Available online at www.sciencedirect.com

SCIENCE DIRECT

Clinical Immunology 116 (2005) 42–53

Clinical IMMUNOLOGY

www.elsevier.com/locate/yclim

Expression of cutaneous lymphocyte-associated antigen (CLA) in tonsillar T-cells and its induction by in vitro stimulation with alpha-*streptococci* in patients with pustulosis palmaris et plantaris (PPP)

Hayabusa Nozawa, Kan Kishibe, Miki Takahara, Yasuaki Harabuchi*

Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan

Received 9 April 2004; accepted with revision 12 January 2005
Available online 22 April 2005

Abstract

Pustulosis palmaris et plantaris (PPP) is known to be a one of the tonsil-related diseases because tonsillectomy is quite effective in curing this condition. However etiological association between tonsils and PPP have not fully clarified yet. Cutaneous lymphocyte-associated antigen (CLA) is known to be a specific homing receptor that facilitates T-cell migration into skin. In this study, we investigated the expression of CLA on T-cells in tonsil, peripheral blood, and skin from patients with PPP. Two-color flow cytometric and two-color immunohistological analyses revealed that the numbers of CLA/CD3 double-positive cells in freshly isolated tonsillar mononuclear cells (TMC) and in tonsillar tissues were significantly higher in patients with PPP than in patients without PPP ($P < 0.01$, each). In vitro stimulus with α-streptococcal antigens enhanced CLA expression of tonsillar T-cells and TGF-β production of TMC in patients with PPP ($P < 0.01$, each), but did not in patients without PPP. In peripheral blood from PPP patients, the number of the CLA/CD3 double-positive cells significantly decreased at 6 months after tonsillectomy ($P < 0.05$). The CLA/CD3 double-positive cells and the postcapillary venule that expressed with a ligand of CLA, E-selectin, were found more frequently in the plantar skin from patients with PPP as compared to that from healthy volunteers ($P < 0.01$, each). These data suggest that a novel immune response to α-*streptococci* may enhance CLA expression on tonsillar T-cells through TGF-β production in patients with PPP, resulting in moving of CLA-positive tonsillar T-cells to skin and tissue damages. This may play a key role in pathogenesis of PPP.

Keywords: Cutaneous lymphocyte-associated antigen (CLA); Tonsil; Tonsillitis; Pustulosis palmaris et plantaris (PPP); α-*streptococci*; IL-6; TGF-β; E-selectin

Introduction

Pustulosis palmaris et plantaris (PPP) is characterized by symmetrical, erythematous, scaly plaques with numerous, sterile non-bacterial, pinpoint pustules that are restricted to the palms and soles [1,2]. PPP is reported to arise in about 0.05% of all population [3]. PPP is seen more frequently in women and is most frequent between the ages of 30 and 60 years. The disease tends to have an unpredictable course with exacerbations that often occur during acute tonsillitis [1,4]. PPP often disappears after tonsillectomy [4–6]. Therefore, PPP is considered one of the tonsil-related diseases. The etiology and pathogenesis of PPP remain unclear.

Previous studies have demonstrated increased serum levels of immune complexes and anti-keratin antibody in PPP; however, these return to normal after tonsillectomy with subsequent improvement in the skin lesions [6]. An animal model with PPP-like skin lesions has been established by reconstituting severe combined immunodeficiency (SCID) mice with tonsillar mononuclear cells (TMC) from patients with PPP [7]. Recently, Yamanaka et al. [8] grafted human PPP skin onto SCID mice and simultaneously injected them with TMC or peripheral blood lymphocytes (PBL) from subjects with PPP. A larger number of TMC infiltrated the area around the papillary dermis of the PPP

* Corresponding author. Fax: +81 166 68 2559.
E-mail addresses: hayabusan4@aol.com (H. Nozawa), kkishibe@asahikawa-med.ac.jp (K. Kishibe), Miki@asahikawa-med.ac.jp (M. Takahara), hyasu@asahikawa-med.ac.jp (Y. Harabuchi).

1521-6616/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2005.01.009

skin than PBL, suggesting that TMC selectively home to the skin lesions of PPP. Furthermore, the mice exhibited increased level of human anti-keratin antibody in mice with the TMC [8]. These findings suggest a close association between tonsils and PPP.

Tonsils play an important role in the defense mechanism of the upper respiratory airway against foreign substances, especially bacteria. However, tonsils may play a pathogenic role in response to bacteria in several diseases. In patients with PPP, α-*streptococci* have been detected at high levels in tonsils from subjects with PPP [9]. Serum antibody levels to α-*streptococci* are also high in patients with PPP, especially in subjects who improve after tonsillectomy [9,10]. Our laboratory demonstrated that TMC from subjects with PPP exhibit increased proliferation and proinflammatory cytokine production including IFN-γ, TNF-α, and IL-6 in response to α-streptococcal stimulation in vitro [10]. These data suggest that hyper-immune responses to α-*streptococci* may be present in subjects with tonsil-related disease PPP.

