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Review

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

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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

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].

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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

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prediction of peptide-binding to HLA is carried out using Position Specific Scoring Matrices (PSSMs) derived from peptides that are known to bind to the relevant HLA molecule. In this study, if peptides received a score higher than the Binding Threshold (BT), then they were considered to bind HLA-DQ7 (DQβ1*0301). Further details are described by Reche et al. [41–43].

3. Results

3.1. Human Leukocyte Antigen (HLA) Class II Gene Associations with Pg

A. HLA Class II Genes in Patients with BP: HLA Class II gene associations were studied in 21 patients with BP. The results show a statistically significant association with DQβ1*0301 ($P<0.05$) [31].
B. HLA Class II Genes in Patients with MMP and OCP: HLA Class II gene associations were studied in 100 patients with MMP and OCP. The results show a statistically significant association with DQβ1*0301 ($P<0.05$) [34–36].
C. HLA Class II Genes in Patients with OP: HLA Class II gene associations were studied in 22 patients with OP. The results show a statistically significant association with DQβ1*0301 ($P<0.05$) [34].

3.2. Serological studies

Serological studies in patients with BP included detection of antibodies to BPAG1 and BPAG2 by an ELISA. Based on the instructions from the manufacturer, antibody levels above 9 units/mL are considered positive [37,38]. In 21 patients with BP, using the ELISA, antibodies to BPAG1 was detected in 18 patients. Antibodies to BPAG2 were detected in all 21 patients. Antibodies to both BPAG1 and BPAG2 were found in 18 patients.

Using the immunoblot assay, all the patients with Oral Pemphigoid in this study had antibodies to α6 integrin as determined by the presence of a 120 kDa band. The positive control (GoH3) monoclonal antibody to α6 also demonstrated an identical 120 kDa band. The sera of the pemphigus vulgaris patient bound to a 160 kDa protein (Desmoglein 3). No binding was observed by the normal human sera.

Using the immunoblot assay, all the patients with Ocular Cicatricial Pemphigoid and Mucous Membrane Pemphigoid in this study had antibodies to β4 integrin as determined by a 205 kDa band. The positive control (UM-SCC-20) monoclonal antibody to β4 also demonstrated an identical 205 kDa band. The sera of the pemphigus vulgaris patient bound to a 160 kDa protein (Desmoglein 3). No binding was observed by the normal human sera.

3.3. Molecular analysis of the potential antigen binding sites on HLA-DQ7(DQβ1*0301)

Using the computer models, we found that HLA-DQ7(DQβ1*0301) will bind on the following peptides on BP180: amino acid 505–513, 635–643, 765–773, 841–849, 1055–1063, 1192–1210, 1283–1291, and 1320–1328 among others (Fig. 1).

Using the computer models, we found that HLA-DQ7(DQβ1*0301) will bind on the following peptides on BP240: amino acid 209–217, 547–555, 925–933, 1295–1303, 2029–2038, and 2366–2374 among others (Fig. 2).

Using the computer model, we found that HLA-DQ7(DQβ1*0301) will bind on the following peptides on α6 integrin: amino acid 34–42, 341–349, 503–511, and 495–453 among others (Fig. 3).

Using the computer model, we found that HLA-DQ7(DQβ1*0301) will bind on the following peptides on β4 integrin: amino acid 617–625, 890–898, 1148–1156, and 1304–1312 among others (Fig. 4).

4. Discussion

In this study, we presented 21 patients with BP, 100 patients with MMP and OCP, and 22 patients with OP, all with a highly statistically significant association with the HLA-DQβ1*0301 allele. Moreover, all 21 of the patients with BP, on serological studies, demonstrated binding of antibodies to BPAG2, and 18 of the patients with BP had antibodies to BPAG1. All the patients with OP demonstrated binding of

NP_000485.3 alpha 1 type XVII collagen.

Bullous pemphigoid autoantigen BP180

Epitopes predicted to bind to HLA-DQ7(DQβ*0301) are represented in yellow.

```
1 mdvtkknkrdgtevterivtetvttrltslppkggtsngyaktaslgggsrlekqslthg
61 ssgyinstgstrghastssyrrahspastlpnspgstferkthvtrhayegsssgnsspe
121 yprkefassstrgrsqtreseirvrlqsaspstrwteldddvkrllkgsrsasvsptrnss
181 ntlpipkkgtvetkivtassqsvsgtydatildanlpshvwsstlpagssmgtyhnnmtt
241 qsssllntnaysagsvfgvpnnmascsptlhpglstsssvfgmqnnlapslttlshgttt
301 tstaygvkknmpqspaavntgvstsaacttsvqsddllhkdckflilekdntpakkemel
361 limtkdsgkvftaspasiaatsfsedtlkkekqaaynadsglkaeangdlktvstkgktt
421 tadihsygssggggsggggvggagggpwgpapawcpcgsccswwkwllglllwllllg
481 llfglialaeevrklkarvdelerirrsilpygdsmdriekdrlqgmapaagadldkigl
541 hsdsqeelwmfvrkklmmeqengnlrgspgpkgdmgspgpkgdrgfpgtpgipgplghpg
601 pqgpkgqkgsvgdpgmegpmgqrgregpmgprgeagppgsgekgergaagepgphgppgv
661 pgsvgpkgsssgspgpqgppgpvglqglrgevglpgvkgdkgpmgppgpkgdqgekgprgl
721 tgepgmrglpgavgepgakgamgpagpdghqgprgeqgltgmpgirgppgpsgdpgkpgl
781 tgpqgpqglpgtpgrpgikgepgapgkivtsegssmltvpgppgppgamgppgppgapgp
841 agpaglpghqevlnlqgppgppgprgppgpsipgppgprgppgeglpgppgppgsflsns
901 etflsgppgppgppgpkgdqgppgprghqgeqglpgfstsgsssfglnlqgppgppgpqg
961 pkgdkgdpgvpgalgipsgpseggssstmyvsgppgppgppgpgsisssgqeiqqyise
1021 ymqsdsirsylsgvqgppgppgppgpvttitgetfdyselashvvsylrtsgygvslfss
1081 sissedilavlqrddvrqylrqylmgprgppgppgasgdgsllsldyaelssrilsymss
1141 sgisiglpgppgppglpgtsyeellsllrgsefrgivgppgppgppgipgnvwssisved
1201 lssylhtaglsfipgppgppgppgprgppgvsgalatyaaensdsfrselisyltspdvr
1261 sfivgppgppgpqgppgdsrllstdashsrgsssssshsssvrrgssysssmstggggags
1321 lgaggafgeaagdrgpygtdigpggyyaaaeggmyagnggllgadfagdldynelavrv
1381 sesmqrqgllqgmaytvqgppgqpgpqgppgiskvfsaysnvtadlmdffqtygaiqgpp
1441 gqkgemgtpgpkgdrgpagppghpgppgprghkgekgdkgdqvyagrrrrrsiavkp
```

