ELSEVIER

Journal of Autoimmunity 27 (2006) 196–202

journal of autoimmunity

www.elsevier.com/locate/issn/08968411

Decreased expression levels of CD22 and L-selectin on peripheral blood B lymphocytes from patients with bullous pemphigoid

Makoto Inaoki [a,b,*], Takeshi Echigo [a], Hiroaki Hayashi [b], Tetsuya Nagaoka [a], Minoru Hasegawa [a], Kazuhiko Takehara [a], Wataru Fujimoto [b], Thomas F. Tedder [c], Shinichi Sato [d]

[a] *Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan*
[b] *Department of Dermatology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan*
[c] *Department of Immunology, Duke University Medical Center, Durham, NC 27710, USA*
[d] *Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan*

Received 26 May 2006; revised 8 September 2006; accepted 12 September 2006

Abstract

Bullous pemphigoid (BP), an autoimmune subepidermal-blistering disease of the elderly, is caused by antibodies against BP antigens at the epidermal basement membrane zone (BMZ). CD22 is a B lymphocyte specific response regulator, which is down-regulated after B-cell activation. Old CD22-deficient mice produce class-switched autoantibodies. To assess the role of CD22 in the pathogenesis of BP, we examined CD22 expression on B cells from BP patients and correlated its expression with clinical parameters. B cell expression of CD22 was 20% lower in BP patients when compared to healthy control subjects. In addition, B cells from BP patients showed decreased expression of L-selectin, which is an indicator of leukocyte activation, and CD22 expression levels were correlated with L-selectin expression. These results suggest that the decreased CD22 expression may be associated with the activation of B cells in BP. CD22 expression levels in BP patients did not correlate with the levels of anti-epidermal BMZ antibodies, and old CD22-deficient mice did not develop the anti-epidermal BMZ antibody. These results suggest that a decrease in CD22 expression may not be associated with BP-specific antibody production.

Keywords: Autoantibody; Bullous pemphigoid; CD22; L-selectin; Leukocyte activation

1. Introduction

Bullous pemphigoid (BP) is an autoimmune subepidermal blistering skin disease, usually occurring in the elderly, that is immunopathologically characterized by the linear deposition of immunoglobulin G (IgG) and/or complement C3 along the epidermal basement membrane zone (BMZ) [1]. Indirect immunofluorescence demonstrates that 70 to 80% of BP patients have circulating IgG antibodies against BP antigens expressed at the epidermal BMZ [2,3]. Two different BP antigens with molecular weights of 230 kDa (BP230) [4,5] and 180 kDa (BP180) [6] have been identified. Among the anti-epidermal BMZ autoantibodies, antibodies against the non-collagenous stretch of the BP180 ectodomain (NC16A) are thought to be crucial for initiation of the disease, because IgG antibodies against the murine homolog of BP180-NC16A induce a BP-like disease in the passive transfer mouse model [7]. Pemphigus, another major autoimmune blistering disease, has two subtypes, pemphigus vulgaris (PV) and pemphigus foliaceus (PF) [8]. Both are characterized histologically by intraepidermal blisters and immunopathologically by bound and circulating IgG class antibodies directed against the cell surface of keratinocytes *in vivo* [8]. Blisters occur just above the basal layer of the epidermis in PV, and blisters occur in the granular layer in PF [8].

* Corresponding author. Present address: Department of Dermatology, Kanazawa Medical Center, 1-1 Shimoishibiki-cho, Kanazawa, Ishikawa 920-8650, Japan. Tel.: +81 76 262 4161; fax: +81 76 222 2758.
E-mail address: inaoki-m@kinbyou.hosp.go.jp (M. Inaoki).

0896-8411/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jaut.2006.09.002

Identification of the target antigen is the key to understanding the pathogenesis of an autoimmune disease; thus, the pathogenic antigens of BP have been well characterized. However, the functional abnormality in B lymphocytes, the source of the autoantibodies, remains unclear in BP. Moreover, aging seems to play a role in the pathogenic mechanism of BP because the majority of the BP patients are over 60 years old at disease onset [9]. However, the role of aging in autoantibody production in BP has not yet been determined.

CD22 is a B lymphocyte-specific glycoprotein of the immunoglobulin superfamily expressed in the cytoplasm of pro-B and pre-B cells and on the cell surface of IgD positive mature B cells [10,11]. CD22 is a multifunctional receptor that regulates B cell function and development through ligand-dependent and -independent mechanisms. CD22 ligand binding regulates the expression of CD22 and IgM, B cell survival, and B cell antigen receptor (BCR)-induced proliferation [12]. In contrast, intracellular calcium responses, CD22 phosphorylation, recruitment of SHP-1 to CD22, and B cell migration are all ligand-independent phenomena and may only require the expression of a CD22 cytoplasmic domain [12]. B cells from CD22 knockout ($CD22^{-/-}$) mice generated an augmented calcium response after BCR crossing and have an IgM-low, major histocompatibility complex class II-high phenotype that is characteristic of stimulated B cells [13–16]. B cells from $CD22^{-/-}$ mice also have a shorter life span and enhanced apoptosis [12,13,16]. Cell surface CD22 on human B cells is down-regulated after BCR-mediated activation [17]. Thus, decreased CD22 expression may be an indicator of B-cell activation.

