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CUTANEOUS BASEMENT MEMBRANE ZONE STRUCTURE, ANTIGENICITY AND THE DIAGNOSTIC SIGNIFICANCE OF SPLIT-SKIN TECHNIQUE.

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INTRODUCTION

Basement membranes are an integral structural component of most tissues. They are composed of thin sheets of extracellular matrices synthesized and secreted by a variety of cells. Basement membranes separate the epithelial, endothelial, muscular and neural tissue from the adjacent connective tissue stroma[1]. During the past years there has been an enormous increase in our knowledge relating to the structure and chemical composition of the epidermal basement membrane zone. The modern concept of the epidermal basement membrane is that of a highly complex group of molecules about 120-150 nm thick, the anatomic detail of which can only be accurately resolved by means of electron microscopy[2]. Ultrastructurally, it appears to be composed of four components proceeding from the epidermis to dermis (Figs. 1, 2).

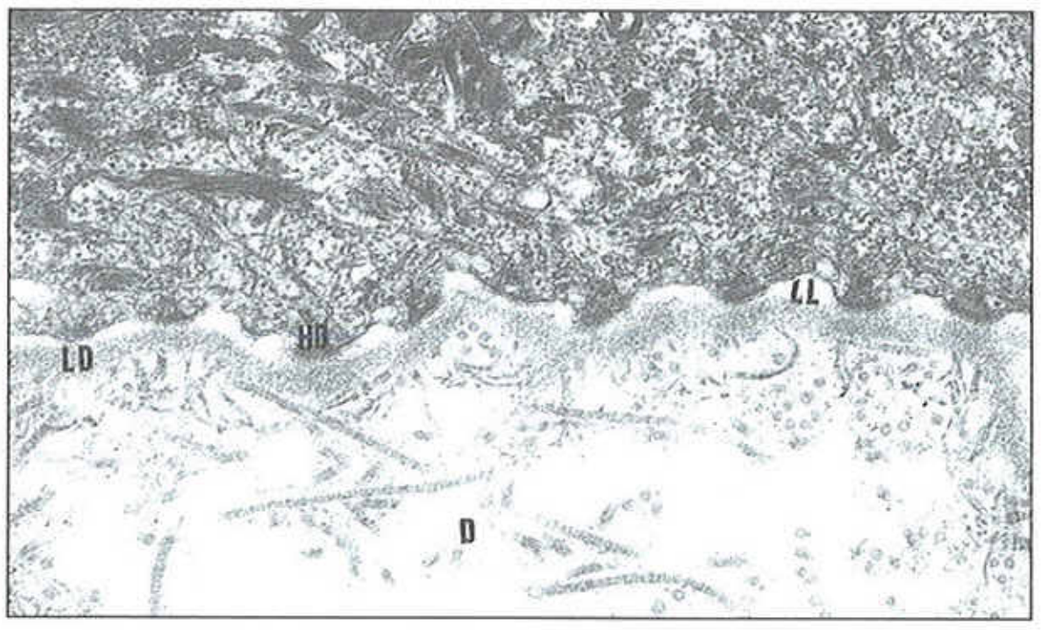

Fig 1: Electron micrograph of epidermal basement membrane zone showing hemidesmosome (HD), lamina lucida (LL), lamina densa LD), and dermis (D). Original magnification 33,200.

