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The Uses of Immunofluorescence Techniques in the Diagnosis and Management of the Autoimmune Bullous Diseases

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SUMMARY

In recent years great progress has been achieved in the understanding of the pathogenic mechanisms of the autoimmune bullous diseases. This progression is based on advances in our understanding of the clinical features, immunological, biochemical, ultrastructural, and molecular biological techniques for differentiation and diagnosis of these diseases. One of the earliest methods developed for investigating the immunological basis of bullous diseases was the use of immunofluorescence techniques on skin biopsy specimens and patient's sera. In the early 1960s immunofluorescence methods began to be applied to the study of diseases of the skin. In 1964, Ernst Beutner[1] described the presence of circulating autoantibodies against epidermal intercellular antigens in the sera of patients with pemphigus vulgaris, after which the field of immunodermatology was expanded.

Immunofluorescence is a microscopic method for the detection of immunoglobulins, complement components and fibrin. There are three basic types of immunofluorescence used in dermatology: direct immunofluorescence for demonstrating antibodies in tissue biopsy specimens and indirect immunofluorescence for demonstrating antibodies in serum or fluids, and complement-binding indirect IF. Immunofluorescence studies are very useful procedures in diseases of the skin. In some diseases the immunofluorescence findings are disease specific and diagnostic but in others the immunofluorescence results can also be prognostic, as in pemphigus. In other immunodermatologic diseases the immunopathologic findings may be characteristic and consistent with a particular disease process but not diagnostic, as is the case of lichen planus.

This paper proposes to discuss immunofluorescence techniques and the ability to differentiate between the autoimmune bullous diseases by their application.

METHODS AND TECHNIQUES

A. Skin Biopsy

Skin biopsies are required for direct immuofluorescence (IF) studies. Optimal result of direct IF studies can be obtained only by appropriate selection of biopsy site[2]. In

bullous diseases two biopsies should be taken. The first from the clinically normal skin, and the second from the edge of the blister which should include adjacent skin. a single biopsy alone of a blister is not suitable for IF study since tissue structures are destroyed. For mucous membrane, biopsy specimen should be taken from periphery of a fresh lesion, since the epithelium is usually eroded in lesional areas. The biopsy should be taken from a fresh blister not older than 24 to 48 hours. A punch biopsy is adequate for immunofluorescence studies of skin or mucosal lesions[3].

The biopsy specimens are immediately snap frozen, this can be performed by immersing the biopsy specimen either in liquid nitrogen or in cold carbon dioxide/hexane bath [3].

If the skin biopsy needs to be transported and liquid nitrogen or carbon dioxide snow are available, the biopsy can be transported in a preservative liquid medium described by Michel et al.[4]. The biopsies received in Michel medium should be washed in phosphate buffer saline (PBS), then mounted in tissue embedding compound (OCT) and frozen as described above, then stored at -25 ^{o}C.

B. Serum Sample

Sample of about 10ml of clotted blood should be taken in a plain vacutainer tube and the serum should be separated and sent to the laboratory for analysis. If not immediately tested it should be stored at 20^{o}C.

C. Immunofluorescence Technique

C1. Direct Immunofluorescence

Direct IF is a one step technique used to detect immunoglobulins, complement and fibrin deposits in the patient's skin. Sections 4 to 6 microns thick of the frozen biopsy are cut on a cryostat and then overlaid with a solution of antihuman FITC (isothiocyanate) conjugated antibodies with defined specificity (anti-IgG, anti-IgM, anti-IgA). The sections are then washed and a cover slip applied with a mounting medium appropriate for fluorescence microscopy[5]. The fluorescence dye attached to the antibody is excited and emits an apple - green colourfluorescence. The degree of fluorescence (weak, moderate orstrong) can only be approximated by the microscopist. The antibody titer cannot be determined by direct immunofluorescence alone.

The pattern of immunofluorescence deposits in a specific area is of diagnostic significance. Deposits at the basement membrane zone area may be granular and appear to be composed of discrete small particles of varying size. Other deposits are fine, smooth and linear, as seen in bullous pemphigoid or continuous like a bands as in lupus erythematosus. In epidermis, the most important areas are the intercellular areas between keratinocytes.

