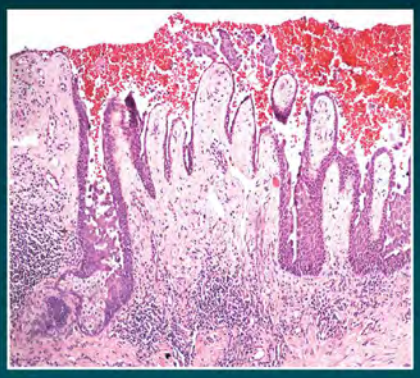
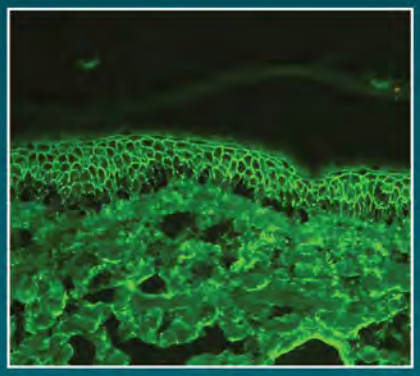
ers.com, for detailed information on dermatology books, visit our website www.jaypeebrothers.com, for detailed information on dermatology boo

Under the Aegis of IADVL Academy


IADVL Textbook of Pemphigus and other Autoimmune Bullous Diseases

Sujay Khandpur
Raghavendra Rao
Vinay Keshavamurthy
Atiya Yaseen
Saritha Mohanan

Foreword
Takashi Hashimoto

JAYPEE

Contents

Section 1: Basic Science in Pemphigus and other Autoimmune Bullous Diseases

Chapter 1: **Ultrastructure of the Skin in Relation to Pemphigus and other Autoimmune Bullous Diseases** 3
Rajat Choudhary, Neha Taneja

Chapter 2: **Principles of Immunology** 14
Vishakha Hooda, Alpana Sharma

Chapter 3: **Etiopathogenesis of Pemphigus** 23
Sunil Dogra, Shikha Shah

Chapter 4: **Etiopathogenesis of Autoimmune Bullous Diseases excluding Pemphigus** 30
Laxmisha Chandrashekar

Section 2: Clinical Aspects of Pemphigus and other Autoimmune Bullous Diseases

Chapter 5: **Clinical Classification of Pemphigus and other Autoimmune Bullous Diseases** 45
Atiya Yaseen

Intraepidermal Autoimmune Bullous Diseases

Chapter 6: **Pemphigus Vulgaris** 51
Rhea Ahuja, Sujay Khandpur

Chapter 7: **Pemphigus Foliaceus** 65
Ajithkumar Kidangazhiathmana, Geethanjali S

Chapter 8: **Paraneoplastic Pemphigus (Paraneoplastic Autoimmune Multiorgan Syndrome)** 71
Vishal Gaurav, Deepika Pandhi, Kyle T Amber

Chapter 9: **Other Variants of Pemphigus** 84
Saritha Mohanan, C Udayashankar

Subepidermal Autoimmune Bullous Diseases

Chapter 10: Bullous Pemphigoid and the Pemphigoids 91
Geeti Khullar, Luca Borradori

Chapter 11: Mucous Membrane Pemphigoid, Anti-laminin 332 Pemphigoid, Epidermolysis Bullosa Acquisita, Bullous Systemic Lupus Erythematosus 102
Neirita Hazarika, Riti Bhatia

Chapter 12: Linear Immunoglobulin A Disease, Chronic Bullous Disease of Childhood, Dermatitis Herpetiformis 113
Chandana Shajil, Dharshini Sathishkumar, Minu Jose Chiramel

Scoring Systems and Clinical Approach to Pemphigus and other Autoimmune Bullous Diseases

Chapter 13: Scoring Systems and Quality of Life Indices 125
Abir Saraswat, Swastika Suvirya, Sonal Sachan

Chapter 14: Clinical Approach to Autoimmune Bullous Diseases 138
Vignesh Narayan R, Rashmi Sarkar

Section 3: Diagnostic Aspects of Pemphigus and other Autoimmune Bullous Diseases

Chapter 15: Histopathological Aspects 149
Neha Taneja, Rhea Ahuja, M Ramam

Chapter 16: Immunofluorescence and Immune Electron Microscopy 158
Raghavendra Rao, Pallavi Hegde

Chapter 17: Newer Diagnostic Techniques 167
Ananya Sharma, Sujay Khandpur

Chapter 18: Immunoblot Assay and Immunoprecipitation 182
Sujay Khandpur, Xiaoguang Li, Takashi Hashimoto, Vinay Keshavamurthy

Chapter 19: Diagnostic Approach to Autoimmune Bullous Diseases 191
Anuradha Bishnoi, Shikha Shah, Debajyoti Chatterjee

Section 4: Therapeutic Strategies for Pemphigus and other Autoimmune Bullous Diseases

Chapter 20: Topical Therapy and Oral Corticosteroids 203
Sanjeev Handa, Shikha Shah

Chapter 21: Oral Anti-inflammatory and Immunosuppressive Therapies 209
Chitra Shivanand Nayak, Gayatri Vitthal Gund

Chapter 22: Pulse Therapy and its Modifications **218**
Binod K Khaitan, Vishal Gupta, Bhavya Swarnkar

Chapter 23: Rituximab in Pemphigus and other Autoimmune Bullous Diseases **225**
Hitaishi Mehta, Vinay Keshavamurthy

Section 5: Other Therapeutic Interventions for Pemphigus and other Autoimmune Bullous Diseases

Chapter 24: Intravenous Immunoglobulin, Omalizumab, Newer Therapeutic Interventions including Emerging Therapies **237**
Rajat Choudhary, Vishal Gupta

Chapter 25: Miscellaneous Therapies **245**
Krina Bharat Patel, Pooja Desai

Chapter 26: Treatment Guidelines, Consensus Statements, Appraisal of Evidence for Pemphigus and other Autoimmune Bullous Diseases, Management in COVID Era **250**
Dipankar De, Hitaishi Mehta, Rahul Mahajan

Chapter 27: Nursing Care in Autoimmune Bullous Diseases **257**
Rochelle C Monteiro, Amitha Ramesh, Ramesh Bhat M

Index **267**

CHAPTER 4

Etiopathogenesis of Autoimmune Bullous Diseases excluding Pemphigus

Laxmisha Chandrashekar

- Animal models in autoimmune bullous diseases
- Pathogenesis of:
  - Bullous pemphigoid
  - Pemphigoid gestationis
  - Lichen planus pemphigoides
  - Epidermolysis bullosa acquisita
  - Mucous membrane pemphigoid
  - Dermatitis herpetiformis
  - Linear IgA dermatosis
  - Bullous systemic lupus erythematosus
  - Anti p200 pemphigoid (anti-laminin γ1 pemphigoid)

INTRODUCTION

Autoimmune bullous diseases (AIBDs) are characterized by blisters and erosions which involve the skin and mucosae. The subepidermal AIBDs are characterized by autoantibodies against components of the basement membrane zone (BMZ). Central to the pathogenesis is the loss of immunological tolerance leading to the production of autoreactive T and B cells. Loss of immune tolerance could be due to a genetic predisposition or infections, drugs, malignancies, etc. There are also associated abnormalities in T regulatory cells. Autoreactive B cells produce pathogenic antibodies against components of the BMZ. Activation of complement results in activation and recruitment of effector cells which release inflammatory mediators and cytokines, leading to tissue damage. In some cases, complement-independent blistering can also occur.

ANIMAL MODELS IN AUTOIMMUNE BULLOUS DISEASES

As discussed in Chapter 3, the animal models are of two types—(1) passive disease model and (2) active model.

Passive Model

Neonatal and Adult Mice

The animal is injected with autoantibodies (either from serum of patients with disease, or from generation of autoantibodies in rabbit). This is useful for understanding the antibody-mediated effects and tissue damage **(Figs. 1A to C)**.

Administering immunoglobulin G (IgG) from individuals with pemphigus vulgaris (PV) into newborn BALB/c mice caused the mice to develop blisters. This is an example of passive model. However, it is not ideal for studying long-term effects or therapeutic interventions.

Passive models involve both neonatal and adult mice. The quantity of antibodies needed to elicit a reaction in adult mice is higher compared to neonatal mice.

Human Skin/Humanized Mice

This enables the replication of human disease. This is done either by using a humanized mouse or by transplantation of human skin in immunodeficient mice with severe combined immunodeficiency (SCID). This technique is used to study the role of anti-laminin 332 antibodies in disease pathogenesis. Human anti-bodies fail to bind to the skin of neonatal mice. Hence, SCID mice with transplanted human skin from a healthy volunteer are used. The injection of either rabbit anti-laminin 332 or patient IgG results in blisters without inflammation.