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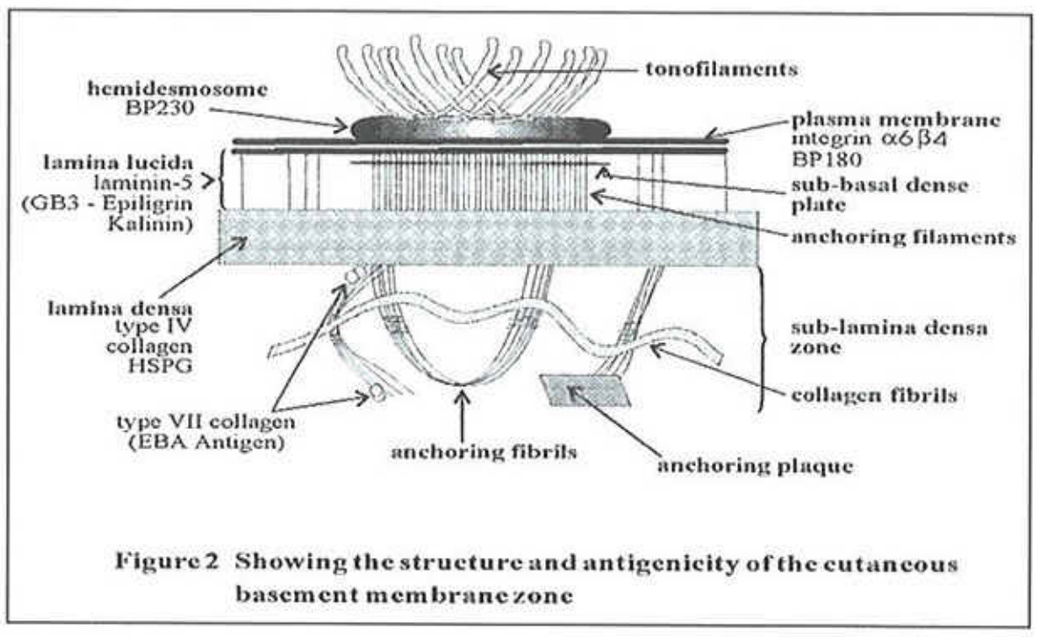

Figure 2 Showing the structure and antigenicity of the cutaneous basement membrane zone

Fig 2: Electron micrograph showing the structure and antiginicity of cutaneous basement membrane zone.

Plasma Membrane:

The first component is the plasma membrane of the basal cell that is situated along the basement membrane, with its special structures known as hemidesmosomes. The plasma membrane is approximately 7-9 nanometers (nm) thick and is composed of three asymmetrical layers[3,4] (Figs. 2, 3).

Hemidesmosome:

The hemidesmosomes are electron-dense linear condensations which are located along the inner aspect of the cell membrane of the basilar keratinocyte. They are considered as junctional complexes which connect basal epidermal keratinocytes to the basement membrane[2,6]. Ultrastucturally the hemidesmosomes appear to consist of two electron dense plaques termed inner and outer plaques[6]. The outer plaque is known as the attachment plaque and appears to be present on the cytoplasmic surface of the plasma membrane[3]. The inner plaque is associated with the keratin intermediate filaments also known as tonofilaments. These filaments are located inside the cell and form part of the cytoskeleton (Figs. 2, 3).

Sub Basal Dense Plate (SBDP):

The SBDP is a thin electron dense linear band. It is located in the upper part of the lamina lucida and runs parallel to the outer plaque of the hemidesmosomes[5,6] (Figs. 2, 3).

Lamina Lucida:

Lamina lucida also referred as lamina rara. It is

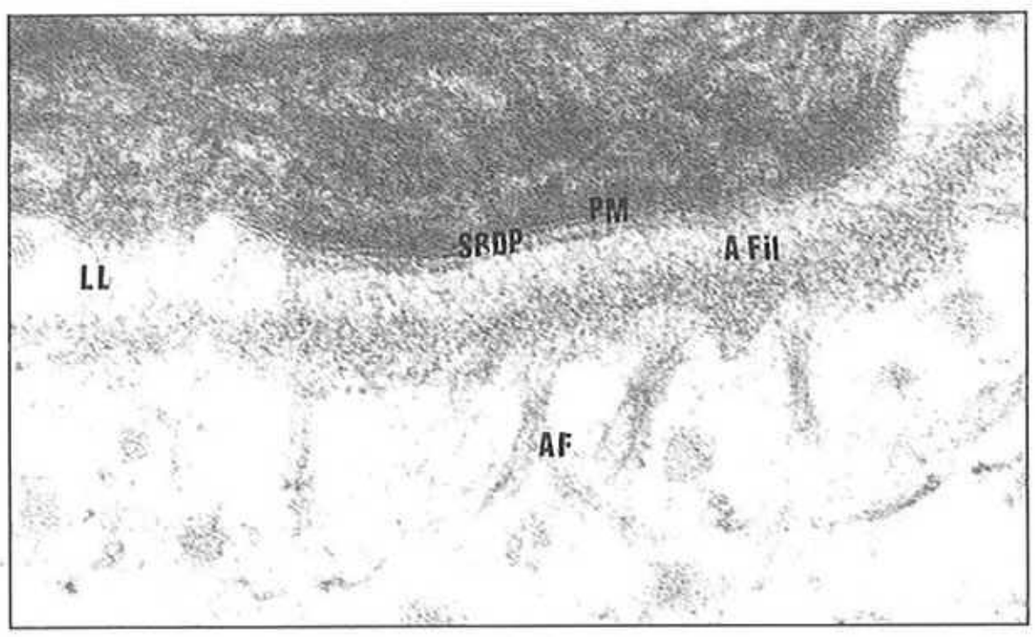

Fig 3: Electron micrograph showing plasma membrane (PM), sub-basal dense plate (SBDP), lamina Lucida (LL), anchoring filaments (A Fil), and anchoring fibrils (AF). Original magnification 140,000.