Bullous pemphigoid autoantigen BP180 binding to HLA-DQ7(DQβ*0301)

Consensus sequence: IWHAVHAWH Optimal Score (%OPT.): 45.671
Binding Threshold: 11.70 All rows highlighted in red represent predicted binders

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	505	LER	IRRSILPYG	DSM	1056.29	19.68	43.09 %
2	1055	GET	FDYSELASH	VVS	1050.11	19.381	42.44 %
3	1283	RLL	STDASHSRG	SSS	898.89	16.697	36.56 %
4	211	DAT	ILDANLPSH	VWS	961.09	16.398	35.90 %
5	841	PGP	AGPAGLPGH	QEV	757.85	16.234	35.55 %
6	220	PSH	VWSSTLPAG	SSM	876.01	15.634	34.23 %
7	1320	GAG	SLGAGGAFG	EAA	717.78	13.849	30.32 %
8	765	MPG	IRGPPGPSG	DPG	818.94	13.625	29.83 %
9	199	VTA	SSQSVSGTY	DAT	896.91	13.531	29.63 %
10	247	SLL	NTNAYSAGS	VFG	865.85	13.391	29.32 %
11	80	TSS	YRRAHSPAS	TLP	1026.14	12.928	28.31 %
12	1201	VED	LSSYLHTAG	LSF	930.03	12.403	27.16 %
13	423	TTA	DIHSYGSSG	GGG	903.91	12.396	27.14 %
14	837	PPG	APGPAGPAG	LPG	675.75	11.938	26.14 %

Fig. 1. Binding sites in Bullous Pemphigoid Antigen 2 (BP180) for DQ7(DQβ1*0301). Bullous Pemphigoid Antigen 2 (BP180) peptide antigens predicted to bind to HLA-DQ7 (DQβ1*0301) are shown in yellow. All shown peptides exceed the Binding Threshold (see Methods). POS. = Position; N = N-terminal; C = C-terminal; MW = Molecular Weight (Daltons); %OPT. = Optimal Score.

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NP_899236.1 . Bullous Pemphigoid antigen 240. Dystonin isoform 1
Description : Bullous Pemphigoid antigen 240.
Transcript Variant: This variant (1) represents the longest transcript and encodes the longest isoform (1), also known as dystonin-1.