Active Model

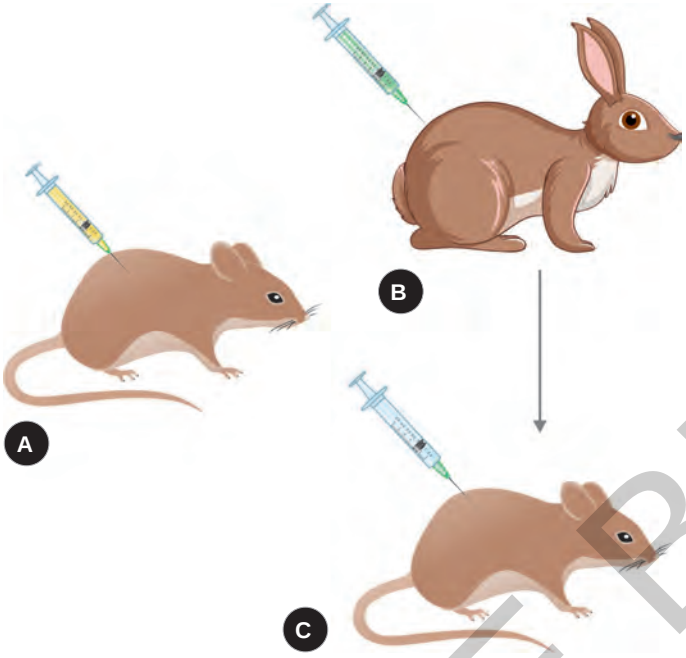
Active models include transgenic models, forced immunization models, and autoreactive lymphocyte transfer.

- *Transgenic model*: These are genetically modified mice. These include the introduction of human autoantigens, knockout of its genes and change of function of endogenous genes, e.g., the production of XVII collagen by transgenic mice. This skin when grafted to syngeneic wild mice produces autoantibodies to collagen XVII.
- *Forced immunization model*: Here, the wild or transgenic mice are injected with an autoantigen to generate an immune response to produce antibodies, e.g., the injection of the NC-1domain of murine collagen into

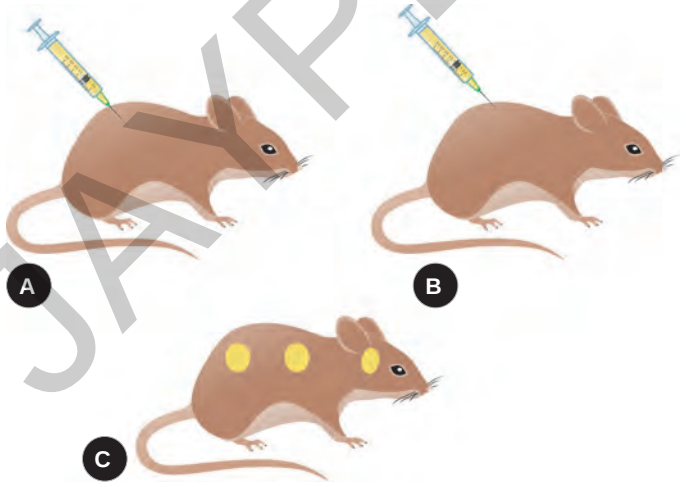
JAYPEE BROTHERS

mice. With further boosts, autoantibody is produced against collagen VII in the mice **(Figs. 2A to C)**.

- *Autoreactive lymphocyte transfer*: These models are used to overcome self-tolerance which prevents the mouse's immune system from reacting with the human antigen, e.g., the generation of a knockout mouse lacking the autoantigen followed by immunization with the autoantigen. The splenocytes containing both B and T cells are transferred to Rag2 (immunocompromised mice) knockouts expressing the autoantigen, which initiates an immune response.



Figs. 1A to C: Passive animal model: (A) mouse is being injected with human IgG, (B) rabbit is injected with murine antigen (C) antibody to the murine protein is injected in to the mouse.



Figs. 2A to C: Active animal model: (A and B) Mouse is injected with repeat dose of autoantigen to produce autoantibodies (C) erosions and blisters noted over the mouse following development of autoantibodies.

PATHOGENESIS OF AUTOIMMUNE BULLOUS DISEASES

Bullous Pemphigoid (BP)

In bullous pemphigoid (BP), the blisters are caused by autoantibodies directed against the hemidesmosome components.

- *Genetic factors* include human leukocyte antigen (HLA) DQB1*0301 in Caucasians and DRB1*04, DRB1*1101, and DQB1*0302 among the Japanese. Gene polymorphisms [cytokine genes interleukin-8 (IL-8) and IL-1β, MT-ATP8] are also associated with BP.
- *Triggers*: Drugs, vaccines, viral infections, radiotherapy, and neurological and neuro-degenerative diseases are associated with BP.
- *Antigens*: BP180 (NC16A) and BP 230 are the antigens.

  BP180 or collagen XVII is a transmembrane protein with the N-terminal within the hemidesmosome plaque and the C-terminal in the lamina lucida. The non-collagenous domain (NC16A) is the target of most autoantibodies. BP 230 belongs to the plakin family and connects the keratin intermediate filaments with the hemidesmosomes **(Figs. 3 and 4)**.

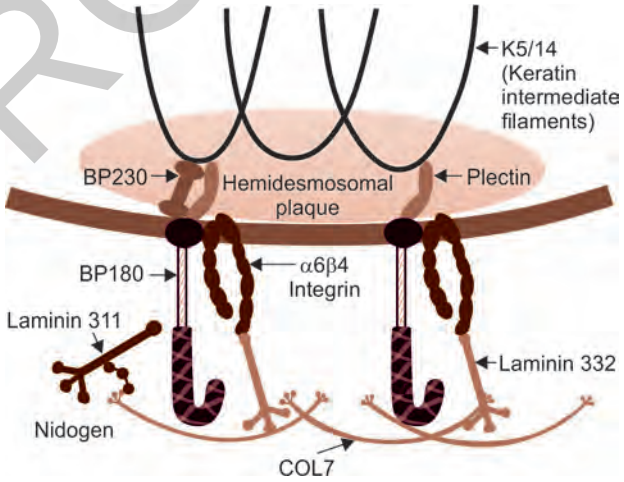


Fig. 3: Structure of basement membrane zone.

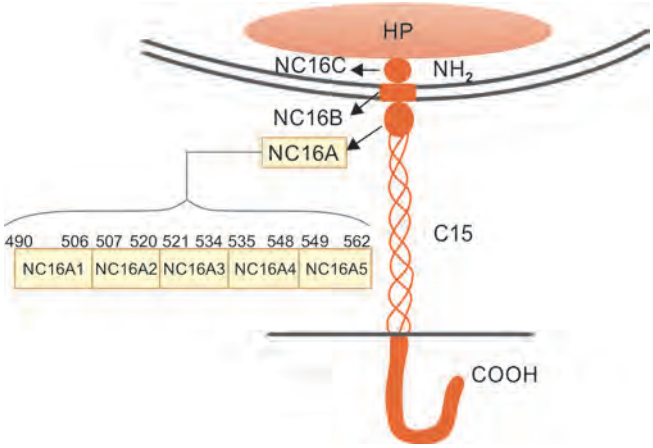


Fig. 4: Structure of BP180 showing the main antigen target (NC16A). (HP: hemidesmosomal plaque; NC: non-collagenous)

JAYPEE BROTHERS

- *Autoantibodies*: IgG and/or IgE directed against BP180 (NC16A) and BP 230.

Mouse Models

Passive model: The major limitation of using patient serum is the lack of cross-reactivity between the human BP 180 and the murine analog. To overcome this, rabbits are first immunized with the murine fusion protein (mus 180A). This leads to antibody production in the rabbit, which is injected into neonatal mice. This results in the development of redness and slight epidermal change on mild pressure. Histology of the skin reveals a subepidermal blister, and there is deposition of IgG and complement at the BMZ.

Cobra venom was used to study the effect of complement. When mice complement was depleted by cobra venom and then injected with the murine antibody, there were no skin changes. The disease could not be replicated in knockout models of C5 deficient and FcyR111 (receptor on neutrophils) mice. This highlights the role of complement and neutrophils in disease pathogenesis.

Active model: One of the limitations of animal models is that they are transient and cannot fully replicate human disease. This is overcome through the use of an active model using knockouts and adoptive transfer.

