Journal of Dermatological Science 68 (2012) 187–193

Contents lists available at SciVerse ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

Sialic acid cyclization of human Th homing receptor glycan associated with recurrent exacerbations of atopic dermatitis☆

Keiichiro Sakuma [a], Takuya Furuhashi [b], Sachiko Kondo [a,c,d], Uichiro Yabe [d], Katsuyuki Ohmori [e], Hidemi Ito [f], Masahiro Aoki [a], Akimichi Morita [b], Reiji Kannagi [a,g,h,*]

[a] Division of Molecular Pathology, Aichi Cancer Center, Nagoya, Aichi 464-8681, Japan
[b] Department of Geriatric and Environmental Dermatology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi 467-8601, Japan
[c] Department of Structural Biology and Biomolecular Engineering, Nagoya City University Graduate School of Pharmaceutical Sciences, Nagoya, Aichi 467-8603, Japan
[d] GLYENCE Co., Ltd, Nagoya, Aichi 464-0858, Japan
[e] Department of Clinical Pathogenesis, Kyoto University School of Medicine, Kyoto 606-8501, Japan
[f] Division of Epidemiology and Prevention, Aichi Cancer Center, Nagoya, Aichi 464-8681, Japan
[g] Research Complex for Medical Frontiers, Aichi Medical University, Aichi 480-1195, Japan
[h] Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan

ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form 16 August 2012
Accepted 18 September 2012

Keywords:
Atopic dermatitis
Homing receptor
Helper T cell
Recurrence
Selectin-ligand glycan
Sialic acid cyclization

ABSTRACT

Background: The molecular pathogenesis underlying recurrent exacerbations of atopic dermatitis (AD) is unclear. Some peripheral CCR4$^+$ and CCR7$^+$ helper memory T cells express the specific homing receptor, sialyl 6-sulfo Lewis X (G152 glycan). This glycan loses receptor activity via cyclization of its sialic acid moiety, thus becoming cyclic sialyl 6-sulfo Lewis X (G159 glycan). These findings suggest that the disordered expression of G152 and G159 glycans may be associated with recurrent exacerbations of AD.
Objective: To assess the possible association of G152 and G159 glycans, which are expressed on peripheral helper T (Th) cells, with frequency of exacerbations.
Methods: The percentage of glycan-expressing cells among peripheral blood CD4$^+$CD45RO$^+$ lymphocytes was determined by flow cytometry. The association of glycans with the frequency of exacerbations determined by recurrence scores as well as with current disease activity was statistically tested.
Results: Current disease activity was significantly associated with CCR4$^+$CCR7$^-$ memory Th cells expressing CSLEX-1 glycan, the conventional skin-trafficking receptor without sialic-acid-cyclization activity. In contrast, the frequency of exacerbations was positively and negatively associated with CCR4$^+$CCR7$^+$ memory Th cells expressing G152 and G159 glycans, respectively. Receiver operating characteristics analyses indicated that the ratio of the G152$^+$/G159$^+$ cell percentages discriminated patients with highly recurrent AD with the best accuracy.
Conclusion: Flow cytometric determination of G159 and G152 glycans on peripheral helper memory T cells may be clinically useful for identifying patients with highly recurrent AD. Disordered sialic acid cyclization of G152 glycan may underlie highly recurrent AD, which may provide a novel therapeutic approach.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin. The prevalence of AD is estimated to be as high as 10–20%, and has increased by 2–3-fold during the past three decades, particularly in industrialized countries [1,2]. Although AD is characterized by relapsing symptoms, including pruritus, eczema, and dermatitis, the molecular pathogenesis underlying recurrent exacerbations is unclear. Topical corticosteroids are the standard medications for AD; however, the effect is limited temporally and spatially. Systemic administration of immunosuppressive agents may have a more extensive effect, but systemic immunosuppressive agents are not suitable for consecutive use because of severe side effects. Thus,

☆ This work was supported by Grants-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science (20790583 and 22790774), a grant from the Japan Science and Technology Agency (Potentiality Verification Stage of Collaborative Development of Innovative Seeds), Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (21590324 and on priority areas 23112520), Grants-in-Aid for the Third-Term Comprehensive Ten-year Strategy for Cancer Control from the Ministry of Health and Welfare, and a grant from the Uehara Memorial Foundation, Japan.
* Corresponding author at: Research Complex for Medical Frontiers, Aichi Medical University, 21 Karimata, Yazako, Nagakute-cho, Aichi-gun, Aichi 480-1195, Japan. Tel.: +81 561 62 3311x1436; fax: +81 561 61 1896.
E-mail address: kannagi-gi@umin.ac.jp (R. Kannagi).

0923-1811/$36.00
http://dx.doi.org/10.1016/j.jdermsci.2012.09.015

identification of the key molecules regulating recurrent exacerbations might provide novel therapeutic approaches.

Helper T (Th) cells play significant roles in the pathogenesis of AD [2,3]. The disordered immunological pathways in AD are initiated by an increased infiltration of circulating T cells into the skin. Hamid et al. [4] demonstrated that the clinically unaffected skin of AD patients contains increased numbers of Th cells expressing IL-4 and IL-13 mRNAs. These Th cells engage Langerhans cells (LCs) bearing allergen-triggered IgE and mast cells in the skin, then recirculate into the peripheral blood. Subsequent skin injury by allergens, scratching, or microbial toxins can result in the development of acute lesions [2,3]. Thus, skin-homing Th cells play a central role in the early steps of AD exacerbations.