Cutaneous lymphocyte-associated antigen (CLA), a glycoprotein recognized by a monoclonal antibody HECA452, is known to be a specific homing receptor that facilitates T-cell migration into skin lesions [11]. The CLA expression on T-cells is reported to be up-regulated by TGF-β and IL-6 [12]. Previous reports demonstrated that the CLA expression on T-cells infiltrating skin lesions was increased in skin diseases such as cutaneous T-cell lymphoma [11], atopic dermatitis [11], and psoriasis [13–15]. E-selectin is a ligand of CLA and is predominantly expressed in postcapillary venules stimulated by inflammation [16]. E-selectin expression as well as expression of CLA is up-regulated in skin lesions of chronic leg ulcers [17]. These data suggest that CLA expressing T-cells, which infiltrate skin lesions through the E-selectin expressing postcapillary venules, play a key role in skin tissue damage in various skin diseases. There is no information regarding CLA expression in tonsil-related disease PPP.

In this study, we performed tonsillectomy in patients with PPP for treatment of PPP and investigated (i) how CLA is expressed in tonsillar T-cells, (ii) how CLA expression of peripheral T-cells changes after tonsillectomy, (iii) how CLA and its ligand E-selectin is expressed in skin lesions, (iv) whether CLA expression of tonsillar T-cells is enhanced by in vitro stimulation with α-streptococcal antigens, and (v) whether TMC produce CLA-related cytokines TGF-β and IL-6 in response to in vitro stimulation with α-streptococcal antigens.

Materials and methods

Patients and samples

All patients were studied at Asahikawa Medical College. The study groups were composed of 2 groups, the PPP group and non-PPP group, undergoing tonsillectomy. The non-PPP group was composed of patients with recurrent tonsillitis (RT) or obstructive sleep apnea syndrome (OSAS). Patients with RT required tonsillectomy because of recurrent episodes (more than three times per year) of acute tonsillitis. Patients with OSAS underwent uvulopalatopharyngoplasty together with tonsillectomy. Any patients with RT or OSAS had not skin diseases. PPP was diagnosed by dermatologists in our hospital on the basis of the findings characterized by symmetrical, erythematous, scaly plaques with numerous, sterile, pinpoint pustules restricted to the palms and soles and by no psoriasis skin lesions elsewhere on the body. The effects of tonsillectomy, based on the changes of skin condition 6 months after tonsillectomy, were judged into 6 grades, “disappeared”, “remarkably effective”, “effective”, “partially effective”, “no changed” and “worsened”, as described previously [4]. All patients signed informed consent for therapy and tissue studies. The study was approved by the Institutional Review Board.

Cell preparation

Tonsillar and peripheral mononuclear cells were isolated from tonsils and peripheral blood by the gradient centrifugation method using Ficoll Paque Plus® (Amersham Pharmacia Biotech, Piscateway, NJ, USA), as described previously [18]. The cells were washed three times with sterile phosphate buffered saline (PBS), suspended in RPMI-1640 medium (GIBCO, Grand Island, NY, USA) and counted. The viability of the cell suspensions were over 95%.

Preparation of bacterial antigens

Frozen whole cell preparations from *Streptococcus* (*S.*) *sanguis* ATCC10556, *S. salivarius* ATCC7073, and *S. mitis* NCTC3265 were used in this study [10,19,20]. Bacteria were grown over night at 37°C in Todd-Hewitt Broth (DIFCO laboratories, Detroit, MI, USA) and incubated for 40 min at 60°C. The heat-inactivated cells were harvested by centrifugation at 9000 × *g* for 30 min at 4°C, resuspended in PBS, and centrifuged in the same conditions. The pellets were then lyophilized overnight in PBS and stored at −80°C until used.

Cell culture

The tonsillar mononuclear cells (TMC) were suspended at a concentration of 1×10^6/ml in 3 ml of RPMI-1640 culture medium (GIBCO) containing 10% fetal calf serum (FCS; GIBCO), 100 U/ml of penicillin, and 100 μg/ml of streptomycin. The cells were then cultured without any mitogens or antigens, with 5 μg/ml phytohemagglutinin (PHA; Sigma-Aldorich, St. Louis, MO, USA), or with 100 μg/ml of lyophilized *streptococci* in 6-well culture plates in an atmosphere with 5% CO2 at 37°C. After 3 days in