C2. Indirect Immunofluorescence

Indirect IF is a two step serological technique for detecting antibodies in a patient's serum or other tissue fluids. In this technique normal skin or monkey oesophagus is used as substrate[6]. It has been shown that good quality sections of monkey oesophagus or other monkey stratified squamous cell mucosac are the antigenic substract of choice for the indirect IF detection of the antibodies of pemphigus and pemphigoid[7]. The frozen specimen of normal human skin is sectioned in the cryostat to a thickness of 5 microns. Sections are overlaid with dilutions of serum so that any antibody against normal tissue components will bind to them. Control sera with known positive and negative antibody reactivity arerun simultaneously. Specimens are then washed to remove excess antibodies that are no bound. FITC-labelled antihuman immunoglobulin, most commonly an animal antihuman IgG, is then overlaid on the tissue section. If one is looking for the presence of circulating anti-IgA antibody. Sections are finally washed and then examined under the fluorescence microscope.

This technique can detect circulating antibodies directed against the intercellular area in the epidermis, and can also detect

circulating antibodies in the patient's serum directed against the different antigens in the basement membrane zone. In cases of severe bullous eruptions in which diagnosis is not certian, immunofluorescence studies of both biopsy specimens and serum are indicated.

C.3 Complement Indirect Immunofluorescene

Complement indirect immunofluorescence is a modification of indirect immunofluorescence for demonstrating complement - fixing antibodies in serum. It is more sensitive than usual indirect immunofluorescence and is a three stage technique. The tissue sections of normal human skin are incubated with the patient's serum. Sections are then washed and incubated with complement (normal human serum diluted in complement fixation test diluting buffer) and then incubated with FITC-labelled antihuman C3 antibodies, which will bind to the C3 molecules generated. After washing sections are examined under the fluorescence microscope.

The complement indirect immunofluorescence technique is useful in the diagnosis of pemphigoid gestationis. In this disease, so few IgG antibodies bind to tieeue antigen that they can not be detected by classic indirect immunofluorescence.[8] Since these molecules generate many C3 complement molecules, complement indirect immunofluorescence will show fluorescence at the site of antigen-antibody binding, demonstrated as a fine linear deposition of C3 at the basement membrane zone of substrate.

C4. Saline-Split Skin Techniques

i-Indirect Saline-Split Skin

This technique is used for differentiating between pemphigoid and EBA antibodies. the rationale of this technique lies in splitting the BMZ through the lamina lucida. This is achieved by incubating skin in[1] molar sodium chloride solution for approximately 72 hrs at 4°C[9]. Saline-split sections are useful in diseases in which antibasement membrane zone antibodies are present. The incubation and subsequent splitting induce a greater sensitivity because more epitopes on the basement membrane zone are exposed. Some diseases produce circulating antibodies that bind to the lamina lucida and a higher level in the basement membrane zone. Other diseases are characterized by anti BMZ antibodies that bind to only the dermal side of the saline-split BMZ[10].

ii-Direct Saline-Split Skin

In this technique specimen is first incubated in 1 molar sodium chloride solution for 48 to 72 hrs at 4°C to induce intra-lamina lucida cleavage, and then stained by the direct immunofluorescence technique to determine in which portion of the separated BMZ the tissue bound immunoglobulin is located. To eliminate the need for a repeat biopsy in such cases, the efficacy of splitting normal perilesional skin biopsies, which had previously been frozen for direct immunofluorescence studies, can be utilised, for the location of antibodies as a method to differentiate between bullous pemphigoid and EBA

AUTO-IMMUNE BULLOUS DISEASES

1. Pemphigus

Pemphigus is an intraepidermal blistering disease characterised by autoantibodies that are directed to the intercellular areas of stratified epithelia of mucous membranes and skin[11] resulting in loss of adhesion between epidermal cells (acantholysis) and blister formation. The group includes pemphigus vulgaris (PV), with a suprabasal acantholysis, pemphigus foliaceus (PF) with acantholysis in the upper parts of the epidermis and paraneoplastic pemphigus. The reasons for the differences in clinical presentation between these disorders, despite the fact that the target antigens are expressed in a similar distribution is unknown.

