive cells in the sinus mucosa was significantly larger in the allergic than in the non-allergic group. The expression level of interleukin-12 receptor β2 subunit messenger ribonucleic acid was virtually equivalent in both groups. These results suggest a T-helper 2-dominated mucosal reaction in the allergic group compared to the nonallergic group, and indicate ubiquitous T-helper 1 activity in the sinus mucosa in both allergic and nonallergic groups. The ubiquity of T-helper 1 cells suggests that they play a role in maintaining local mucosal defences against foreign antigens, which are continually entering the upper respiratory tract.

References