Epitopes predicted to bind to HLA-DQ7(DQβ*0301) are represented in yellow.

```
1 magylspaaylyveeqeylqayedvlerykderdkvqkktftkwinqhlmkvrkhvndly
61 edlrdghnlislllevlsgdtlprekgrmrfhrlqnvqialdylkrrqvklvnirndditd
121 gnpkltlgliwtiilhfqisdihvtgesedmsakerlllwtqqategyagircenfttcw
181 rdgklfnaiihkyrpdlidmntvavqsnlanlehafyvaekigvirlldpedvdvsspde
241 ksvityvsslydafpkvpeggegigandvevkwieyqnmvnyliqwirhhvttmsertfp
301 nnpvelkalynqylqfketeippketekskikrlyklleiwiefgriklllqgyhpndiek
361 ewgkliiamlerekalrpeverlemlqqianrvqrdsvicedklilagnalqsdskrles
421 gvqfqneaeiagyilecenllrqhvidvqilidgkyyqadqlvqrvaklrdeimalrnec
481 ssvyskgriltteqtklmisgitqslnsgfaqtlhpsltsgltqsltpsltsssmtsgls
541 sgmtsrltpsvtpaytpgfpsglvpnfsssgvepnslqtlklmqirkpllkssllddqnlte
601 eeinmkfvqdllnwvdemqvqldrtewgsdlpsveshlenhknvhraieefesslkeaki
661 seiqmtaplkltyaeklhrlesqyakllntsrnqerhldtlhnfvsratneliwlnekee
721 eevaydwserntniarkkdyhaelmreldqkeeniksvqeiaeqlllenhparltieayr
781 aamqtqwswilqlcqcveqhikentayfeffndakeatdylrnlkdaiqrkyscdrsssi
841 hkledlvqesmeekeellqykstianlmgkaktiiqlkprnsdcplktsipikaicdyrq
901 ieitiykddecvlannshrakwkvisptgneamvpsvcftvpppnkeavdlanrieqqyq
961 nvltlwheshinmksvvswhylineidrirasnvasiktmlpgehqqvlsnlqsrfedfl
1021 edsqesqvfsgsditqlekevnvckqyyqellksaereeqeesvynlyisevrnirlrle
1081 ncedrlirqirtplerddlhesvfriteqeklkkelerlkddlgtitnkceeffsqaaas
1141 ssvptlrselnvvlqnmnqvysmsstyidklktvnlvlkntqaaealvklyetklceeea
1201 viadknnienlistlkqwrsevdekrqvfhaledelqkakaisdemfktykerdldfdwh
1261 kekadqlverwqnvhvqidnrlrdlegigkslkyyrdtyhpldwiqqvettqrkiqenq
1321 pensktlatqlnqqkmlvseiemkqskmdecqkyaeqysatvkdyelqtmtyramvdsqq
1381 kspvkrrrmqssadliiqefmdlrtrytalvtlmtqyikfagdslkrleeeeksleeekk
1441 ehvekakelqkwvsnisktlkdaekagkppfskqkisseeistkkeqlsealqtiqlfla
1501 khgdkmtdeernelekqvktlqesynllfseslkqlqesqtsgdvkveekivaerqqeyk
1561 eklqgicdlltqtenrlighqeafmigdgtvelkkyqskqeelqkdmqgsaqalaevvkn
1621 tenflkengeklsqedkalieqklneakikceqlnlkaeqskkeldkvvttaikeetekv
1681 aavkqleesktkienlldwlsnvdkdseragtkhkqvieqngthfqegdgksaigeedev
1741 ngnlletdvdgqvgttqenlnqqyqkvkaqhekiisqhqaviiatqsaqvllekqgqyls
1801 peekeklqknmkelkvhyetalaesekkmkltshslqeelekfdadytefehwlqqseqel
1861 enleagaddinglmtklkrqksfsedvishkgdlryitisgnrvleaaksccskrdggkvd
1921 tsathrevqrkldhatdrfrslyskcnvlgnnlkdlvdkyqhyedascgllaglqaceat
1981 askhlsepiavdpknlqrqleetkalqgqissqqvaveklkktaevlldargsllpaknd
2041 iqktlddivgryedlsksvnerneklqitltrslsvqdgldemldwmgnvesslkeggqv
2101 plnstalqdiisknimleqdiagrqssinamnekvkkfmettdpstasslqakmkdlsar
2161 fseashkhketlakmeelktkvelfenlseklqtfletktqaltevdvpgkdvtelsqym
2221 qestseflehkkhlevlhslllkeisshglpsdkalvlektnnlskkfkemedtikekkea
2281 vtscqeqldafqvlvkslkswikettkkvpivqpsfgaedlgksledtkklqekwslktp
2341 eiqkvnnsgislcnlisavttpakaiaavksggavlngegtatnteefwankgltsikkd
2401 mtdishgyedlglllkdkiaelntklsklqkaqeessammqwlqkmnktatkwqqtpapt
2461 dteavktqveqnksfeaelkqnvnkvqelkdkltelleenpdtpeaprwkqmlteidskw
2521 qelnqltidrqqkleessnnltqfqtveaqlkqwlvekelmvsvlgplsidpnmlntqrq
2581 qvqillqefatrkpqyeqltaaqqgilsrpgedpslrgivkeqlaavtqkwdsltgqlsd
2641 rcdwidqaivkstqyqsllrslsdklsdldnklssslavsthpdamnqqletaqkmkqei
2701 qqekkqikvaqalcedlsalvkeeylkaelsrqlegilksfkdveqkaenhvqhlqsaca
2761 sshqfqqmsrdfqawldtkkeeqnkshpisakldvleslikdhkdfsktltaqshmyekt
2821 iaegenlllktqgsekaalqlqlntiktnwdtfnkqvkerenklkeslekalkykeqvet
2881 lwpwidkcqnnleeikfcldpaegensiaklkslqkemdqhfgmvellnntansllsvce
2941 idkevvtdenksliqkvdmvteqlhskkfclenmtqkfkefqevskeskrqlqcakeqld
3001 ihdslgsqaysnkyltmlqtqqkslqalkhqvdlakrlaqdlvveasdskgtsdvllqve
3061 tiaqehstlsqqvdekcsfletklqgighfqntiremfsqfaefddeldsmapvgrdaet
3121 lqkqketikaflkklealmasndnanktckmmlateetspdlvgikrdlealskqcnkll
3181 draqareeqvegtikrleefysklkefsillqkaeeheesqgpvgmetetinqqlnmfkv
3241 fqkeeieplqgkqqdvnwlgqqliqsaakststqglehdlddvnarwktlnkkvaqraaq
3301 lqeallhcgrfqdalesllswmvdteelvanqkppsaefkvvkaqiqeqkllqrllddrk
3361 stvevikregekiattaepadkvkilkqlslldsrweallnkaetrnrqlegisvvaqqf