Pemphigus vulgaris is the most common

form of pemphigus. The disease has a peak incidence in patients between the fourth and sixth decade. PV patients form extensive erosions and crusts on mucosa and skin. In more than 50 per cent of cases the disease begins with oral lesions which may precede the cutaneous lesions by several months[12]. Other mucous membranes affected include conjunctivae, larynx, esophagus[13], vulva and urethera. Cutaneous lesions in PV can be localised or generalised and present as flaccid vesicles or bullae that rupture easily and produce painful raw areas. The blisters may develop on normal skin or an erythemtous plaque. Lateral traction on normal skin may cause the epidermis to shear off and produce additional erosions (Nikolsky's sign). Neonatal pemphigus is caused by maternal IgG crossing the placenta and cause blistering in the new-born infant.

Pemphigus vegetans is a rare form of pemphigus vulgaris. Patients are usually younger than those with PV. This type can be divided into the Neumann and Hallopeau. Lesions of the Neumann subtype start as vesicles then progress to become large verrucous, vegetating lesions localised mainly to the flexural and intertriginous areas. Pemphigus vegetans of Hallopeau present with pustules that develope into vegetating plaques.

Another type of pemphigus is pemphigus foliaceus, in which the blistering process occurs in the mid to upper epidermis resulting in a very superficial split, the majority of the patients presenting with crusted or scaly shallow erosions. Lesions are distributed over the face, scalp and trunk while mucosae are spared. Patients often have a more chronic, less severe course than those with PV.

Brazilian pemphigus (Fogo Selvagem) is a variant of pemphigus foliaceus. It occurs in endemic foci, and affects those living in rural areas of South America suggesting that an arthropod vector may play a role in transmission of this disease. Thymosine alpha 1 which is elevated inspecifically in patients, with viral infections, has been found to be elevated in these patients, which suggest possible viral etiology of Fogo Selvagem[14]. Pemphigus erythematosus (Senear - Usher syndrome) is a variant of pemphigus foliaceous in which the clinical features of PF overlap with the clinical and immunological features of lupus erythematosus[15]. It is characterized by well-demarcated, scaly, erythematous patches that often present on the malar area of the face. This variant is a less severe and more localized form of pemphigus.

Some cases of pemphigus have been associated with the administration of drugs, particularly thiol-containing compounds, such as D-penicillamin and Captopril[16]. Other drugs are ampicillin, penicillin and rifampicin. The majority of cases are identical to pemphigus foliaceus, and some are identical to pemphigus vulgaris. Burns, ultraviolet light and ionizing radiation have been reported to induce pemphigus, by alteration of epidermis so that the antigen is exposed[17].

Paraneoplastic pemphigus is a newly described variant in whcih all patients have an associated neoplasm[18,19]. Most of these neoplasms are non-Hodgkin's lymphomas, thymomas or haematological malignancies.

The tendency of pemphigus patients to develop malignancy could be related to immunosuppressive treatment. In these patients indirect immunofluorescence negative on normal human skin and weakly positive on monkey esophagus substrate. There was a positive indirect IF when rat bladder epithelium was used as the substrate.

Pemphigus vulgaris has been associated with other diseases such as mysthenia gravis, pernicious anaemia, thyroiditis, bullous pemphigoid and lichen planus.

Direct immunofluorescence demonstrates intercellular IgG (Fig.1) in lesional and normal perilesional skin in all variants of pemphigus[20]. Complement 3 can also be detected in about 50 percent of cases. In addition 20 per cent of patients will have IgM or IgA deposits. The presence of intercellular