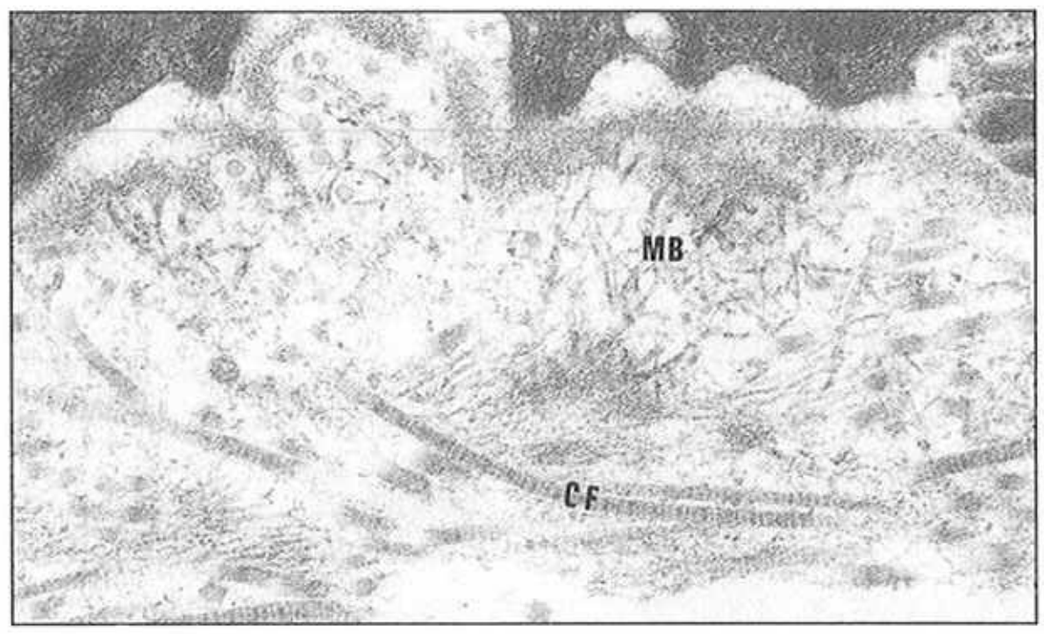

Fig 4: Electron micrograph showing microfibrils bundle (MB) and collagen fibers (CF). Original magnification 49,400.