Steps

- Rag2 deficient mice crossed with COL XV11-humanized mice (lack mouse COL XV11, but have human XV11).
- Produce Rag2-/-/COL XV11 humanized mice.
- Grafting (immunization) is performed from COLXVII-transgenic mice onto matched 6-week-old wild-type mice.
- Isolation of splenocytes from the grafted wild-type mice.
- Injection of these splenocytes into the Rag2-/-/COL XV11 humanized mice.
- Recipient mice produce antibodies to collagen XV11 and develop blisters.
- Depletion of CD4+ cells results in no antibody production to collagen XV11.

Steps in Disease Pathogenesis (Fig. 5)

Complement Dependent Pathway:

- Loss of immune tolerance.
- This leads to the production of autoantibodies against BP180 and 230.
- IgG1 and IgG3 autoantibodies along with complement activate effector cells (neutrophils and eosinophils).
- IgE antibodies to NC16A activate and recruit eosinophils, causing them to degranulate and help in blister formation.
- Release of proteolytic enzymes [matrix metalloproteinase-9 (MMP-9) and neutrophil elastase], cytokines, and chemokines.
- Inflammation and blister formation.

The classical and alternate pathways (lesser extent) are involved in blister formation. IL-17 is elevated in the serum of patients and plays a vital role in inducing blister formation. Eosinophils activate tissue factors which further activate the coagulation pathway, contributing to blister formation. Monocytes increase the capacity of neutrophils to release MMP-9. Among the other proteolytic enzymes, granzyme B also has been reported to be elevated in BP and contributes to blistering.

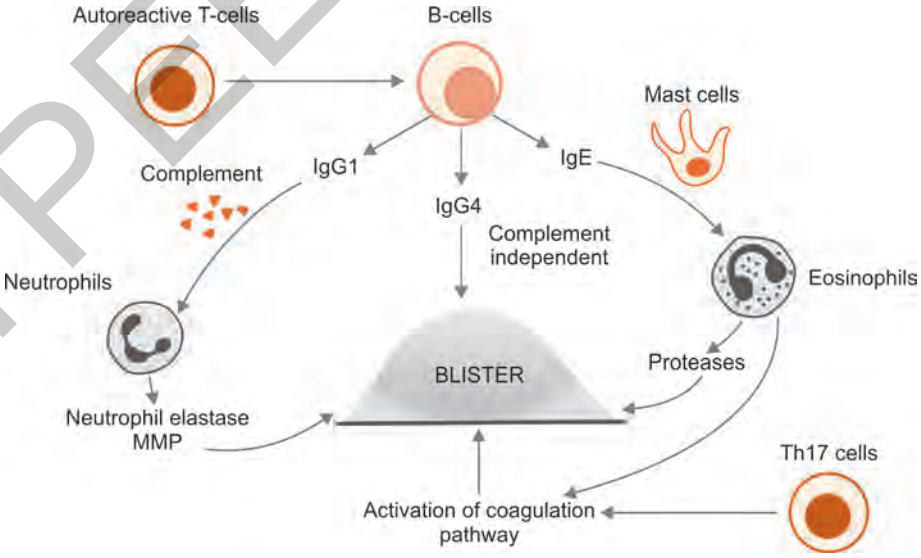


Fig. 5: Pathogenesis of bullous pemphigoid: Showing both complement-mediated and independent mechanisms of blistering. *Image courtesy*: Dr Vinupriya S, Assistant Professor, Department of Dermatology, KMCH Institute of Sciences and Research, Coimbatore, Tamil Nadu, India.
(MMP: matrix metalloproteinase; Th17: T helper 17)

JAYPEE BROTHERS

Complement-independent Mechanisms

Macropinocytosis, and proteasome- and ubiquitin-mediated degradation of BP 180-The production of anti-BP180 induces internalization of the antigen complexed with antibody through pinocytosis, which eventually leads to blister formation **(Fig. 6)**.

Probable steps:

- Internalization of BP180 with antibody by pinocytosis
- Weakening of BMZ
- Degradation of these by proteasomes and/or lysosomes
- Release of cytokines (IL-6 and IL-8) by keratinocytes which help in blister formation. IL-8 released from keratinocytes recruits and activates neutrophils. IL-6 contributes to tissue damage.
- *IgE and eosinophils*: Anti BP180 IgE and BP180-IgE complexes bind to the BMZ and FcεR1 receptors on mast cells, eosinophils, and basophils. This causes degranulation and release of pro-inflammatory mediators [eosinophil granule proteins and reactive oxygen species (ROS)]. Keratinocytes also release eotaxin-1 and IL-5 resulting in a positive loop contributing to inflammation and blister formation.
- IgG4 antibodies are capable of inducing non-inflammatory BP in the absence of complement.

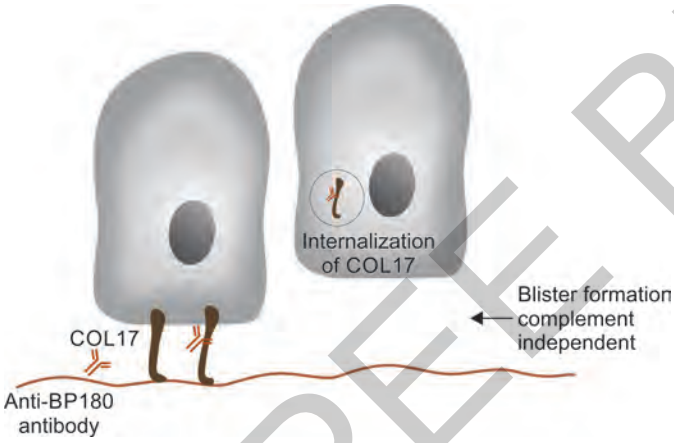


Fig. 6: Pathogenesis of bullous pemphigoid: Production of anti-BP180 induces internalization of the antigen complexed with antibody through pinocytosis, which eventually leads to blister formation.

Associations

- *Neurologic diseases:* Patients with neurologic diseases have a higher risk of developing BP. One hypothesis is that in neurologic diseases, there is a loss of self-tolerance to BP 180, and a degree of crosstalk exists between brain BP180 and skin BP180.
- *Thrombosis*: There is an increased risk of thrombosis and thrombo-embolism among patients with BP. Inflammation in BP triggers the coagulation cascade. D-dimer and prothrombin fragment (F1+2) have been reported to increase in patients with BP and parallel autoantibody BP180 level.
- *Vitamin D deficiency*: Patients with BP have a higher prevalence of vitamin D deficiency.

Drug-induced Pemphigoid (Dipeptidyl Peptidase-4 Inhibitors)

Dipeptidyl peptidase-4 (DPP) inhibitors, also known as gliptins, are commonly used anti-diabetic medications. Numerous studies have established a link between drug use and risk of BP.

DPP-4 (CD26) is part of the prolyloligopeptidase group of enzymes, which breaks down numerous bioactive peptides. It is widely expressed in different cell types, including keratinocytes and T-lymphocytes. Studies have reported that the expression of DPP-4/CD26 is increased in BP patients regardless of prior treatment with gliptins. Inhibition of DPP-4 increases the pro-inflammatory cytokines with eosinophilic stimulation, and an impact on plasminogen activation and plasmin formation.

Among the Japanese, specific unique immunological characteristics have been described. They are predominantly non-inflammatory and associated with the HLA-DQB1*03:01 allele. However in Europeans, no such differences have been reported.

Pemphigoid Gestationis

It is a specific pregnancy dermatosis. It typically appears during the third trimester but can also occur in the second trimester or after delivery. It is mostly self-limiting and disappears by 6 months post-delivery. If a woman has pemphigoid gestationis (PG) during one pregnancy, there is a 90% chance that it will recur in future pregnancies and be more severe. However, if the woman changes partner, the risk of the condition occurring in subsequent pregnancy drops to 5%. It is associated with hyperthyroidism, molar pregnancy, trophoblastic tumors, and choriocarcinoma.

Genetic Factors

- *Major histocompatibility complex (MHC):* MHC class II plays a crucial role in the development of PG. Maternal HLA-DRB1*0301 and DRB1*0401/040X alleles are strongly associated with PG. Both haplotypes are reported in 45% of cases and 3% of controls. Trophoblast and stromal amniochorionic cells express these haplotypes.
- *Aberrant complement system*: C4*QO is present in 90% of PG cases which may hinder the removal of immune complexes and facilitates deposition. This allele is in strong linkage disequilibrium with the DR-3 and DR-4 alleles; hence, pathogenicity is in doubt.
- *Autoantibody target*: IgG antibodies (predominantly IgG1 subclass) directed against NC16A BP180. Antibodies to full-length BP180 and BP230 have also been reported.
- *Modulation*: Progesterone and estrogen modulate disease activity. Disease severity decreases in late pregnancy on account of high progesterone. Postpartum disease severity increases accompanied by reduced progesterone and increased estrogen levels.