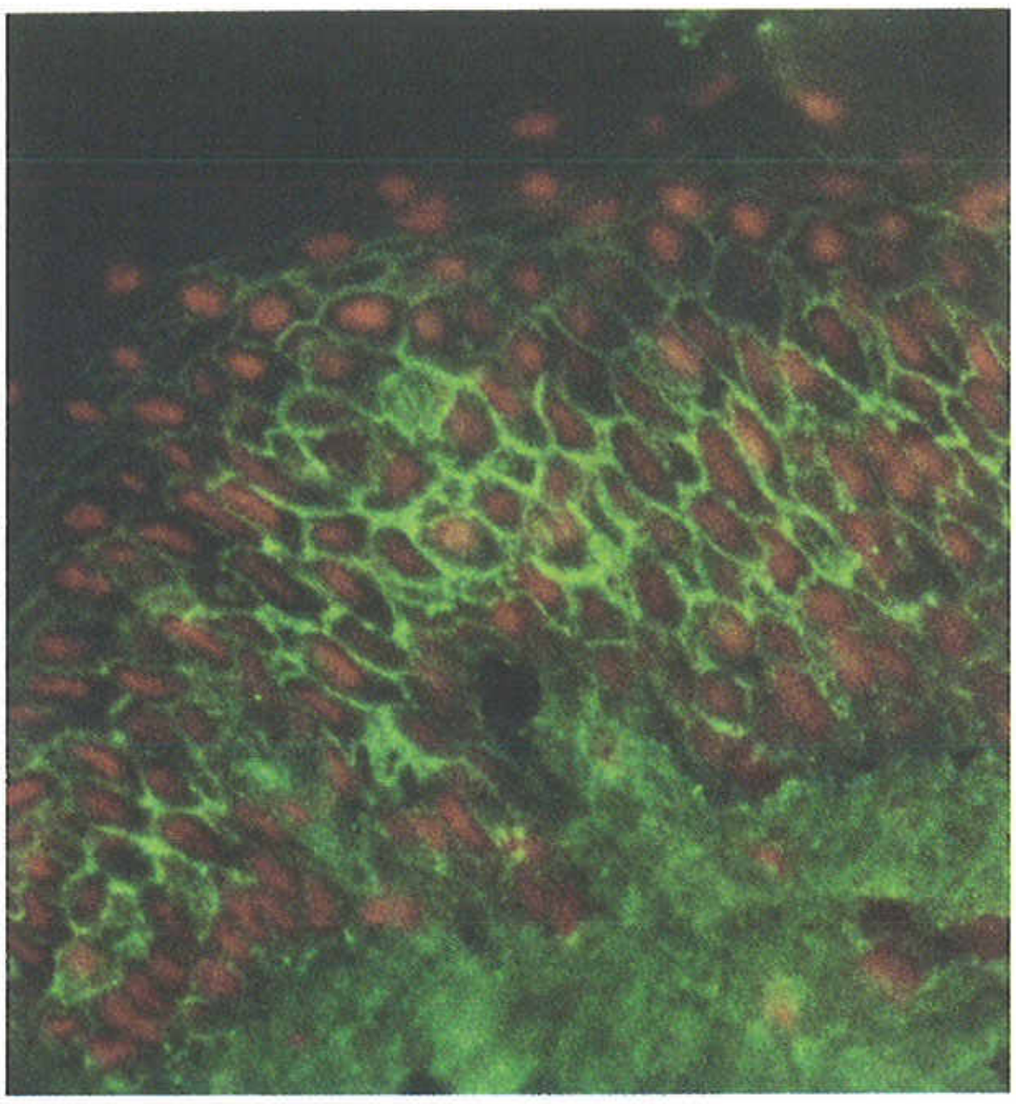

Fig.1. Direct IF of pemphigus showing intercellular IgG fluorescence.

deposits of complement in the absence of similar deposits of IgG does not establish a diagnosis of pemphigus[8]. In pemphigus erythematosus direct IF shows intercellular IgG and C3 as well as granular IgG and C3 at the dermo-epidermal junction (Fig.2).

Indirect IF in patients with pemphigus shows circulating IgG[21] specific for an antigen detected on the surface of stratified squamous epithelium (anti - ICS). All four subclasses of IgG have been found. IfG4 appears to be the predominant subclass of circulating autoantibodies[22]. It was found that the circulating autoantibody titer correlates with disease activity in patients with pemphigus vulgaris[23]. Follow us serum studies at 2 to 4 weeks intervals during the active phase of the disease and six monthly intervals during remission provide a useful prognostic guide. Low or undetectable antibody titers in patients with pemphigus usually occur when the disease is in an early stage or when it affects only localised areas such as the scalp or the mouth.