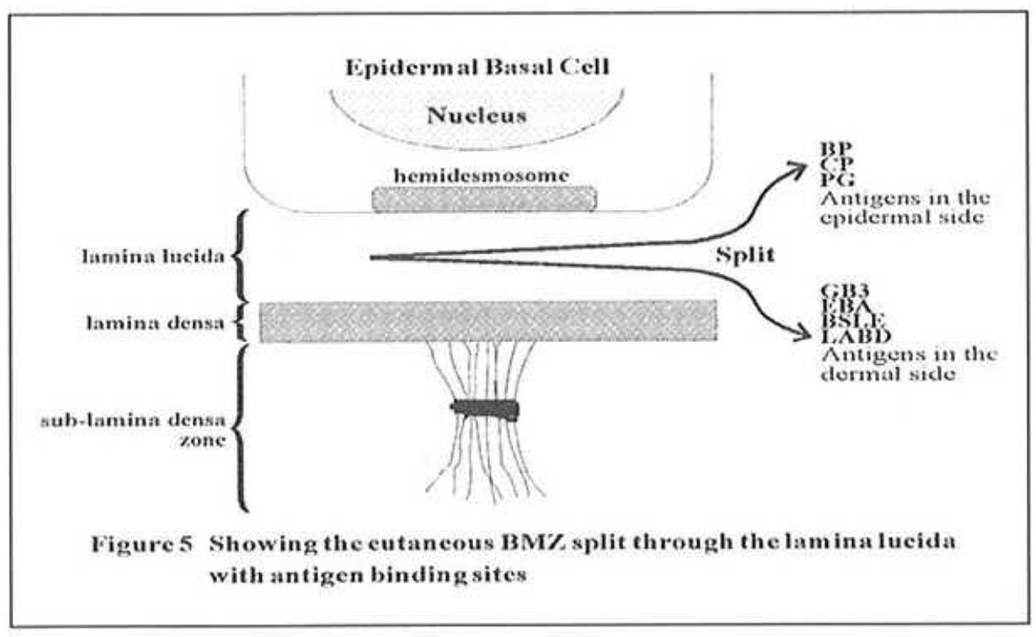

Fig 5: Electron micrograph showing the cutaneous BMZ split through the lamina lucida with antigen binding sites.

situated immediately below plasma membrane of the basal epidermal cells. As its name implies, the lamina lucida is electron-lucent, about 20-40 nm in thickness[4,7]. The lamina lucida is probably the weakest structure of the dermo-epidermal junction and has been considered to be the most frequently involved in various subepidermal blistering disorders[8]. Very fine thread-like structures transvere the lamina lucida, these are known as anchoring filaments[1] (Figs. 2, 3).

Lamina Densa:

Lamina densa appears as a fine granular amorphous electron-dense structure approximately 30 to 60 nm wide7 (Fig.2).

Sub Lamina Densa:

Sub lamina densa region is situated immediately below the lamina densa and is composed of three different fibrous structures.Anchoring fibrils, microfibril bundles and collagen fibres[7] (Figs. 2,3).

Anchoring Fibrils:

Anchoring fibrils have a unique structure which consist of a central irregular cross-banding and a fan like projections of smaller fibrillar components extending from both ends[5]. The upper projections appear to embed into lower most part of the lamina densa. While the lower projections either loop around the collagen fibres and back into the lamina densa or form an interlocking meshwork of fibrils. At times they appear to attach directly to an irregular shaped electron-dense condensation known as anchoring plaques (Figs. 2, 3).

Microfibril Bundles:

Microfibril bundles appear as parallel fibres which pass deep into the dermis perpendicularly from the lamina densa[4] (Fig. 4).

Collagen Fibres:

Collagen fibres are randomly oriented and usually appear singly or occasionally in groups of several fibres[3,4] (Figs. 2, 4).

Antigenicity of Epidermal Basement Membrane Zone:

During the last two decades, our understanding of the biochemical composition of the basement membrane zone has been considerably extended. This has been mainly due to the generation of monoclonal and polyclonal antibodies to normal components of the basement membrane. Epidermal basement membrane zone is known to contain the target

antigens in several autoimmune bullous diseases. Thus knowledge of the molecular composition of the basement membrane zone is important in improving our understanding of the genetic and immunologically mediated blistering skin diseases (Fig. 2).

Bullous Pemphigoid Antigen:

Bullous pemphigoid antigen was the first basement membrane protein identified within human skin[9]. It was defined by the presence of linear homogeneous (tubular) deposists of IgG and C3 along the demo-epidermal junction of perilesional skin from patients with bullous pemphigoid[10]. This antigen is synthesized by epidermal basal cells and has been produced by keratinocytes in culture[11,12].

Bullous Pemphigoid Antigen 1:

Immunoprecipitation studies have shown that up to 97% of bullous pemphigoid autoantibodies recognize a 230 kD protein. This protein is now known as bullous pemphigoid antigen 1 (BPAg1)[13]. Immunoelectron microscopy studies show that it is localized to the hemidesmosomes[14]. The gene encoding BPAg1 has been localized to the short arm of human chromosome 6 at 6p11-6p12 and northern analysis has determined that the full length mRNA encoding BPAg1 is 9 kb[15,17].

On the basis of entire DNA sequence, it has been proposed that BPAg1 has a central rod structure flanked by terminal globular domains[17,18]. Interestingly, primary sequence analysis of DNA also shows that it is an intracellular protein that revealed significant homology to desmoplakin I and plectin [1,14,18].