JAYPEE BROTHERS

Steps in disease pathogenesis:

- Loss of immune privilege of the fetoplacental unit
- BP180 is presented to MHC class II
- Recognized as foreign
- Formation of autoantibody
- Autoantibody binds to amniotic membrane.
- The placental basement membrane undergoes structural changes.
- Anti-BP180 antibodies are deposited at the dermo-epidermal junction (DEJ).
- Activate complement
- Recruit effector cells (eosinophils and neutrophils)
- Proteases are released.
- Blister formation

The primary event starts with loss of immune privilege due to aberrant maternal MHC class II expression. BP180 is presented to MHC complex in the presence of paternal MHC class II allele. BP180 is recognized as foreign, producing anti-BP180 antibodies that affect the placenta and skin. In addition to anti-BP180, antibodies against paternal MHC antigens are also produced, the significance of which is unclear. The anti-BP180 antibodies are deposited at the DEJ which results in complement activation and effector response. The activated eosinophils and neutrophils degranulate releasing proteases leading to blister formation.

Lichen Planus Pemphigoides

It is a subepidermal bullous disorder showing overlap of lichen planus and BP. It is first characterized by lichenoid inflammation, followed by the development of subepidermal blisters. The bullae develop over both lichenoid areas and normal skin. Medication has been reported to trigger lichen planus pemphigoides (LPP). Recently, it has been reported as a complication of programmed cell death protein and programmed death-ligand 1 inhibition checkpoint blockage.

Autoantibody targets: BP180 (NC16A, C terminal epitope), BP230, desmoglein-1, 130 kDa, and 200 kDa.

Steps in disease pathogenesis:

- Cytotoxic CD8+ cells (lichen planus)
- Apoptosis of basal cells
- Exposure of DEJ antigens
- Autoantibodies against collagen XVII
- Activation of both complement and non-complement blistering mechanisms
- Effector cell activation
- Activation of proteases
- Bulla formation

Anti-BP180 (NC16A) and anti-BP230 antibodies are associated with a clinical presentation similar to BP; however, those with anti-BP180 (C-terminal portion of BP180) have predominant mucosal lesions akin to mucous membrane pemphigoid (MMP).

Epidermolysis bullosa Acquisita

Epidermolysis bullosa acquisita (EBA) is characterized by autoantibodies to collagen VII. It clinically presents as inflammatory or non-inflammatory forms.

Structure of Collagen VII

Collagen VII is essential for skin stability. Anchoring fibrils contain collagen VII which bind molecules in the basal lamina to the underlying connective tissue. It has a central collagenous domain flanked by a large non-collagenous domain (NC1) and a smaller non-collagenous domain (NC2) **(Fig. 7)**. NC1 binds to other proteins such as laminin 332, collagen 1, and collagen IV.

Autoantigen

The predominant antigen is the epitope in NC1 domain of collagen VII. Rarely, reactivity to NC2 and collagen domain has been reported.

Autoantibody Type

IgG (most common), followed by IgA (10%), and rarely IgE and IgM are the autoantibodies seen.

These are pathogenic, based on clinical and experimental data. In an ex vivo human model, it was observed that anti-collagen VII (COLVII) could recruit neutrophils resulting in DEJ separation. In both passive transfer (antibody to COLVII) and injection mouse models (COLVII antigen), it was possible to replicate the inflammatory EBA-like presentation. Anti-COLVII (NC1) antibodies correlate with disease activity among EBA patients.

Genetic Factors

Association with HLA-DR2, HLA-DRB1*13 and HLA-DRB1*15:03, has been reported. Supporting evidence also comes from the immunization mouse model. There is a strong association of disease susceptibility (inflammatory EBA) with the MHC H2s haplotype.

Cutaneous Microbiome

Rich and diverse skin microbiome prior to immunization in mice can prevent or modulate blister formation. In an immunized genetically identical mouse model (SJL/J), 20%

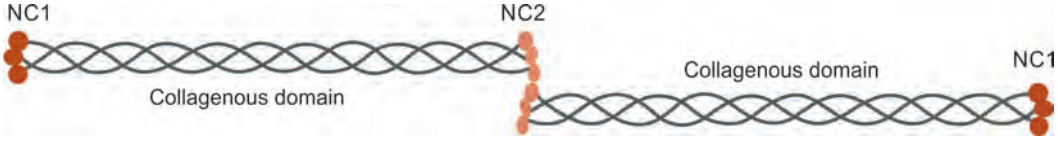


Fig. 7: Structure of collagen VII.
(NC: non-collagenous)

JAYPEE BROTHERS

of the mice failed to produce blisters. It is postulated that the effect of innate immune response (skin microbiome) modulates this effect.

Associations

Diabetes mellitus, autoimmune thyroiditis, inflammatory bowel disease, rheumatoid arthritis, and hepatitis C infection

Steps in Disease Pathogenesis (Fig. 8)

- Loss of immune tolerance (genetic and environmental causes)
- Afferent phase: Production of autoantibodies against COLVII
- Maintenance of autoantibody
- Efferent phase: Activation of complement and recruitment of neutrophils followed by activation of proteases leading to inflammation.

Experimental Data

Data from the immunization mouse model suggests that anti-COLVII antibody production is T cell-dependent. Mice deficient in T cells do not produce these autoantibodies. Depletion of CD4+ cells in mouse models delays autoantibody production and clinical disease. In an immunization mouse model (injected with COLVII) depleted of B cells, CD4+ T cell response to the antigen was not observed. Development of these COLVIICD4+ cells needs B cells, dendritic cells, and macrophages. Autoantibodies were not observed in granulocyte macrophage colony-stimulating factor (GM-CSF)-depleted mice and with neutrophil depletion, suggesting their key role in autoantibody production.

Maintenance of Autoantibody

Disease activity and progression are affected by the autoantibody's half-life and immunoglobulin pathogenicity.

- *Neonatal Fc receptor (FcRn)*: It controls the half-life of autoreactive antibodies. It prevents the catabolism of IgG. This has been demonstrated with intravenous immunoglobulin (IVIg), which inhibits FcRn, thereby reducing autoantibody level **(Fig. 9)**.
- *Differential glycosylation*: It affects the pathogenicity of the autoantibody. Agalactosylated IgG antibodies are pro-inflammatory. In contrast, sialylated IgG antibodies are anti-inflammatory.

Efferent Phase

- Activation of complement (C3a and C5a)
- Recruitment of neutrophils and mast cells to the skin (upregulation of CD18 and ICAM 1)
- Bind Fc domain of IgG through Fc gamma receptor (FcgR) expressed on the cell surface of neutrophils **(Fig. 10)**.
- Neutrophil activation
- Release of elastase and gelatinase
- Activates matrix metalloproteinases and produces reactive oxygen species.
- Damage to the BMZ and bullae formation.
- Inflammation at BMZ further activates keratinocytes and mast cells.
- Production of inflammatory cytokines and chemokines
- Perpetuate inflammation

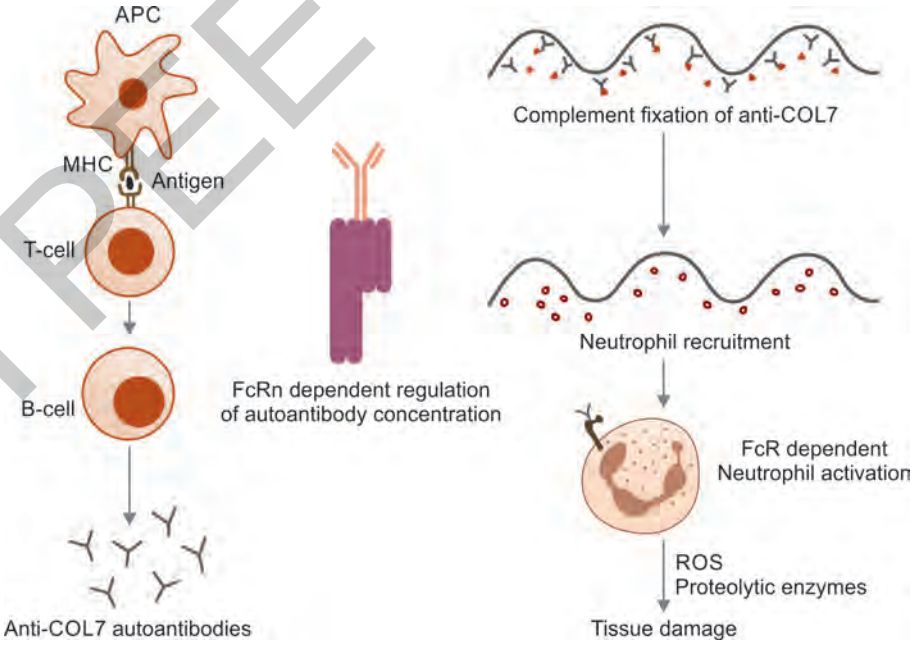


Fig. 8: Pathogenesis of epidermolysis bullosa acquisita (EBA): Loss of tolerance, followed by an afferent phase, maintenance of autoantibody, and efferent phase.