3421 hetleplnewlttiekrlvncepigtqaskleeqiaqhkaleddiinhnkhlhqavsigq
3481 slkvlssredkdmvqskldfsqvwyieiqekshsrsellqqalcnakifgedevelmnwl
3541 nevhdklsklsvqdysteglwkqqselrvlqedillrkqnvdqallnglellkqttgdev
3601 liiqdkleaikarykditklstdvaktleqalqlarrlhsthleelctwldkvevellsye
3661 tqvlkgeeasqaqmrpkelkkeaknnkalldslnevssallelvpwrareglekmvaedn
3721 eryrlvsdtitqkveeidaailrsqqfdqaadaelswitetekklmslgdirleqdqtsa
3781 qlqvqktftmeilrhkdiiddlvksghkimtacseeekqsmkkkldkvlknydticqins
3841 erylqleraqslvnqfwetyeelwpwltetqsiisqlpapaleyetlrqqqeehrqlrel
3901 iaehkphidkmnktgpqllelspgegfsiqekyvaadtlysqikedvkkravaldeaisq
3961 stqfhdkidqilesleriverlrqppsisaevekikeqisenknvsvdmeklqplyetlk
4021 qrgeemiarsggtdkdisakavqdkldqmvfiwenihtlveereaklldvmelaekfwcd
4081 hmslivtikdtqdfirdledpgidpsvvkqqqeaaetireeidglqeeldivinlgseli
4141 aacgepdkpivkksidelnsawdslnkawkdridkleeamqaavqyqdglqavfdwvdia
4201 ggklasmspigtdletvkqqieelkqfkseayqqqiemerlnhqaelllkkvteesdkht
4261 vqdplmelkliwdsleeriinrqhklegallalgqfqhaldellawlthteqllseqkpv
4321 ggdpkaieielakhhvlqndvlahqstveavnkagndliessageeasnlqnklevlnqr
4381 wqnvlekteqrkqqldgalrqakgfhqeiedlqqwltdterhllaskplgglpetakeql
4441 nvhmevcaafeakeetykslmqkgqqmlarcpksaetnidqdinnlkekwesvetklner
4501 ktkleealnlamefhnslqdfinwltqaeqtlnvasrpslildtvlfqidehkvfanevn
4561 shreqiieldktgthlkyfsqkqdvvliknllisvqsrwekvvqrlvergrslddarkra
4621 kqfheawsklmewleeseksldseleiandpdkiktqlaqhkefqkslgakhsvydttnr
4681 tgrslkektsladdnlkldlddmlselrdkwdticgksverqnkleeallfsgqftdalqal
4741 idwlyrvepqlaedqpvhgdidlvmnlidnhkafqkelgkrtssvqalkrsareliegsr
4801 ddsswvkvqmqelstrwetvcalsiskqtrleaalrqaeefhsvvhallewlaeaeqtlr
4861 fhgvlpddedalrtlidqhkefmkkleekraelnkattmgdtvlaichpdsittikhwit
4921 iirarfeevlawakqhqqrlasalagliakqelleallawlqwaettltdkdkevipqei
4981 eevkaliaehqtfmeemtrkqpdvdkvtktykrraadpsslqshipvldkgragrkrfpa
5041 sslypsgsqtqietknprvnllvskwqqvwllalerrrklndaldrleelrefanfdfdi
5101 wrkkymrwmnhkksrvmdffrridkdqdgkitrqefidgilsskfptsrlemsavadifd
5161 rdgdgyidyyefvaalhpnkdaykpitdadkiedevtrqvakckcakrfqveqigdnkyr
5221 fflgnqfgdsqqlrlvrilrstvmvrvgggwmaldeflvkndpcrakgrtnmelrekfil
5281 adgasqgmaafrprgrrsrpssrgaspnrstsvssqaaqaaspqvpatttpkilhpltrn
5341 ygkpwltnskmstpckaaecsdfpvpsaegtpiqgsklrlpgylsgkgfhsgedsglitt
5401 aaarvrtqfadskktpsrpgsragskagsrassrrgsdasdfdiseiqsvcsdvetvpqt
5461 hrptpragsrpstakpskiptpqrkspaskldksskr
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BPA240 epitopes predicted to bind HLA-DQ7(DQβ1*0301)

Consensus sequence: IWHAVHAWH Optimal Score: 45.671

Binding Threshold: 11.70 All rows highlighted in red represent predicted binders.

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	2505	PAK	AIAAVKSGG	AVL	754.88	20.494	44.87 %
2	4035	KDI	IDDLVKSGH	KIM	965.07	19.992	43.77 %
3	5650	TSV	SSQAAQAAS	PQV	801.82	17.945	39.29 %
4	3969	VEE	IDAAILRSQ	QFD	968.13	17.195	37.65 %
5	299	IQW	IRHHVTTMS	ERT	1063.23	15.434	33.79 %
6	3858	ALQ	LARRLHSTH	EEL	1072.24	15.425	33.77 %
7	1543	LKD	AEKAGKPPF	SKQ	926.09	15.061	32.98 %
8	1363	LKY	YRDTYHPLD	DWI	1161.25	14.818	32.45 %
9	574	TSR	LTPSVTPAY	TPG	930.07	14.805	32.42 %
10	5722	RLP	GYLSGKGFH	SGE	947.06	14.776	32.35 %
11	1540	SKT	LKDAEKAGK	PPF	941.09	14.556	31.87 %
12	534	NSG	FAQTLHPSL	TSG	995.15	14.356	31.43 %
13	2376	LKE	ISSHGLPSD	KAL	893.96	14.332	31.38 %
15	4674	LDG	ALRQAKGFH	GEI	1009.18	13.651	29.89 %
19	2146	LLD	ARGSLLPAK	ND2	894.09	13.333	29.19 %
20	5765	RPG	SRAGSKAGS	RAS	801.86	13.219	28.94 %
21	526	ISG	ITQSLNSGF	AQT	948.04	12.997	28.46 %
22	5500	YYE	FVAALHPNK	DAY	978.16	12.924	28.30 %
23	3464	QGL	IQSAAKSTS	TQG	873.96	12.923	28.30 %
24	5084	RTS	SVQALKRSA	REL	941.1	12.679	27.76 %
25	2775	LRG	IVKEQLAAV	TQK	952.16	12.355	27.05 %
26	218	QSN	LANLEHAFY	VAE	1059.2	12.316	26.97 %
27	5576	STV	MVRVGGGWM	ALD	951.19	12.215	26.75 %
28	970	WKV	ISPTGNEAM	VPS	901.0	12.06	26.41 %
30	4038	IDD	LVKSGHKIM	TAC	994.25	11.771	25.77 %