IgA Pemphigus

A number of reports have described a pruritic intraepidermal vesiculobullous eruption characterized by intercellular IgA but not IgG. This disease is known as IgA pemphigus. Patients with IgA pemphigus are divided into two types: The first is a subcorneal pustular dermatosis-type, with pustule formation and IgA deposition in the upper epidermis. The second is an intraepidermal neutrophilic IgA dermatosis type, with intraepidermal pustule formation and IgA deposition in the epidermis. IgA pemphigus occurs in middle aged and elderly individuals. Several cases have also been reported in children. The primary lesion is a flaccid vesicle, pustule or bulla on an erythematous or normal base. Nikolsky's sign is negative. The lesions of the foliaceus type

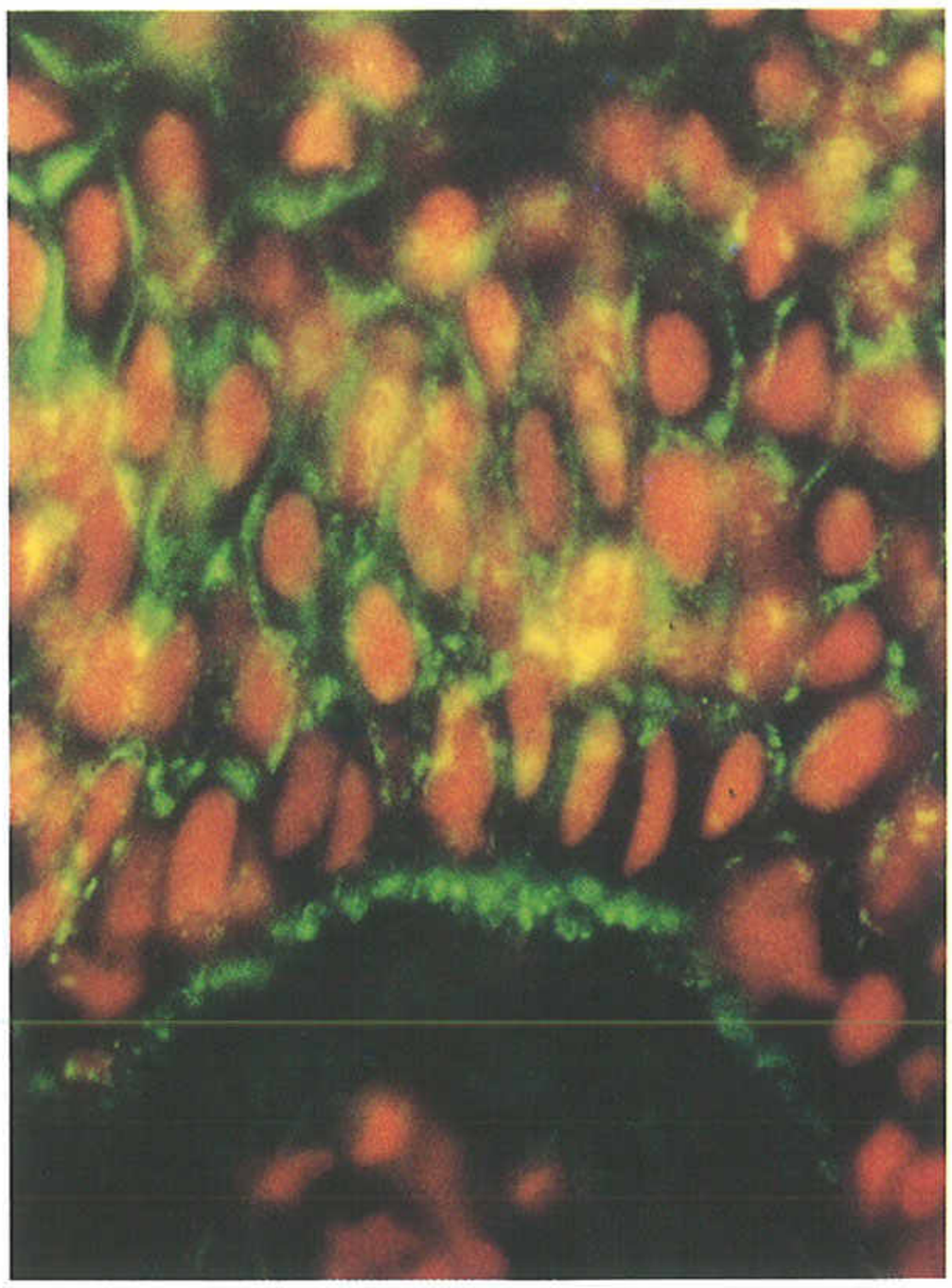

Fig.2. Direct IF of pemphigus erythematosus showing mixed staining pattern. Intercellular and granular BMZ fluorescence with IgG.

appear mostly on the axilla, trunk and limbs while the lesions of the intraepidermal pustule type tend to be widespread. Mucosal surfaces are rarely involved.

Direct IF of perilesional and normal skin of these patients shows intercellular IgA deposition usually in the upper part of epidermis. Complement components are not detected. Circulating intercellular IgA antibodies have been detected by indirect IF on monkey oesophagus and guinea pig in low titer in less than 50 per cent of cases.

2. Bullous Pemphigoid

Bullous permphigoid is an autoimmune subepidermal blistering disease of the skin. It is usually a disease of the elderly, with the majority of patients affected in their sixth or seventh decades. It rarely occurs in childhood[24,25]. The skin lesions are vesicles and bullae that may occur on normal skin or an erythematous base. In addition to blisters and erosions most patients have erythematous and oedematous macules and plaques. Lesions often occur on flexor surfaces of the upper extremities, thighs, axillae and lower abdomen. Pruritus of variable severity is the major problem[26]. Mucosal involvement is not usually seen, but when it does occur the oral mucosa is most frequently involved.

A number of clinical variants of bullous pemphigoid have been described. These include localised, vesicular[27], atrophic, cicatricial, nodular and juvenile. The most common variant is localised BP[28] in which lesions are usually limited to the lower extremities or occasionally the palms and soles resembling a dyshidrotic eczema and named dyshidrosiform pemphigoid[29].