Bullous Pemphigoid antigen 2 (BPAg2):

BPAg2 has a molecular weight of 180 kD and is referred to as the minor bullous pemphigoid antigen. Immunoblot studies showed that approximately 50% of patients with bullous pemphigoid have circulating IgG autoantibodies that recognize a 180 kD protein[16,19]. Circulating autoantibodies from patients with herpes gestationis also recognize this antigen (85 to 90 % cases)[20,21]. Ultrastructuraly BPAg2 is localized to both the hemidesmosome and the upper lamina lucida. The gene encoding BPAg2 has been localized to the long arm of chromosome 10 at locus 10q24.3 and northern analysis has determind that the full length mRNA encoding BPAg2 is 6 kb[22]. Therefore it is evident that BPAg2 and BPAg1 are distinct gene products.

Cicatricial Pemphigoid Antigen:

For many years there has been controversy regarding the nature of the antigen that react with autoantibodies in cicatricial pemphigoid. Direct immunoelectron microscopy studies reveal localization of the immunoreactants within the lamina lucida of the basement membrane zone[23,24]. Bernard et al.[25] reported that a 180 kD protein was the major cicatricial pemphigoid target antigen.Very recently Domloge-Hultsch et al.[26] described a group of patients who had circulating autoantibodies against epiligrin.

Alpha 6 Beta 4 Integrins (64 Integrin):

Integrins are heterodimeric transmembrane receptors which promote cell-cell and cell-matrix interactions[27]. Ultrastructurally 64 integrin is located in hemidesmosomes, closely associated with bullous pemphigoid antigen[28]. The extracellular domain of 64 integrin is considered an important receptor for adhesion ligands in the lamina lucida of the basement membrane zone[28].

Recently, Niessen et al.[29] reported that laminin type 5 and laminin type 1 are specific ligands for 64 in basal keratinocytes.

19-DEJ-1 Antigen:

This antigen is present within the mid or upper lamina lucida solely underneath the hemidesmosomes on the anchoring filaments[18,30]. Preliminary reports show that the 19-DEJ-1 antigen represents a protein with a molecular weight of approximately 165 kD[5,18]. The 19-DEJ-1 antigen appears to be absent in Junctional Epidermolysis Bullosa (JEB), a disease characterized by skin cleavage within the lamina lucida and associated with absence or decreased numbers of hemidesmosomes[30].

Laminin 5 (Kalinin or Nicein):

Laminin 5 is a recently identified laminin isoform in the basement membrane zone of stratified epithelia[31]. It has a molecular weight of 440 kD in its non-reduced state. It is rod shaped and composed of three separate subunits of molecular weights 165, 155, and 140 kD[14,18]. Indirect immunofluorescence (IIF) technique with 1M NaCL split-skin shows that antigen binds to the dermal portion[18]. In human

epidermal basement membrane, laminin 5 has been shown to localize to anchoring filaments. Laminin 5 is the currently designated term for both kalinin, nicein and epiligrin which appear to be the same protein[1].

Laminin 6 (K-Laminin):

Marinkovich and co-worker[32] in 1992 reported the identification of a new laminin variant to the basement membrane zone of skin. Preliminary reports suggest that laminin 5 and K-laminin are joined by a disulfide bond in anchoring filaments[1,14].

Epiligrin:

Epiligrin is a glycoprotein complex in the extracellular matrix and it is the major integrin ligand of human epidermal cells[33,34]. Epiligrin is found in the lamina lucida closely associated with anchoring filaments. Recently, it has been reported that epiligrin and laminin 5 are probably identical[34].

Laminin 1:

Laminin 1 is the most abundant non-collagenous glycoprotein found in the basement membrane zones[35] This glycoprotein is known as a classical laminin, has a molecular weight of 800 kD and is composed of three distinct polypeptide chains (1,1,1). The 1 chain has a molecular weight of 400 kD while both 1 and 1 chains are 200 kD[14]. It is now quite clear that laminin 1 is not a single molecule but rather a family of heterotrimeric isoforms.

GB3 Antigen:

GB3 antigen is a 600 kD glycoprotein which has recently been renamed as BM-600[36]. The GB3 monoclonal antibody recognizes several different sized protein chains (93.5, 125, 130 and 150 kD)[3,7]. It is suggested that most of these smaller proteins serve as a subunits of larger (600 kD) macromolecule[18]. Split-skin with 1M NaCL shows that GB3 antigen is present along the dermal side of separation[18]. GB3 antigen resides within the lamina lucida of the epidermal basement membrane zone[37].

AA3 Antigen:

AA3 antigen was defined with a polyclonal antibody produced in a rabbit by immunization with human amnion[38]. The distribution of AA3 antigen is similar to that of GB3[18]. The molecular weight of AA3 antigen is 37 kD and it also localizes most probably within the lamina lucida[39].

