Autoimmunity Reviews 11 (2011) 40–47

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Role of MHC Class II Genes in the pathogenesis of pemphigoid☆,☆☆,★

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ARTICLE INFO

Article history:
Received 15 June 2011
Accepted 6 July 2011
Available online 18 July 2011

Keywords:
Bullous Pemphigoid
Mucous membrane pemphigoid
MHC-HLA Class II Genes
Anti-BMZ autoantibodies
Pemphigoid antigens

ABSTRACT

Pemphigoid (Pg) is an autoimmune subepidermal blistering disease that affects the elderly population. The phenotype can be Bullous Pemphigoid (BP), which primarily involves the skin, or Mucous Membrane Pemphigoid (MMP), which primarily involves mucus membranes. Ocular Cicatricial Pemphigoid (OCP) and Oral Pemphigoid (OP) are subsets of MMP. The known antigens in BP are Bullous Pemphigoid Antigen 1 (BPAG1, also known as BP230), Bullous Pemphigoid Antigen 2 (BPAG2, also known as BP180), and subunits of human integrins α6 and β4. The Human Leukocyte Antigen (HLA) allele HLA-DQβ1*0301 has been reported to be associated with enhanced susceptibility to all of these subsets. Sera of patients with the four subsets are characterized by the presence of anti-Basement Membrane Zone (anti-BMZ) antibodies. In this manuscript, we present a model in which relevant portions of the four different antigens involved in pemphigoid have potential sites that could be presented by an antigen presenting cell (APC) in conjunction with DQβ1*0301 to a T cell receptor to initiate the process that results in anti-BMZ antibody production. Thus, this model provides a hypothetical computer-based mechanism to explain how a single HLA allele can be associated with the production of antibodies to four different antigens that result in four different subsets of a disease with four different clinical profiles and prognoses.

Contents

1. Introduction . . . 40
2. Methods . . . 41
 2.1. Patients . . . 41
  2.1.1. Inclusion criteria . . . 41
 2.2. Determination of T cell epitopes in relevant antigens . . . 41
3. Results . . . 42
 3.1. Human Leukocyte Antigen (HLA) Class II Gene Associations with Pg . . . 42
 3.2. Serological studies . . . 42
 3.3. Molecular analysis of the potential antigen binding sites on HLA-DQ7(DQβ1*0301) . . . 42
4. Discussion . . . 44
Take-home messages . . . 46
References . . . 46

1. Introduction

Pemphigoid (Pg) is a potentially fatal subepidermal blistering autoimmune disease. The majority of the patients are elderly [1]. Pg has two major phenotypes, Bullous Pemphigoid (BP) and Mucous Membrane Pemphigoid (MMP), also referred to as Cicatricial Pemphigoid (CP) [2].

BP characteristically affects elderly patients who present with large tense bullae on the entire skin and frequently the extremities [3,4]. Oral involvement is infrequently observed [3,4]. Pruritus may be significant [3]. The blisters rupture easily leaving large denuded surfaces, which can be easily infected since the blister fluid has a composition very similar to serum [5,6]. The mortality rate can vary from 19 to 30% [4]. Lesions of BP heal without scarring, but tend to leave post-inflammatory hypo- or hyper-pigmented macules [4].

☆ There are no funding sources.
☆☆ The authors have no conflict of interest or competing interests to disclose.
★ This manuscript had not been previously presented.
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doi:10.1016/j.autrev.2011.07.002

MMP affects the mucous membranes of the oral cavity, conjunctiva, nose, esophagus, pharynx, larynx, genitalia, anal canal, and the skin [3,6–11]. The lesions with MMP, upon healing, result in irreversible scarring [3,6–11]. This scarring can have catastrophic and very significant influences on the patients' quality of life. Scarring of the larynx can result in sudden asphyxiation, scarring of the esophagus requires repeated dilatation, and scarring of the anal canal, penile, and vaginal mucosa can significantly affect activities of daily living [6].

There are two subsets of MMP that deserve special mention because of the striking differences in their clinical presentation and prognosis. When MMP or CP involves predominantly or exclusively the conjunctival mucosa, it is referred to as Ocular Cicatricial Pemphigoid (OCP) [4]. The most concerning aspect of ocular involvement is that it can lead to blindness in spite of the most aggressive immunosuppressive treatment [12]. Oral Pemphigoid (OP) is that subset of MMP where the disease process is limited only to the oral cavity, and usually does not involve any other mucosa [13]. While OP is usually not fatal, eating, swallowing, and maintaining adequate nutritional levels can be both challenging and difficult [6].

The hallmark of both BP and MMP (including the subsets) is that these patients have circulating antibodies to molecules in the Basement Membrane Zone (BMZ) of the skin or the mucosal tissues [4]. These antibodies may be detected by Indirect Immunofluorescence (IIF) using a variety of substrates, the most common of which is monkey esophagus [4]. The histology of lesions from both variants of Pg shows a subepidermal vesicle with a dermal infiltrate that may be eosinophilic, neutrophilic, or mixed [2,11,14]. Direct Immunofluorescence studies of perilesional tissue in both variants demonstrate deposition of Immunoglobulin (Ig) G and/or complement along the BMZ [2,8,11].