(APC: antigen presenting cell; COL7: collagen 7; FcRn: neonatal crystallizable fragment receptor; MHC: major histocompatibility complex; ROS: reactive oxygen species)

JAYPEE BROTHERS

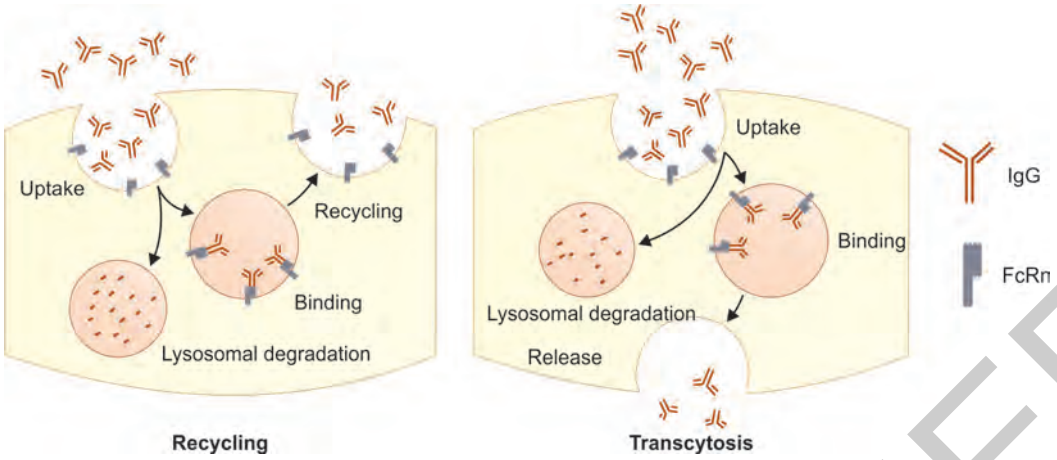


Fig. 9: Fc neonatal receptor (FcRn) and epidermolysis bullosa acquisita (EBA): Neonatal Fc receptor preventing catabolism of autoantibodies.

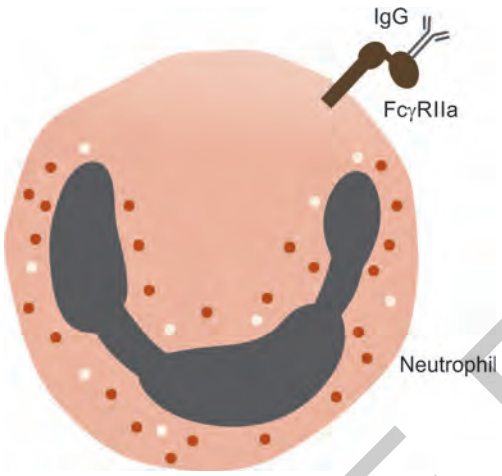


Fig. 10: Fcγ receptor and epidermolysis bullosa acquisita (EBA): IgG antibody binding to neutrophil induces crosslinks in FcγR.

FcγR and EBA

FcγR plays an essential role in the pathogenesis of EBA **(Fig. 10)**. Autoantibody binding to effector cells (neutrophils) induces crosslinks in the FcγR. This results in a downstream cascade leading to inflammation. In an inflammatory milieu, activating FcγR (FcγRI, FcγRIII, and FcγRIV) are upregulated, and inhibitory FcγRIIB are down-regulated. In a passive transfer mouse model, mice deficient in the common gamma chain of the activating FcγR region, failed to develop the disease even in the presence of anti-COLVII antibodies. In a knock-out mice model, only mice with knock-out FcγR1V failed to produce any clinical or histological evidence of disease.

Resolution

Flightless I, an actin-remodeling protein, modulates skin blistering in experimental EBA. Expression of flightless 1 is accompanied by a decrease in the tight junction (claudin 1 and 4) expression, resulting in delayed healing.

Mucous Membrane Pemphigoid

It predominantly affects the mucosae. The conjunctival and oral mucosae are commonly involved. It is characterized by both blistering and scarring. The difficulties in diagnosis of MMP are due to multiple antigen targets and low titers of circulating autoantibodies.

Genetic Factors

HLA-DQB1*0301 has been reported to be associated with MMP. In ocular MMP, association with HLA-DR4, HLA-DQw3 and HLA-DQβ*10301 has been reported.

Target Antigens

BP180 (C terminal), BP 230, Laminin 332, type VII collagen, and α6-β4 integrin are the various antigen targets.

Autoantibodies

They belong to IgG and IgA subtypes.

Pathophysiology

Laminin 332 Variant of MMP

- *Associations*: Vaccination (diphtheria-tetanus vaccine), tumors (pancreatic, colonic, gastric tumors, and lymphoma), mercury poisoning, and amalgam teeth procedures
- *Pathogenic steps*:
  - Loss of immune tolerance to laminin 332
  - Inflammation
  - Decreased adhesion to the basement membrane

In an antibody transfer mouse (anti-laminin 332 antibody transfer) model experiment, it was reported that mice injected with the recombinant antibody showed erosions and crusts predominantly on the head and neck along with oral involvement. Control mice who received rabbit IgG did not develop clinical or histological alteration. Histology of the experimental mouse showed a subepithelial separation and inflammatory cells in the upper dermis. In addition, the experimental group had a greater density

JAYPEE BROTHERS

of collagen fibers. The role of complement is not clearly established in mouse models. In another mouse model, injection of rabbit anti-laminin 332 against the immunodominant region produced lesions similar to MMP, which was both Fc and complement-dependent.

COL17-type MMP

COL17 directly binds to COL4 in the keratinocytes from skin and oral mucosa.

Pathogenic steps ***(Fig. 11)***:

- IgG produced against C-terminal of COL17
- Disrupts binding of COL17 to COL4
- Non-inflammatory blister formation

Another mechanism probably is due to internalization of COL17 on autoantibody binding to the C-terminus of collagen 17. This plays a vital role in MMP; however, all anti-MMP antibodies did not show this.

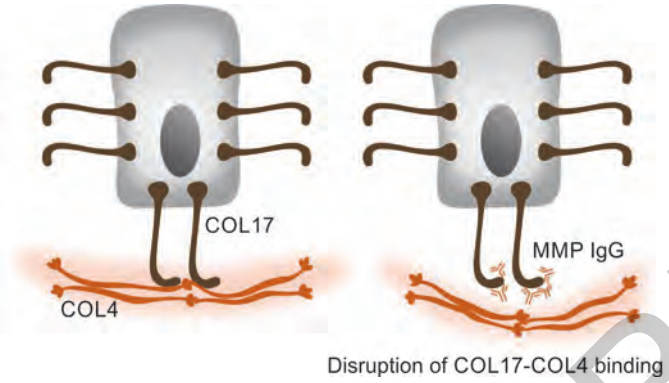


Fig. 11: Pathogenesis of mucous membrane pemphigoid (MMP): Disruption of COL17-COL4 binding by autoantibody, leading to non-inflammatory blister formation.
(COL: collagen; MMP: mucous membrane pemphigoid)

Ocular MMP

- *Genetic association*: DQw7, HLA A2, HLA B*49, HLA B*35, HLA B*8, DR-2, and DR-4 have been reported with ocular MMP.
- *Associations*: Topical timolol, pilocarpine, epinephrine, and practolol, have been reported with ocular MMP.
- *Antigen targets*: They include α6β4 integrin, BP180, laminin 332, COLVII.
- *Autoantibody*: They belong to IgG and IgA class.