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BMZ antibodies to α6 integrin. Patients with MMP and OCP demonstrated binding of BMZ antibodies to β4 integrin. Patients with MMP and OCP were grouped together for two reasons. The first is that many of the OCP patients had extraocular disease [44]. The second reason is that both subsets are characterized by the presence of anti-β4 integrin antibodies in their sera [19,30]. Moreover, a major aspect of this study was to examine whether epitopes that bind to T cell receptors in the various molecules in the BMZ, that are associated with the pathogenesis of pemphigoid, are similar in the computer model used in this study to those observed in in-vitro experiments. There is data in the literature to indicate that such models are effective in these predictions [45].

The role of BPAG1 and BPAG2 antigens in BP has been studied to understand the involvement of autoreactive T cells [20–22,26]. While most studies focus on the NC16A domain of the BPAG2 antigen, there are reports that implicate the domains other than NC16A in BPAG2 and others in BPAG1 that may be recognized by the autoreactive T cells [20,21]. After expressing the NC16A-mimicking residues 490–562 of the BP180 antigen extracellular domain as fusion proteins with Glutathione S-transferase (GST), some studies showed that T cells react with the entire sequence, as well as sequences 490–534 and 507–534 [22,26]. Interestingly, the computer model in this manuscript also demonstrates that sequence 505–513 among others is a T cell epitope for BPAG2.

Employing a different experimental technique, Thoma-Uszynski et al. have identified seven epitopes in Bacculovirus generated proteins to which autoreactive T cells bind to BPAG2 [20]. These epitopes were present in the extracellular domain of BP180 and are as follows: AA residues 490–1465, AA 490–812, AA 467–567, AA 1048–1465, AA 1352–1465, and AA 809–1106 [20]. Another study also reported T cell reactivity against residues 804–1430, indicating epitopes within the extracellular domain beyond the NC16A domain [21]. The computer model reported in this study demonstrates that the potential binding sites within BPAG2 are the following sequences: 505–513, 635–643, 765–773, 841–849, 1055–1063, 1192–1210, 1283–1291, and 1320–1328 among others. It is note-worthy that these sequences detected by the computer model were all present in the peptides created in the baculovirus system by previous investigators. This observation, in some significant measure, validates the accuracy and utility value of the computer model.

The BPAG1 antigen appears also to have epitopes recognized by the T cells. However, the T cell response to the BPAG1 antigen is somewhat less reproducible than that of the BPAG2 antigen [20]. In these experiments, peptides BP230-N (residues 1–1307), BP230-C1 (residues 1881–2649), and BP230-C2 (residues 2077–2649), produced in the baculovirus system, stimulated autoreactive T cells in patients with BP [20]. The computer model reported in this study demonstrates that the potential binding sites within BPAG1 are the following sequences: 209–217, 547–555, 925–933, 1295–1303, 2029–2038, and 2366–2374 among others. Of note is the observation that the T cell epitopes detected by earlier investigators were similar to those predicted by the computer model. As in the case of BPAG2, our observations in BPAG1 demonstrate similar T cell epitopes, validating the utility, value, and high degree of predictability of the computer model.