Fibronectin:

Fibronectin is a high molecular weight glycoprotein which is found in plasma, connective tissue and as a component of the basement membrane zone[4]. It thought to be an important component in many interesting biologic activities including wound healing, cell adhesion and motility[2].

Type IV Collagen:

Collagen IV is a major extracellular matrix protein component of the skin basement membrane zone. It is present exclusively within lamina densa[40]. Type IV collagen has a molecular weight of approximately 400 kD and is composed of three polypeptide chains each with a molecular weight of 180 kD[4,18].

Type V Collagen:

Collagen V is localized within the lamina lucida region and on the polar aspect of the basal cell keratinocyte plasma membrane[18,41].

Heparan Sulfate Proteoglycan (HSPG):

HSPG is a large molecular weight (>400kD) proteoglycan which is present within all skin basement membrane zones[18]. The core protein of HSPG localizes primarily within lamina densa[42]. However , lesser amounts are also present within the lamina lucida and beneath the lamina densa. Thus, based upon these finding, this molecule presumably serves as one of the key molecular bridges which can span the entire basement membrane zone[18].

Chondroitin 6-Sulfate Proteoglycan (C6SPG):

C6SPG has been demonstrated within all skin basement membrane zones with molecular weight of approximately 400 kD. It has been localized to the lamina densa of the epidermal basement membrane zone[18,43].

Entactin/Nidogen:

Entactin is a sulphated glycoprotein with molecular weight of approximately 150-160 kD which is present in all tissue basement membrane zones[44]. Previously it was felt to be unrelated to nidogen, a self aggregating protein which binds strongly to laminin[42]. This has now been shown to be the same as entactin. Entactin/Nidogen has a high affinity to

form complexes with laminin and is usually expressed together with laminin within basement membrane zones[18].

KF1 Antigen:

KFI antigen has a tissue distribution which is restricted to the basement membrane of stratified squamous epithelia[7]. It is localized to the lamina densa of dermo-epidermal junction and has a molecular weight of approximately 72 kD[7,45].

LDA-1:

This antigen has a low molecular weight of about 51.5 kD and placenta appears to be the richest available tissue source for this antigen[18,43]. It is localizes primarily within the lamina densa, although smaller amounts are also noted in the adjacent sublamina densa region[46].

Type VII Collagen:

Collagen VII is one of the newly identified members of the collagen family. It is also known as the epidermolysis bullosa acquisita antigen[47]. Type VII localizes within anchoring fibrils which are situated immediately beneth the lamina densa of many epithlia[48]. It consists of three identical chains each of which consists of a 145 kD N-terminal non-collageneous domain and a 145 kD C-terminal collageneous domain[18]. Type VII collagen is altered in its distribution in Recessive Dystrophic Epidermolysis Bullosa, thus it is believed that collagen VII may play a critical role in the maintenance of adhesion of the lamina densa to the underlying dermis[18].

AF1/AF2 Antigen:

AF1 and AF2 antigens are localised to the area immediately subjacent to the lamina densa[49]. However AF1 and AF2 antigen appear to decorate anchoring fibrils rather than lamina densa itself[18].

Diagnostic significance of split-skin technique in subepidermal bullous diseases:

Subepidermal bullous diseases are characterized by autoantibodies to the basement membrane zone of stratified squamous epithelium. Recent studies have shown that the antibodies in each disease have characteristic ultrastructural and antigenic binding properties. Because of similarities in the clinical, histologic and immunohistologic features of some of the diseases it may be necessary to determine the ultrastructural or antigenic binding properties of the antibodies to distinguish between the diseases. Immunofluorescence microscopy is used to detect basement membrane zone autoantibodies.

It is usually applied to tissue substrates with an intact basement membrane zone. Those substrates are limited because autoantibody binding cannot always be detected and because autoantibodies with different ultrastuctural and antigenic properties cannot easily be distinguished from each other. Split-skin refers to skin that has been artificially separated through the lamina lucida (Fig. 5). It has been recently used as a substrate for detecting and characterizing basement membrane zone autoantibodies by immunofluorescence[50]. Recent studies show that split-skin is more sensitive than intact skin for detecting basement membrane zone antibodies and that antibodies with different ultrastructural binding sites can often be differentiated on split-skin[50]. The most common method for splitting skin is incubating it in 1M NaCL[51]. Several other techniques for splitting skin have been reported. These include mechanical[52] (suction), enzymatic[53-55], and heat[56]. We are currently standarizing these methods to establish the best technique for splitting the skin. In this article we will review the findings using the 1M NaCL split-skin technique in subepidermal bullous disorders.