Pathogenesis: There are three steps in the pathogenesis of ocular MMP **(Fig. 12)**. It includes the injury phase, inflammatory phase, and fibrotic phase. A two-hit hypothesis has been proposed to explain the findings in ocular MMP. The first hit is the genetic predisposition and the second hit is probably an unknown trigger.

1. *Injury phase*: It is characterized by the following processes:
   a. Two-hit process
   b. Autoantibodies against target antigens
   c. Produce specific B cell clones
   d. Production of autoantibodies which target BMZ
   e. Activate complement
2. *Inflammatory phase*: It is characterized by the activation of effector cells resulting in cell damage and activation of fibrosis.
   a. Activation of inflammatory cells (granulocytes, mast cells, T lymphocytes and macrophages)
   b. Migrate to substantia propria
   c. Tissue damage and release of inflammatory and profibrotic cytokines (Th1 and Th2)
   d. Cell damage and stimulation of fibroblasts

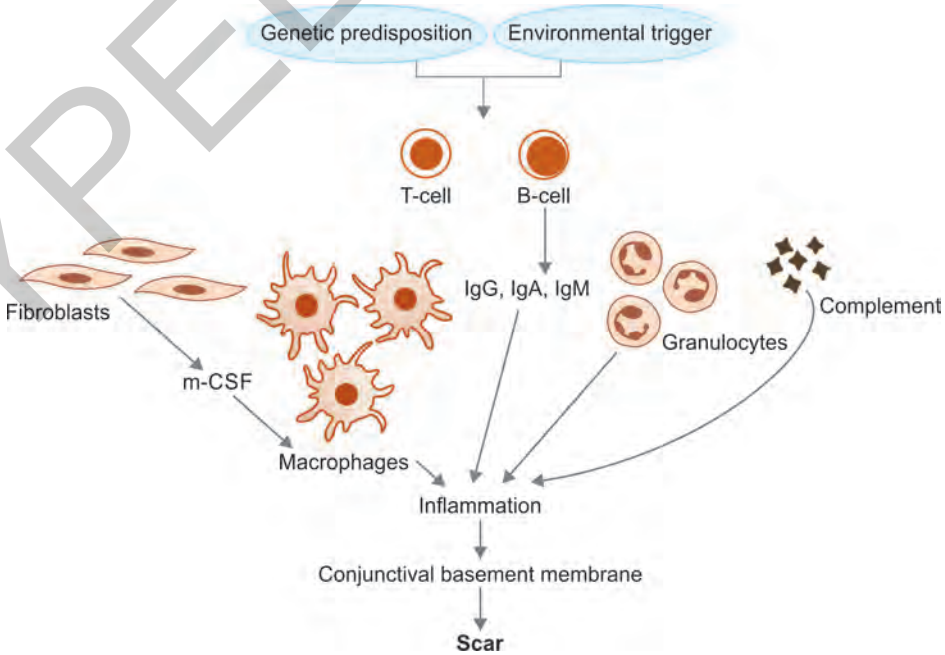


Fig. 12: Pathogenesis of ocular mucous membrane pemphigoid (MMP): Injury phase, inflammatory phase, and fibrotic phase.
(m-CSF: macrophage-colony stimulating factor)

JAYPEE BROTHERS

3. *Fibrotic phase*: It is characterized by activation of fibroblasts that results in inflammation and scarring. Steps in the process are:
   a. Activated conjunctival fibroblasts
   b. Produce cytokines and extracellular matrix components
   c. Endothelial cells proliferate and form fibrotic granulation issue
   d. Subconjunctival scarring

Dermatitis Herpetiformis

It is an AIBD characterized by associated gluten sensitivity. It is a specific manifestation of celiac disease, presenting as itchy polymorphic lesions which are symmetrically distributed over the extensor aspects.

- *Genetic factors*: Dermatitis herpetiformis (DH) is associated with HLA-DQ2 (85% of cases) and DQ8 (15%).
- *Triggers*: They include gluten, potassium iodide, gastrointestinal surgery, drugs, gastrointestinal infection, and hormonal therapy.
- *Autoantigen*: Epidermal transglutaminase (eTG or TG3)—it is usually expressed in the spinous layers and is involved in the terminal differentiation of keratinocytes. Tissue transglutaminase (tTG or TG2) is the primary autoantigen of celiac disease.
- *Autoantibodies*: They belong to the IgA class directed against eTG. There is conflicting literature regarding pathogenicity. Passive transfer (SCID mouse model with human skin grafts) using anti-eTG or human serum from DH patients failed to elicit any blisters, though granular IgA deposits were detected.

Steps in Disease Pathogenesis (Fig. 13)

- tTG/TG2 catalyzes the deamidation of gluten
- Increased gluten peptide binding to HLA-DQ2 and DQ8 expressed on the antigen presenting cells (APC).
- Immune response against tTG and gliadin. This response is mediated by both Th1 and Th2.
- Gut inflammation (formation of anti-tTG IgA deposits and increased IL-8 enhanced selectin and GM-CSF)
- Autoimmunity against eTG/TG3 is probably due to epitope spreading

Epitope spreading: The development of autoantibodies targeting eTG is unclear in patients with celiac disease. It could be due to epitope spreading in view of the homology between tTG and eTG. Other observations in support of this hypothesis include a low prevalence of these antibodies in children compared to adult cases of DH.

Formation of skin eTG/IgA aggregates: One hypothesis is that epidermal trauma leads to eTG shedding into the dermis, where it binds to circulating anti-eTG IgA. Another hypothesis is that these aggregates (eTG/IgA) exist as circulatory immune complexes.

- eTG/IgA in skin activates fibrin found at the tips of dermal papillae.
- This results in blister formation and activates effector cells (predominantly neutrophils).
- Activated neutrophils migrate to the skin.
- Release granzyme B and elastase.
- Cleave collagen BMZ at lamina lucida (probably through destruction of laminin 332).
- Further release of proteases from basal keratinocytes.
- Blister formation and pruritus (mediated by neuropeptides, corticotropin-releasing factor, and IL-31).

Linear IgA Dermatosis

It is characterized by linear deposits of IgA along the BMZ. It occurs both in children and adults.

There are three patterns of IgA deposition in linear IgA dermatosis (LAD)—(1) lamina lucida type (most common),

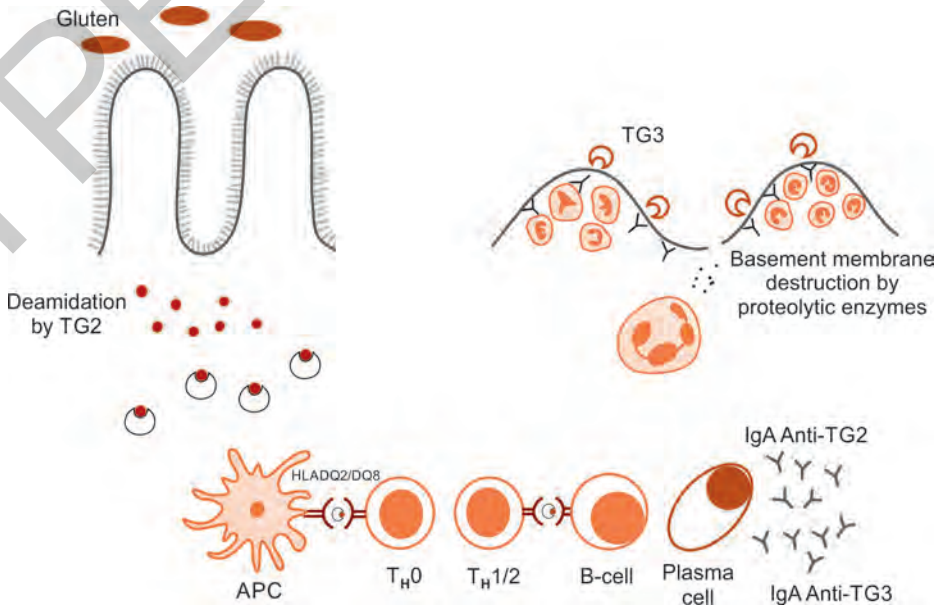


Fig. 13: Pathogenesis of dermatitis herpetiformis.
(APC: antigen presenting cell; TG2: tissue transglutaminase; TG3: epidermal transglutaminase; T_H: T helper)

JAYPEE BROTHERS

(2) sublamina densa type, and (3) combined pattern. There is significant overlap with BP, MMP, and EBA.

Genetic Factors

Associated with HLA-B8, HLA-CW7, DR3, and tumor necrosis factor 2 (TNF2).