The computer model presented in this manuscript demonstrates that the relevant epitopes present in BPAG1 and BPAG2 can be presented to HLA-DQβ1*0301 and then be presented to T cell receptors and thus produce the necessary T cell responses. The sites on human integrin β4 subunit that bind to the T cell receptor in MMP or OCP have not been described. Similarly, the binding sites for the T cell receptor on integrin subunit α6 in patients with OP have not been described. However, given that the computer model has predicted T cell receptor binding sites within BPAG1 and BPAG2, that were similar to those found in the literature [20], it can be anticipated that, in all likelihood, the T cell epitopes predicted by the computer in human α6 and β4 integrin subunits will be similar to those done in experimental studies using human T cells from patients with OP, MMP, and OCP.

In the literature, there are three studies on patients with BP in whom HLA-MHC II genes have been reported [46–48]. In two of these studies containing 97 patients, a statistically significant increased incidence of HLA-DQB1*0301 has been observed [46,48]. In a study of 25 Northern Chinese patients with BP, no statistically significant frequency of DQβ1*0301 was observed [47]. This difference reflects the difference in the genetic background between Chinese and the patients in the other studies.

Four studies, which included 224 Caucasian patients with MMP reported documented a statistically significant incidence of HLA-DQB1*0301 [32,33,49,50]. Two studies on 20 Caucasian patients with OCP reported a statistically significant increased frequency of OCP with DQβ1*0301 [33,51]. One study on 20 Caucasian patients with OP showed a statistically significant association with DQβ1*0301 [49]. In another study of 11 Caucasian patients with OP, 64% carried DQβ1*0301 allele [33]. However, the authors were unable to demonstrate a statistically significant observation primarily because of the use of inappropriate controls.

The cumulative literature demonstrates that the HLA Class II DQβ1*0301 allele is the most frequently observed HLA Class II allele in patients with all the clinical variants or subsets of pemphigoid disease in a statistically significant correlation.

In several autoimmune diseases, studies on the immunogenetics have provided significant information in understanding their pathogenesis [52–55]. In some autoimmune diseases with multiple subtypes, a common HLA allele association has been observed. HLA-DRβ1*1501 has been shown to be associated with both benign and malignant multiple sclerosis [56]. DRβ1*0405-DQβ1*0401/ DRβ1*0802-DQβ1*0302 genotype was shown to be associated with both acute-onset and slowly progressive type 1diabetes, while fulminant diabetes was associated with DRβ1*0405-DQβ1*0401/DRβ1*0405-DQβ1*0401 genotype, as shown by a study on a Japanese population [57].

This study has provided a computer-based model, which partially explains the mechanisms by which a single HLA allele (DQβ1*0301) present in all subsets of Pg patients, is capable of binding to multiple T cell epitopes within BPAG1, BPAG2, α6 integrin, and β4 integrin. This binding to the T cell receptor is capable of stimulating antigen specific T cells. These T cells will interact with B cells through the CD40-CD40L to produce four distinct anti-BMZ antibodies with different specificities. These four different anti-BMZ antibodies will bind to their specific target antigen and through a series of biochemical phenomena, result in the production of a subepidermal blister. In BP, such blisters will heal without scar formation. Patients with OP recover from their blistering disease without any scar formation. In contrast, patients with MMP and OCP, usually, during the healing process, develop irreversible scar formation. This scarring process can cause significant morbidity, compromise the quality of life, and in patients with ocular involvement, can result in blindness [12]. Thus, the above described process results in four distinct clinical entities, each with a different clinical outcome as demonstrated in Fig. 5. The authors recognize that while the phenotypic presentation of Pg may be influenced by different genetic factors, non-genetic factors, and soluble and insoluble mediators of immune and inflammatory processes, non-the-less HLA-DQβ1*0301 may play an important role in the pathogenesis of these clinical variants of pemphigoid.