Previous studies have suggested that decreased expression of CD22 is associated with autoimmunity. One study has revealed that 5-month-old $CD22^{-/-}$ mice produce autoantibodies against double-stranded (ds) DNA and the dsDNA-histone complex of IgM class, predominantly [14]. Another study has revealed that $CD22^{-/-}$ mice with either a C57BL/6 or BALB/c genetic background develop high-affinity, IgG class anti-dsDNA autoantibodies, particularly after 8-months of age, and some $CD22^{+/-}$ heterozygous mice also produce IgG class anti-dsDNA autoantibodies by 12-months of age [18]. In addition, old $CD22^{-/-}$ mice produce IgG-class autoantibodies against cardiolipin or myeloperoxidase [18]. Another study has revealed that $CD22^{+/-}$ C57BL6 mice carrying the autoimmune acceleration gene, *Yaa*, have significantly increased production of IgG anti-DNA autoantibodies [19]. These observations suggest that a deficiency in CD22 predisposes to the development of autoimmunity when combined with increasing age or other disease susceptibility loci. In human autoimmune disease, however, decreased levels of CD22 expression on B cells have not yet been reported.

L-selectin (CD62L) is a leukocyte adhesion molecule involved in leukocyte interaction with vascular endothelial cells [20]. L-selectin binds to inducible ligands on the vascular endothelium at sites of inflammation and mediates leukocyte rolling [20,21]. It has been suggested that down-regulated expression of L-selectin on leukocytes reflects the augmented activation of those cells, because cellular activation of leukocytes rapidly induces shedding of the extracellular domain of L-selectin from the cell surface by endoproteolytic cleavage [22,23]. Decreased expression of L-selectin on subsets of leukocytes have been observed in some skin diseases including atopic dermatitis and severe psoriasis, and activation of these cells are suggested [24,25].

In this study, we examined CD22 and L-selectin expression on B cells in patients with BP to assess the role of CD22 in the pathogenesis of BP. Our data demonstrated that expression levels of CD22 on B cells were significantly decreased in BP patients. We then measured L-selectin expression on B cells in BP patients to assess whether the reduced CD22 expression reflects B cell activation. Next, we measured disease-specific antibody levels in BP patients to assess the effect of decreased CD22 expression on autoantibody production. Finally, we measured anti-epidermal BMZ antibodies in the sera of old CD22-deficient mice to determine whether decreased CD22 expression in combination with aging results in the production of autoantibodies specific for BP.

2. Materials and methods

2.1. Patients and controls

The levels of CD22 expression on the surface of circulating B cells were determined in 27 patients with BP (17 female and 10 male; mean age 76 ± 13 years; age range 31–99 years) with clinical, pathological, and immunological features typical of BP. Direct immunofluorescence showed deposition of IgG or C3 along the epidermal BMZ of peribullous skin in all the patients. Indirect immunofluorescence showed 23 of the 27 BP patients had circulating IgG autoantibodies to epidermal BMZ. None of the patients had been treated with systemic corticosteroids or immunosuppressive drugs before entry into the study, and all of the patients had active disease when the study was performed. The clinical severity of BP was evaluated as follows [26,27]: patients with moderate BP ($n = 12$) had some disseminated bullae and erythema, usually accompanied by pruritus, on less than one quarter of the body surface area; patients with severe BP ($n = 15$) had many disseminated bullae, usually accompanied by pruritus, on more than one quarter of the body surface area. Patients with localized pemphigoid were excluded from this study. Flow cytometric analysis was also performed in 9 patients with PF and 4 patients with PV, and 22 healthy control individuals (12 female and 10 male; mean age 71 ± 15 years; age range 33–92 years). The ages ($p > 0.10$) and sex ratios ($p > 0.10$) in normal controls and BP patients were not significantly different. CD40 expression on B cells was examined in 16 of the BP patients, 5 of the PF patients, and 15 of the healthy control subjects. L-selectin expression on B cells was examined in 24 of the BP patients, 8 of the PF patients, 4 of the PV patients, and 22 of the healthy control subjects. Serum levels of IgM, IgG, and anti-BP180 IgG antibodies were also measured in 26 of the BP patients (16 female and 10 male; mean age 76 ± 14 years; age range 31–99 years) and 22 healthy control individuals (11 female and 11 male;