Triggers

They include infections, drugs (vancomycin-50% of cases), vaccination, and autoimmune disorders (Crohn's disease, ulcerative colitis, and autoimmune lymphoproliferative disorders).

Antigen Targets

- *Lamina lucida type*: linear IgA antigen 1 (LAD-1, 120 kDa), linear IgA bullous dermatosis antigen (LABD97, 97 kDa), BP230, BP180, collagen VII, and laminin 332.
- *Sublamina densa type*: Probably collagen VII.

Autoantibody Type

They belong predominantly to IgA1 and sometimes IgG.

Steps in Disease Pathogenesis

- Genetic background with stressors (infections, drugs, vaccination, etc.)
- Production of aberrant IgA1 antibodies
- Bind to antigens at the DEJ (ectodomain of BP180, LAD-1, and LABD 97)
- Activation of neutrophils by these autoantibodies
- Cross-linking of FcαRI on neutrophils with IgA immune complexes
- Release of leukotriene B4, elastase, and reactive oxygen species
- Accumulation of predominantly neutrophils and few eosinophils independent of complement in the skin
- Inflammation and blister formation

Neoepitope Formation

The primary antigenic stimulus is the processed extracellular part of BP180 ectodomains. Proteolytic cleaving of BP180 by ADAM (a disintegrin and metalloprotease) 9, 10, 17 and plasmin produces the soluble ectodomain LAD-1 and LABD 97. The N terminals of both LAD-1 and LABD97 are present within the NC16A domain **(Fig. 14)**. Sera of most react with cleaved BP180 and not the full-length BP180.

Intermolecular Epitope Spreading Phenomenon

Disease progression and inflammation could result in the exposure of intracellular BP230-producing autoantibodies to BP230. These were more frequently detected when BP180 autoantibodies were present (LAD-1 and NC16A).

Complement activation: IgA immune complex can activate neutrophils by binding to and crosslinking with FcαRI, which is complement-independent. IgA activates complement weakly unlike IgG or IgM. Moreover, as it lacks a C1q binding site, it cannot activate the classical complement pathway. However, it can activate the alternate and lectin pathways.

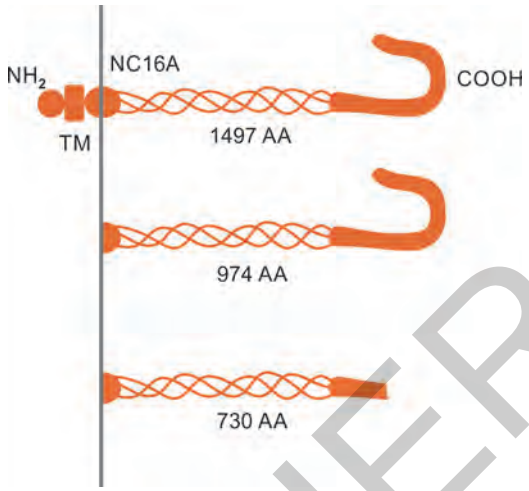


Fig. 14: Pathogenesis of linear IgA dermatosis: Neoepitope formation-proteolytic cleaving of BP180 by ADAM 9, 10, 17, and plasmin produces the soluble ectodomain LAD-1 and LABD 97.

Bullous Systemic Lupus Erythematosus

Bullous lupus erythematosus (BSLE) is a rare AIBD characterized by a subepidermal blister which usually heals without milia or scarring. It occurs in patients with SLE.

Genetic Factors

BSLE has been reported to be associated with HLA-DR2.

Drugs

Methimazole and nivolumab can trigger BSLE.

Antigen Targets

They include type VII (NC1 and NC 2 domain). Other antigens reported are laminin 332, laminin 311, and BPAg1. These are probably due to epitope spreading.

Autoantibody Type

They belong predominantly to IgG2 and IgG3, followed by IgA and IgM.

The autoantibodies are pathogenic. This has been demonstrated in a human cryosection model. Incubation with patient sera in the presence of neutrophils induced a subepidermal split. In this model, the autoantibodies did not fix complement.

Steps in Pathogenesis

- Autoantibodies (predominantly IgG) are produced against collagen VII
- They bind to both NC1 and NC2
- Recruitment of neutrophils and activation of complement
- Blister formation occurs

Anti p200 Pemphigoid (Anti-laminin γ1 Pemphigoid)

It is a rare subepidermal AIBD characterized by autoantibodies against the 200 kD protein present in the lamina lucida.

JAYPEE BROTHERS

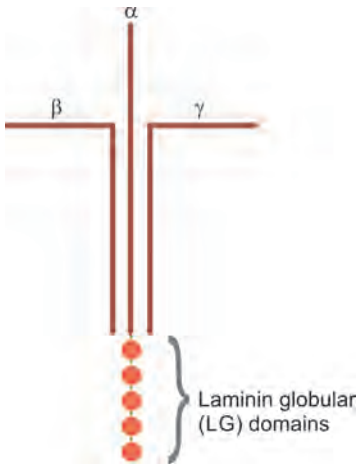


Fig. 15: Structure of laminin heterotrimer.

Antigen Targets

Laminin γ1 (C terminus) is the main antigen. Other antigens reported include BP180, laminin 332, BP230, and COL7 (in about 25% of cases). This is most likely due to epitope spreading. Laminin γ1 is a 200 kD protein. It is part of the laminin heterotrimer **(Fig. 15)** which serves to maintain adhesion outside the hemidesmosome. The C-terminal portion binds to integrins and the arm part binds to nidogen through which it anchors to the BMZ.

Autoantibody Types

They belong to IgG4 type. The antibodies detected in antilaminin γ1 pemphigoid are pathogenic.

Associations

It is associated with psoriasis. It is hypothesized that there is an alteration of the extracellular matrix in psoriatic skin on account of the rapid cell cycle and turnover, which stimulates the production of autoantibodies against the 200 kD protein.

CONCLUSION

The interplay between different components of the innate and adaptive immunity and complement system directed against the components of the basement membrane zone is responsible for various subepidermal AIBDs. Its thorough understanding helps the clinician recognize specific clinical manifestations, undertake relevant investigations and prescribe specific treatment directed against the various pathomechanisms responsible for the disease.

TAKE HOME MESSAGE

- AIBDs are characterized by loss of tolerance either due to a genetic cause or an environmental trigger. Loss of tolerance is associated with activation of the immune system, production of autoantibodies, recruitment of inflammatory cells, and inflammation. These processes are predominantly complement-mediated.
- These blistering disorders are studied with the use of various animal models and sometimes with ex vivo human skin cryosections. These models include both passive and active models.
- In BP, NC16A of BP180 is the predominant antigen target. Pathogenic antibodies are predominantly of IgG and IgE class. There are both complement-dependent and independent mechanisms of blister formation. The main effector cells are eosinophils and neutrophils which release protease and cationic proteins, which contribute to inflammation and blister formation.
- In PG, the primary event starts with loss of immune privilege due to aberrant maternal MHC class II expression. This results in BP180 being presented to the MHC complex in the presence of paternal MHC class II allele. BP180 is recognized as foreign, resulting in the production of anti-BP 180 antibodies that affect both the placenta and skin.
- In LAD, the primary antigenic stimulus is the processed extracellular part of BP180 ectodomain. Proteolytic cleaving of BP180 by ADAM 9, 10, 17, and plasmin produces the soluble ectodomain LAD-1 and LABD 97. In the presence of a genetic background and trigger, aberrant IgA1 antibodies are produced toward the antigen targets. These activate neutrophils which release ROS, leukotriene B4, and elastase, resulting in inflammation and blister formation. IgA1 weakly activates complement.
- In EBA, NC1 domain of COL7 is the main autoantigen. IgG to NC1 is the most common autoantibody. These antibodies are pathogenic. The development of EBA is characterized by loss of immune tolerance, an afferent phase characterized by autoantibodies to COL7, maintenance of autoantibody, and an efferent phase characterized by activation of complement and recruitment of neutrophils followed by activation of proteases leading to inflammation.