Fig. 2. Binding sites in Bullous Pemphigoid Antigen 1 (BP240) for DQ7(DQβ1*0301). Bullous Pemphigoid Antigen 1 (BP240) peptide antigens predicted to bind to HLA-DQ7 (DQβ1*0301) are shown in yellow. All shown peptides exceed the Binding Threshold (see Methods). POS. = Position; N = N-terminal; C = C-terminal; MW = Molecular Weight (Daltons); %OPT. = Optimal Score.

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Integrin alpha chain, alpha 6 isoform a precursor [Homo sapiens].

Epitopes predicted to bind to HLA-DQ7(DQβ*0301) are represented in yellow.

MAAAGQLCLLYLSAGLLSRLGAAFNLDTREDNVIRKYGDPGSLFGFSLAMHWQLQP
EDKRLLLVGAPRAEALPLQRANRTGGLYSCDITARGPCTRIEFDNDADPTSESKED
QWMGVTVQSQGPGGKVVTCAHRYEKRQHVNTKQESRDIFGRCYVLSQNLRIEDDMD
GGDWSFCDGRLRGHEKFGSCQQGVAATFTKDFHYIVFGAPGTYNWKGIVRVEQKNN
TFFDMNIFEDGPYEVGGETEHDESLVPVPANSYLGFSLDSGKGIVSKDEITFVSGA
PRANHSGAVVLLKRDMKSAHLLPEHIFDGEGLASSFGYDVAVVDLNKDGWQDIVIG
APQYFDRDGEVGGAVYVYMNQQGRWNNVKPIRLNGTKDSMFGIAVKNIGDINQDGY
PDIAVGAPYDDLGKVFIYHGSANGINTKPTQVLKGISPYFGYSIAGNMDLDRNSYP
DVAVGSLSDSVTIFRSRPVINIQKTITVTPNRIDLRQKTACGAPSGICLQVKSCFE
YTANPAGYNPSISIVGTLEAEKERRKSGLSSRVQFRNQGSEPKYTQELTLKRQKQK
VCMEETLWLQDNIRDKLRPIPITASVEIQEPSSRRRVNSLPEVLPILNSDEPKTAH
IDVHFLKEGCGDDNVCNSNLKLEYKFCTREGNQDKFSYLPIQKGVPELVLKDQKDI
ALEITVTNSPSNPRNPTKDGDDAHEAKLIATFPDTLTYSAYRELRAFPEKQLSCVA
NQNGSQADCELGNPFKRNSNVTFYLVLSTTEVTFDTPDLDINLKLETTSNQDNLAP
ITAKAKVVIELLLSVSGVAKPSQVYFGGTVVGEQAMKSEDEVGSLIEYEFRVINLG
KPLTNLGTATLNIQWPKEISNGKWLLYLVKVESKGLEKVTCEPQKEINSLNLTESH
NSRKKREITEKQIDDNRKFSLFAERKYQTLNCSVNVNCVNIRCPLRGLDSKASLIL
RSRLWNSTFLEEYSKLNYLDILMRAFIDVTAAAENIRLPNAGTQVRVTVFPSKTVA
QYSGVPWWIILVAILAGILMLALLVFILWKCGFFKRSRYDDSVPRYHAVRIRKEER
EIKDEKYIDNLEKKQWITKWNENESYS

Alpha 6 Integrin binding to DQ7(DQβ*0301)

Consensus sequence: IWHAVHAWH Optimal Score: 45.671
Binding Threshold: 11.70 All rows highlighted in red represent predicted binders

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	34	DNV	IRKYGDPGS	LFG	974.09	14.91	32.65 %
2	341	PQY	FDRDGEVGG	AVY	932.95	14.743	32.28 %
3	503	KSC	FEYTANPAG	YNP	951.01	14.403	31.54 %
4	495	PSG	ICLQVKSCF	EYT	1022.29	14.108	30.89 %
5	1050	YDD	SVPRYHAVR	IRK	1066.24	12.743	27.90 %
6	280	VSG	APRANHSGA	VVL	861.92	12.698	27.80 %
7	174	DWS	FCDGRLRGH	EKF	1042.19	12.271	26.87 %

Fig. 3. Binding sites in human integrin α6 for DQ7(DQβ1*0301). α6 peptide antigens predicted to bind to HLA-DQ7(DQβ1*0301) are shown in yellow. All shown peptides exceed the Binding Threshold (see Methods). POS. = Position; N = N-terminal; C = C-terminal; MW = Molecular Weight (Daltons); %OPT. = Optimal Score.

Epitopes predicted to bind are highlighted in the sequence as yellow.

NP_000204.3 **Integrin beta 4 isoform 1 precursor**
Description
Transcript Variant: This variant (1) encodes the longest isoform (1).

1 magprpspwarlllaalisvslsgtlanrckkapvksctecvrvdkdcayctdemfrdrr
61 cntqaellaagcqresivvmessfqiteetqidttlrrsqmspqglrvrlrpgeerhfel
121 evfeplespvdlyilmdfsnsmsddldnlkkmgqnlarvlsqltsdytigfgkfvdkvsv
181 pqtdmrpeklkepwpnsdppfsfknvisltedvdefrnklqgerisgnldapeggfdail
241 qtavctrdigwrpdsthllvfstesafhyeadganvlagimsrnderchldttgtytqyr
301 tqdypsvptlvrllakhniipifavtnysysyyeklhtyfpvsslgvlqedssnivelle
361 eafnrirsnldiraldsprglrtevtskmfqktrtgsfhirrgevgiyqvqlralehvdg
421 thvcqlpedqkgnihlkpsfsdglkmdagiicdvctcelqkevrsarcsfngdfvcgqcv
481 csegwsgqtcnctgslsdiqpclregedkpcsgrgecqcghcvcygegryegqfceydn
541 fqcprtsgflcndrgrcsmgqcvcepgwtgpscdcplsnatcidsnggicngrghcecgr
601 chchqqslytdticeinysaihpglcedlrscvqcqawgtgekkgrtceecnfkvkmvde