Selectin-mediated cell adhesion is essential in lymphocyte homing and leukocyte recruitment into immune foci [5–11]. Sialyl Lewis X (sLe^x), the best-established carbohydrate ligand for E- and P-selectins, is constitutively expressed on monocytes and granulocytes [12,13]. Most peripheral resting T cells do not express sLe^x; however, they are strongly induced to express sLe^x upon activation [14,15]. In contrast, a subset of peripheral resting memory Th cells express another carbohydrate ligand for E- and P-selectins, sialyl 6-sulfo Le^x [15]. This glycan was first identified on high endothelial venules (HEVs) as the major ligand for **L**ymphocyte selectin [16]. Subsequent studies have demonstrated the binding capacity of sialyl 6-sulfo Le^x for **E**ndothelial selectin and **P**latelet selectin [17] and the expression of sialyl 6-sulfo Le^x on some peripheral $CD4^+CD45RO^+CCR4^+CCR7^+$ lymphocytes, resting skin-homing memory Th cells [15].

Interestingly, the selectin-binding activity of sialyl 6-sulfo Le^x is abrogated via cyclization of its sialic acid moiety [18]. Briefly, *N*-acetylneuraminic acid, which is located at the non-reduced terminus of sialyl 6-sulfo Le^x, is de-*N*-acetylated to form an amino group, followed by a dehydration reaction between the amino and carboxyl groups at the C-1 position of the de-*N*-acetylated neuraminic acid to form cyclized neuraminic acid. Because these reactions occur at the moiety essential for the interaction with selectins, the final product, cyclic sialyl 6-sulfo Le^x, lacks selectin-binding activity. This cyclization does not occur in the sialic acid moiety of sLe^x. Therefore, it is likely that sialyl 6-sulfo Le^x is involved in skin-homing of resting Th cells.

Based on these observations, we assessed possible association of sLe^x, sialyl 6-sulfo Le^x, and cyclic sialyl 6-sulfo Le^x on circulating memory Th cells with the pathogenesis of AD.

2. Materials and methods

2.1. Blood samples

Peripheral blood samples (2 ml) were obtained from 31 AD patients and 16 normal controls at the Nagoya City University Hospital (Nagoya, Japan; Table 1). It was strictly confirmed using detailed questionnaires that normal controls did not have allergic diseases, such as bronchial asthma, allergic rhinitis, drug allergies, and food allergies or infectious diseases. This study was approved by the Ethics Committee of the Nagoya City University Graduate School of Medical Sciences, and all samples were obtained after obtaining informed consent. All investigations were performed according to the Declaration of Helsinki.

2.2. Antibodies

Murine monoclonal antibodies against sialyl 6-sulfo Le^x (G152, IgM), cyclic sialyl 6-sulfo Le^x (G159, IgG1), and sLe^x (CSLEX-1, IgM) were prepared as previously described [15,16,18]. Rat antibody against cutaneous lymphocyte-associated antigen (CLA; HECA-452, IgM) was obtained from BioLegend (San Diego, CA). PE-conjugated anti-CCR4 (205410) and anti-CCR7 antibodies (150503) were obtained from R&D Systems (Minneapolis, MN). PerCP/Cy5.5-conjugated anti-CD4 (OKT4) and allophycocyanin (APC)-conjugated anti-CD45RO antibodies (UCHL-1) were obtained from BioLegend and BD Biosciences (Franklin Lakes, NJ), respectively.

Table 1
Profiles of NCs and AD patients.

NCs	AD	Patients
Number	16	31
Gender		
Male	11	21
Female	5	10
Age (mean ± SD)	35.1 ± 8.9	33.8 ± 11.4
Objective SCORAD scores (mean ± SD)		36.0 ± 16.0
SCORAD scores (mean ± SD)		40.7 ± 17.1
Serum TARC levels (mean ± SD) (pg/ml)	ND	1726 ± 1690
Disease activity		
Severe-to-moderate (objective SCORAD: ≥15)		28 (90.3%)
Mild (objective SCORAD: <15)		3 (9.7%)

Profiles of normal controls (NCs) and patients with atopic dermatitis (AD) entered in this study. SD: standard deviation, SCORAD: Severity Scoring of Atopic Dermatitis, TARC: thymus activation regulated chemokine, ND: not determined.

2.3. Flow cytometry

Total blood cells (100 μl) were incubated with antibodies for 30 min at 4 °C using 10 μl of culture supernatant or commercial antibodies according to the manufacturers' recommendations. After two washes with PBS(−), cells were incubated with a 1:300 dilution of FITC-conjugated secondary antibody (Millipore, Billerica, MA) for 30 min at 4 °C. After hemolysis with FACS lysing solution (BD Biosciences) and two washes with PBS(−), cells were analyzed with a FACSCalibur (BD Biosciences).

2.4. Evaluation of current disease activity of AD

The scores of Severity Scoring of Atopic Dermatitis (SCORAD) and the levels of serum thymus activation regulated chemokine (TARC) were determined on the day when blood samples were obtained. SCORAD scores were determined as previously described [19]. Serum TARC levels were determined by ELISA.

2.5. Statistic analyses

The statistic significance of the differences in the percentage of Th cells between the two groups was assessed by the Mann–Whitney test using StatView (SAS Institute, Cary, NC). Pearson and Spearman correlation coefficients were determined using StatView for assessing the association between the percentage of Th cells and objective SCORAD scores or serum TARC levels and for assessing the association between the percentage of Th cells and recurrence scores, respectively. Receiver operating characteristics (ROC) analyses were performed using Stata 11 (StataCorp, College Station, TX).

3. Results

3.1. Increased proportions of $G152^+$ and $CSLEX\text{-}1^+$ memory Th cells in AD patients

Our first approach to assess the possible association of sialyl 6-sulfo Le^x, cyclic sialyl 6-sulfo Le^x, and sLe^x on peripheral memory Th cells with the pathogenesis of AD was to compare their expression levels between AD patients and normal controls (NCs).