There are two major target antigens in BP, Bullous Pemphigoid Antigen 1 (BPAG1, also known as BP230) and Bullous Pemphigoid Antigen 2 (BPAG2, also known as BP180) [2,14,15]. The major target of the autoantibody in OP is subunits of human integrin α6 [13]. In MMP and OCP, the target antigen is a subunit of human β4 integrin [14,16–18]. Sera of patients with MMP may have autoantibodies that bind BPAG1 and BPAG2, but the levels and presence do not correlate with disease activity [19].

BPAG1 has a molecular weight of 230 kDa [14]. It is a desmoplakin located in the intracellular portion of the hemidesmosome complex [14]. Its gene is located on the short arm of chromosome six [14]. BPAG2 is a transmembrane hemidesmosome with a molecular weight of 180 kDa [14,20]. It has 15 domains that belong to the long carboxy-terminal that spans the lamina lucida, and a non-collagenous 16A (NC16A) domain, found adjacent to the transmembranous aspect of the ectodomain [14,20–24]. The NC16A is known to contain major BP antigenic epitopes [21,22,25–27]. Integrins are heterodimers of α and β subunits in combination, and serve an important function in cell adhesion [16]. The α6β4 heterodimer is found in the hemidesmosomes of skin and mucous membranes [16]. The 120 kDa α6 integrin has been shown to be the target antigen in OP [13]. The titers of antibodies to α6 subunit correlate with disease severity and activity in patients with OP [28]. The α6 subunit contains 1073 amino acids [29]. Antibodies to the β4 integrin subunit correlate with disease severity and activity in the sera of MMP and OCP patients [30].

Many investigators have demonstrated that in patients with BP and all the clinical variants of MMP, there is an increased susceptibility to the disease associated with the HLA- DQβ1*0301 allele [31–34]. Moreover, reports have shown T cell and antibody binding sites in BPAG1, BPAG2, α6, and β4 in patients with Pg [18,20,22,26,29].

The aim of this study was to determine if there existed a possible molecular basis for a single HLA allele binding all four different antigens involved in BP and MMP and presenting them to antigen-specific T and B cells, leading to the production of four distinct antibodies to BMZ, and four distinct clinical phenotypes of pemphigoid.

2. Methods

2.1. Patients

The patients were seen at the Center for Blistering Diseases (CBD) in Boston, MA. 21 patients with BP, 100 patients with MMP and OCP, and 22 patients with OP were enrolled in this study. Some of these patients have been previously reported [31,34–36]. This study was approved by the Institutional Review Board (IRB).

2.1.1. Inclusion criteria

To be included in this study, the patients had to fulfill the following criteria:

2.1.1.1. Clinical profile.

A. Patients with BP had large tense blisters present on the skin and no mucosal disease.
B. Patients with Oral Pemphigoid had erosions on the gingival and other sites in the oral cavity but no disease in any other mucosal tissues or the skin on long-term follow-up (minimum three years).
C. Patients with OCP had scarring in the conjunctiva with symblepharon and ectropion. Some had scarring of the conjunctiva and decreased visual acuity.
D. Patients with MMP had erosive lesions in the oral cavity, pharynx, larynx, esophagus, genitalia, and anal canal. 32% of them had cutaneous involvement.

2.1.1.2. Histology. Biopsy of a fresh lesion demonstrated a subepidermal or subepithelial blister with a mixed cell infiltrate in the dermis or submucosa.

2.1.1.3. Immunopathology. Direct immunofluorescence of perilesional skin or mucosal tissue demonstrated the presence of IgG and complement along the BMZ in a homogenous linear smooth pattern.

2.1.1.4. Serological studies.

A. In patients with BP, antibodies to BPAG1 and BPAG2 were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) [37,38].
B. In patients with OP, antibodies to α6 integrin (105 kDa protein) determined by an immunoblot assay using bovine gingival lysate as substrate [13]. The positive control was GoH3 monoclonal antibody [13] and sera of a patient with active pemphigus vulgaris. The negative control was 25 normal human serum.
C. In patients with OCP and MMP, antibodies to β4 integrin (205 kDa protein) were determined by an immunoblot assay using bovine gingival lysate as substrate [17,30]. The positive control was UM-SCC-20 monoclonal antibody [16] and sera of a patient with active pemphigus vulgaris. The negative control was 25 normal human serum.

2.1.1.5. MHC class II typing. High resolution HLA-MHC II typing was done by site polymerase chain reaction with sequence specific primers (PCR-SSP) [39] on DNA of each patient obtained from peripheral blood.

2.2. Determination of T cell epitopes in relevant antigens

A theoretical computer model was used to predict antigen binding sites for HLA class II in the DQβ1*0301 allele. T cell immune responses are elicited upon the recognition of peptide-antigens bound to HLA Class II molecules. Therefore, T cell epitopes may be surmised through the prediction of antigen-HLA binding [40]. Here, we have used the RANKPEP server (http://imed.med.ucm.es/Tools/rankpep.html), to predict potential T cell epitopes within BP180, BP230, and human Integrin α6 and β4 [41–43] that are restricted by HLA-DQ7, the predominant HLA II molecule whose β chain is DQβ1*0301. The