Bullous Pemphigoid (BP):

Bullous pemphigoid is an acquired blistering disease of the elderly. It is characterized histologically by subepidermal bullae and immunopathologically by in vivo deposition of autoantibodies and complement components along the epidermal basement membrane zone. Circulating antibodies are detected in 70-80% of BP patients, but when 1M NaCL split-skin is used this rate rises to 95%, perhaps because there is an increase in antigen exposure[57]. Further more, it is possible to obtain positive titres with higher dilutions if 1M NaCL split-skin is used[58]. Characteristic immunofluorescence patterns reveal linear homogeneous deposition in the epidermal side of the split only (85%) or both the epidermal and dermal sides of the split (15%)[59].

Cicatricial Pemphigoid (CP):

CP is a chronic bullous disorder of mucous membrane, oral and conjuctiva principally, with cutane-

ous lesions only expressed in one third of patients. Circulating antibodies against basement membrane zone are detected only in 10-30% of cases[60].

Using mucous membrane separated with 1M NaCL indirect immunofluorescence (IIF) sensitivity increased up to 50%.57 CP antibodies usually bind to the epidermal or epidermal and dermal sides of split-skin, but in a few cases they bind only to the dermal side[61].

Pemphigoid (Herpes) Gestationis (PG):

PG is a rare subepidermal bullous disease of pregnancy and the postpartum period. It characteristically develops during the second or third trimester with urticarial papules and plaques around the umbilicus, with subsequent development of both vesicles and bullae. Direct immunofluorescence (DIF) shows linear C3 deposits in the basement membrane zone in all active PG cases. IgG is detected in 30% of cases[62]. By means of IIF in split-skin IgG-C3 deposits are found on the epidermal side. Although freguently conventional IIF is not effective to demonstrate circulating antibodies against basement membrane zone[63]. Nevertheless by complement-binding IIF technique it is possible to demonstrate complement-binding antibodies (PG or HG factor) in the sera of these patients[64].

Epidermolysis Bullosa Acquisita (EBA):

EBA is an acquired autoimmune blistering disease that presents with marked skin fragility, blisters and erosions at sites of trauma and heals with scarring and milia.The usual finding using DIF in involved and perilesional skin is linear IgG deposition in the basement membrane zone as a broad band. Both IgA, IgM and complement are often detected. Circulating antibodies aganist basement membrane zone can be demonstrated by IIF in 25-50% EBA cases espicially in early stages[65,66]. When 1M NaCL split-skin is used as IIF substrate, antibodies bind to the dermal side of the split, thus it can be distinguished from BP (where antibodies bind to the epidermal side).

Recently, it has been described that DIF in perilesional IM NaCL split skin is also a reliable method to differentiate between both entities, IgG deposit in BP always occurs in epidermal side (with or without dermal deposition) while in EBA there are deposits only in dermal side[67,68].

Linear IgA Bullous Dermatosis (LABD):

Chronic bullous disease of childhood and adult LABD have similar clinical, histological and immunopathological findings and it has been suggested that both variants are a different expression of the same disease. DIF shows a homogeneous band of IgA along the basement membrane zone of non-affected skin, and also other immunoglobulins in 10-20% of cases. When intact skin is used as substrate, IIF demonstrate serum IgA antibodies against the basement membrane zone in 72% cases of childhood disease and 20% of adults. When 1M NaCL split-skin is used, positive rates significantly increase up to 92 and 42% in childhood and adult disease, respectively[69].

Bullous Systemic Lupus Erythematosus (Bullous SLE):

Bullous SLE is an acquired subepidermal blistering disease. Typical clinical features include a widespread, nonscarring, vesiculobullous eruption that occurs mainly on sun-exposed skin.DIF reveals linear IgG deposition in the basement membrane zone and occasionally IgM (50%) and IgA (66%). The pattern is homogeneous-linear in most cases, although up to 40% can present granular linear or fibrilar patterns. IIF employing split-skin shows antibodies binding to the dermal side of the split, thus it can be distinguished from BP[70,71].

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