661 lkraeevvvrcsfrdeddctysytmegdgapgpnstvlvhkkkdcppgsfwwlipllll
721 llpllallllcwkycacckaclallpccnrghmvgfkedhymlrenlmasdhldtpmlr
781 sgnlkgrdvvrwkvtnnmqrpgfathaasinptelvpyglslrlarlctenllkpdtrec
841 aqlrqeveenlnevyrqisgvhklqqtkfrqqpnagkkqdhtivdtvlmaprsakpallk
901 ltekqveqrafhdlkvapgyytltadqdargmvefqegvelvdvrvplfirpedddekql
961 lveaidvpagtatlgrrlvnitiikeqardvvsfeqpefsvsrgdqvaripvirrvldgg
1021 ksqvsyrtqdgtaqgnrdyipvegellfqpgeawkelqvkllelqevdsllrgrqvrrfh
1081 vqlsnpkfgahlgqphsttiiirdpdeldrsftsqmlssqppphgdlgapqnpnakaags
1141 rkihfnwlppsgkpmgyrvkywiqgdseseahlldskvpsveltnlypycdyemkvcayg
1201 aqgegpyssIvscrthqevpsepgrlafnvvsstvtqlswaepaetngeitayevcyglv
1261 nddnrpigpmkkvlvdnpknrmllienlresqpyrytvkarngagwgpereaiinlatqp
1321 krpmsipiipdipivdaqsgedydsflmysddvlrspsgsqrpsvsddtgcgwkfeplllg
1381 eeldlrrvtwrlppeliprlsassgrssdaeaphgppddggaggkggslprsatpgppge
1441 hlvngrmdfafpgstnslhrmtttsaaaygthlsphvphrvlstsstltrdynsltrseh
1501 shsttlprdystltsvsshdsrltagvpdtptrlvfsalgptslrvswqeprcerplqgy
1561 sveyqllnggelhrlnipnpaqtsvvvedllpnhsyvfrvraqsqegwgreregvities
1621 qvhpqsplcplpgsaftlstpsapgplvftalspdslqlswerprrpngdivgylvtcem
1681 aqgggpatafrvdgdspesrltvpglsenvpykfkvqarttegfgperegiitiesqdgg
1741 pfpqlgsraglfqhplqseyssittthtsatepflvdgltlgaqhleaggsltrhvtqef
1801 vsrtlttsgtlsthmdqqffqt

Beta 4 Integrin binding to DQ7(DQβ*0301)
Consensus sequence: IWHAVHAWH
Optimal Score: 45.671
Binding Threshold: 11.70
All rows highlighted in red represent predicted binders.

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	1208	FNW	LPPSGKPMG	YRV	865.06	20.182	44.19 %
2	1362	SQP	YRYTVKARN	GAG	1152.32	19.166	41.97 %
3	647	CEI	NYSAIHPGL	CED	953.07	19.047	41.70 %
4	932	VLM	APRSAKPAL	LK9	892.08	18.434	40.36 %
5	771	CWK	YCACCKACL	ALL	959.23	16.829	36.85 %
6	1014	EAI	DVPAGTATL	GRR	825.91	15.922	34.86 %
7	911	QTK	FRQQPNAGK	KQD	1027.15	14.639	32.05 %
9	425	EVG	IYQVQLRAL	EHV	1085.32	14.228	31.15 %
10	1421	FLM	YSDDVLRSP	SGS	1033.12	13.589	29.75 %
11	1689	FRV	RAQSQEGWG	RER	977.04	13.035	28.54 %
12	661	DLR	SCVQCQAWG	TGE	940.09	12.643	27.68 %
13	970	TLT	ADQDARGMV	EFQ	944.03	12.108	26.51 %
14	1060	RIP	VIRRVLDGG	102	966.15	11.829	25.90 %

Fig. 4. Binding sites in human integrin β4 for DQ7(DQβ1*0301). β4 peptide antigens predicted to bind to HLA-DQ7(DQβ1*0301) are shown in yellow. All shown peptides exceed the Binding Threshold (see Methods). POS. = Position; N = N-terminal; C = C-terminal; MW= Molecular Weight (Daltons); %OPT. = Optimal Score.

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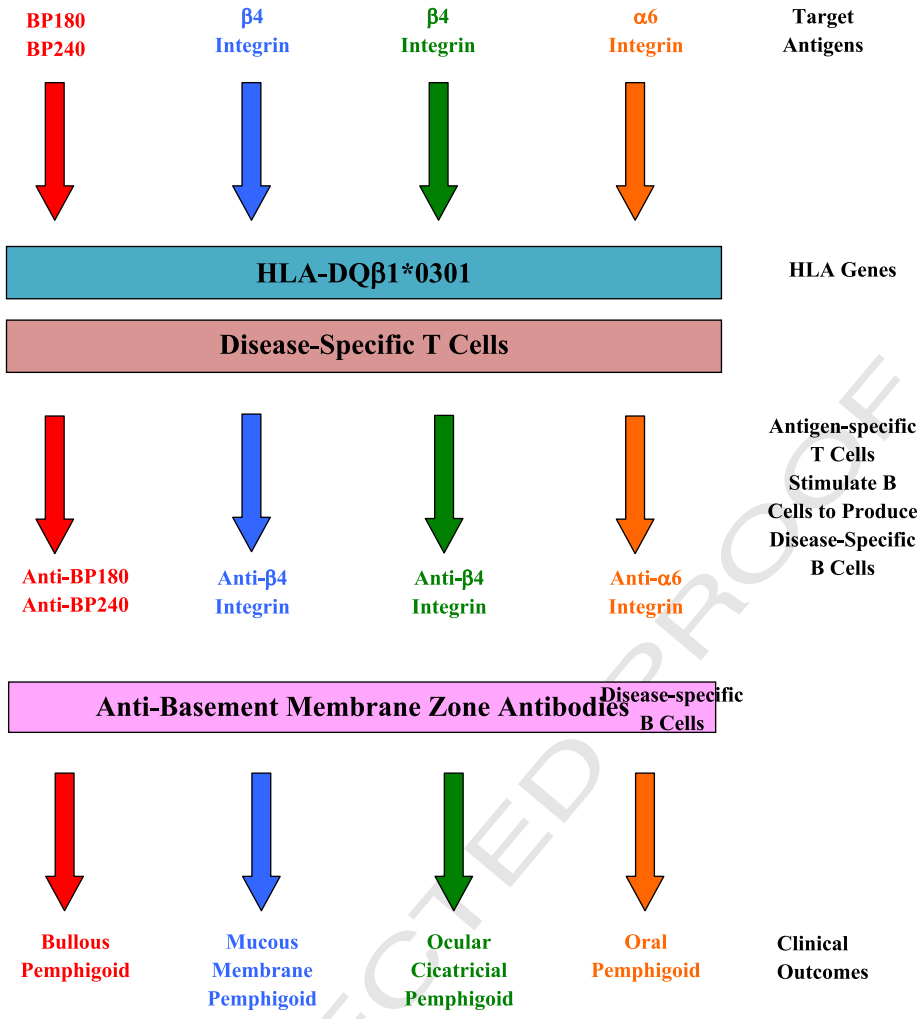

Fig. 5. Summary of proposed mechanism by which a single HLA allele (DQβ1*0301) binds all four different pemphigoid antigens and produces four distinct phenotypes of pemphigoid disease. Variants of pemphigoid disease - bullous pemphigoid, mucous membrane pemphigoid, ocular cicatricial pemphigoid, and oral pemphigoid, all are characterized by anti-BMZ antibodies. Each of these has different antigenic targets. They all have a common HLA MHC Class II allele in high frequency associated with them, and it recognizes epitopes within each of these antigens. Resultantly, a different antibody is produced in each variant producing a different phenotype. (BP240 = Bullous Pemphigoid Antigen 1; BP180 = Bullous Pemphigoid Antigen 2; HLA = Human Leukocyte Antigen).

Take-home messages

- Pemphigoid has four clinically dissimilar variants. These are BP, MMP, OCP, and OP.
- All variants produce anti-BMZ antibodies, each targeting different antigens.
- The antigens: BPAG1 and BPAG2 in BP; β4 integrin in MMP and OCP; α6 integrin in OP.
- All variants have significant enhanced susceptibility associated with HLA-DQβ1*0301.
- T cell epitopes in the antigens bound to DQβ1*0301 by computer model.

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