  Autoantibody binding to effector cells (neutrophils) induces crosslinks in the FcgR. This results in a downstream cascade leading to inflammation. In an inflammatory milieu, activating FcgR (FcgRI, FcgRIII, and FcgRIV) is upregulated and inhibitory FcgR11B is downregulated.
- In MMP, laminin 332, BP180 (C terminal), BP230, COL7, and α6-β4 integrin, are the autoantigens. Autoantibodies belong to IgG and IgA subclass. There are three steps in the pathogenesis of ocular MMP—(1) injury phase, (2) inflammatory phase, and (3) fibrotic phase. A two-hit hypothesis has been proposed to explain the findings in ocular MMP. The first hit is genetic predisposition and the second hit is probably an unknown trigger.
- In BSLE, COL7 (NC1 and NC2 domain) are the main antigenic targets. Others reported are laminin 332, laminin 311, and BPAg1. These are probably due to epitope spreading. Pathogenic autoantibodies belong predominantly to the IgG2 and IgG3 class, followed by IgA and IgM. Binding of autoantibodies results

JAYPEE BROTHERS

in activation of complement and recruitment of neutrophils, which then leads to blister formation.

- In anti p200 pemphigoid (anti-laminin γ1 pemphigoid), the target antigen is laminin γ1 (C terminus). Others reported include BP180, laminin 332, BP230, and COL7 (in about 25% of cases). This is most likely due to epitope spreading. The pathogenic antibodies belong to IgG4 type.

MULTIPLE CHOICE QUESTIONS

1. Which of the following antibody binds weakly to complement?
(a) IgG1
(b) IgG3
(c) IgM
(d) IgA

2. Which of the following is an active mouse model?
(a) Injection of patient serum containing IgG into mice
(b) Injection of rabbit serum containing IgG into mice
(c) Injection of NC-1 domain of murine collagen 7 into mice
(d) Injection of patient serum containing IgG to SCID mice transplanted with human skin

3. Which of the following antibodies is associated with malignancies?
(a) Anti-laminin γ1
(b) Anti-NC16A BP180
(c) Anti-laminin 332
(d) Anti-α6-β4 integrin

4. Which of the following is true regarding neonatal Fc receptor?
(a) It is associated with catabolism of IgG
(b) IVIg stimulates FcRn
(c) It stands for neonatal crystallizable fragment receptor
(d) FcRn stimulators are used to treat autoimmune diseases

5. Autoantibodies in dermatitis herpetiformis predominantly belong to which class?
(a) IgG
(b) IgA
(c) IgM
(d) IgE

6. What is internalization of antigen followed by destruction called?
(a) Osmosis
(b) Pinocytosis
(c) Transcellular elimination
(d) Diffusion

Answers

1. (d) 2. (c) 3. (c) 4. (c) 5. (b) 6. (b)

SUGGESTED READING

1. Pollmann R, Eming R. Research techniques made simple: mouse models of autoimmune blistering diseases. *J Invest Dermatol*. 2017;137:e1-e6.
2. Bieber K, Sun S, Ishii N, Kasperkiewicz M, Schmidt E, Hirose M, *et al*. Animal models for autoimmune bullous dermatoses. *Exp Dermatol*. 2010;19:2-11.
3. Genovese G, Di Zenzo G, Cozzani E, Berti E, Cugno M, Marzano AV. New insights into the pathogenesis of bullous pemphigoid: 2019 Update. *Front Immunol*. 2019;10:1506.
4. Cole C, Vinay K, Borradori L, Amber KT. Insights into the pathogenesis of bullous pemphigoid: the role of complement-independent mechanisms. *Front Immunol*. 2022;13:912876.
5. Yang M, Wu H, Zhao M, Chang C, Lu Q. The pathogenesis of bullous skin diseases. *J Transl Autoimmun*. 2019;2:100014.
6. Ujiie H, Shibaki A, Nishie W, Sawamura D, Wang G, Tateishi Y, *et al*. A novel active mouse model for bullous pemphigoid targeting humanized pathogenic antigen. *J Immunol*. 2010;184:2166-74.
7. Sadik CD, Lima AL, Zillikens D. Pemphigoid gestationis: Toward a better understanding of the etiopathogenesis. *Clin Dermatol*. 2016;34:378-82.
8. Schauer F, Mai S, Hofmann SC, Mai Y, Izumi K, Kern JS, *et al*. Autoreactivity to BP180 neoepitopes in patients with pemphigoid gestationis. *JAMA Dermatol*. 2022;158:212-4.
9. Hübner F, Langan EA, Recke A. Lichen planus pemphigoides: From lichenoid inflammation to autoantibody-mediated blistering. *Front Immunol*. 2019;10:1389.
10. Papara C, Danescu S, Sitaru C, Baican A. Challenges and pitfalls between lichen planus pemphigoides and bullous lichen planus. *Australas J Dermatol*. 2022;63:165-71.
11. Koga H, Prost-Squarcioni C, Iwata H, Jonkman MF, Ludwig RJ, Bieber K. Epidermolysis bullosa acquisita: The 2019 update. *Front Med (Lausanne)*. 2019;5:362.
12. Kamaguchi M, Iwata H. The diagnosis and blistering mechanisms of mucous membrane pemphigoid. *Front Immunol*. 2019;10:34.
13. Branisteanu DC, Stoleriu G, Branisteanu DE, Boda D, Branisteanu CI, Maranduca MA, *et al*. Ocular cicatricial pemphigoid (Review). *Exp Ther Med*. 2020;20:3379-82.
14. Antiga E, Maglie R, Quintarelli L, Verdelli A, Bonciani D, Bonciolini V, *et al*. Dermatitis herpetiformis: novel perspectives. *Front Immunol*. 2019;10:1290.

JAYPEE BROTHERS

15. Kemppainen E, Salmi T, Lindfors K. Missing insight into T and B cell responses in dermatitis herpetiformis. *Front Immunol*. 2021;12:657280.
16. Mori F, Saretta F, Liotti L, Giovannini M, Castagnoli R, Arasi S, *et al*. Linear immunoglobulin A bullous dermatosis in children. *Front Pediatr*. 2022;10:937528.
17. Edwards G, Diercks GFH, Seelen MAJ, Horvath B, van Doorn MBA, Damman J. Complement activation in autoimmune bullous dermatoses: a comprehensive review. *Front Immunol*. 2019;10:1477.
18. Contestable JJ, Edhegard KD, Meyerle JH. Bullous systemic lupus erythematosus: a review and update to diagnosis and treatment. *Am J Clin Dermatol*. 2014;15:517-24.
19. Florea F, Bernards C, Caproni M, Kleindienst J, Hashimoto T, Koch M, *et al*. Ex vivo pathogenicity of anti-laminin γ1 autoantibodies. *Am J Pathol*. 2014;184:494-506.
20. Kridin K, Ahmed AR. Anti-p200 pemphigoid: A systematic review. *Front Immunol*. 2019;10:2466.

JAYPEE BROTHERS

or detailed information on dermatology books, visit our website www.jaypeebrothers.com, for detailed information on dermatology books, visit ou

IADVL Textbook of Pemphigus and other Autoimmune Bullous Diseases

Salient Features

- *IADVL Textbook of Pemphigus and other Autoimmune Bullous Diseases* has been written as a comprehensive textbook catering to dermatology postgraduates and dermatologists actively involved in the management of autoimmune bullous diseases.
- The book has been carefully designed to encompass various features including basic science, clinical, diagnostic, and therapeutic aspects of these diseases.
- The book has contributions from both Indian and International experts in this group of diseases.
- It has been prepared to make it relevant to the Indian readers.
- It has been written very systematically with several images, tables and flowcharts to make the book an easy read.
- It will be a reference book for all dermatologists on the subject of pemphigus and other autoimmune bullous diseases.

Sujay Khandpur MD DNB MNAMS
Professor
Department of Dermatology and Venereology
All India Institute of Medical Sciences
New Delhi, India

Raghavendra Rao MD DNB
Professor and Head
Department of Dermatology, Venereology and Leprosy
Kasturba Medical College
Manipal Academy of Higher Education (MAHE)
Manipal, Karnataka, India

Vinay Keshavamurthy MD DNB MNAMS MRCP FRCP (London)
Associate Professor
Department of Dermatology, Venereology and Leprology
Postgraduate Institute of Medical Education and Research
Chandigarh, India

Atiya Yaseen MD DVD
Medical Officer, Dermatologist
Department of Health and Medical Education
Sub District Hospital Pampore
Jammu & Kashmir, India

Saritha Mohanan MD DNB
Associate Professor
Department of Dermatology, Venereology and Leprosy
Indira Gandhi Medical College and Research Institute
Kathirkamam, Puducherry, India

Printed in India

Available at all medical bookstores or buy online at www.jaypeebrothers.com

JAYPEE BROTHERS
Medical Publishers (P) Ltd.
EMCA House, 23/23-B, Ansari Road,
Daryaganj, New Delhi - 110 002, INDIA
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

Shelving Recommendation
DERMATOLOGY

ISBN 978-93-5696-144-9