Patients with 'pemphigoid nodularis' in which episodes of blistering are followed by intense pruritus and scarring closely resemble prurigo nodularis both clinically and histologically.[30]

Vegetating permphigoid is a rare variant characterised by purulent and verrucous vegetating lesions. Although the lesions most closely resemble those found in pemphigus vegetans, histology and immunofluorescence studies support the diagnosis of bullous pemphigoid[31].

Cicatricial pemphigoid is relatively rare and chronic scarring blistering disease that involves mucosal surfaces of larynx, pharynx, oesophagus, conjunctiva and genitalia[32]. Intact blisters may be seen on the skin surface near mucous membrane surfaces. Lesions head with scarring that cause blindness or oesophageal stenosis[33]. Cicatricial pemphigoid is divided into two subtypes: classical cicatricial pemphigoid (CCP) and the Brunsting - Perry variant of pemphigoid (BPP).

There are reports of an association of bullous pemphigoid with other disorders including diabetes, psoriasis, rheumatoid arthritis and malignancy, of which carcinoma of the lung, breast and gastrointestinal tract are the most common[34].

Direct IF studies of perilesional skin biopsy specimen show linear deposits at the basement membrane zone of complement in 80 to 100 per cent of patients with bullous pemphigoid and IgG in 80 percent of these patients. Subtyping of the IgG deposits shows that IgG4 is found in almost all patients. Linear IgM are found in about one-third of patients. Other classes of immunoglobulins are found less frequently. Previous studies showed that the pemphigoid antigen is expressed more in flexural areas of the arms and legs, and the lowest amounts are found in scalp and facial skin[35] Thus the biopsy of perilesional skin from lesions present near the axilla or anticubital fossa is preferred.

Approximately 70 percent of patients with BP have circulating IgG anti-BMZ antibodies detected by indirect IF. Lower titers of IgE and IgA anti-BMZ antibodies have been reported. In other forms of permphigoid the percentage of seropositive findings is lower. Studies showed that the majority of the circulating auto-antibodies were of the IgG^4 subclass[36]. It is generally accepted that there is no

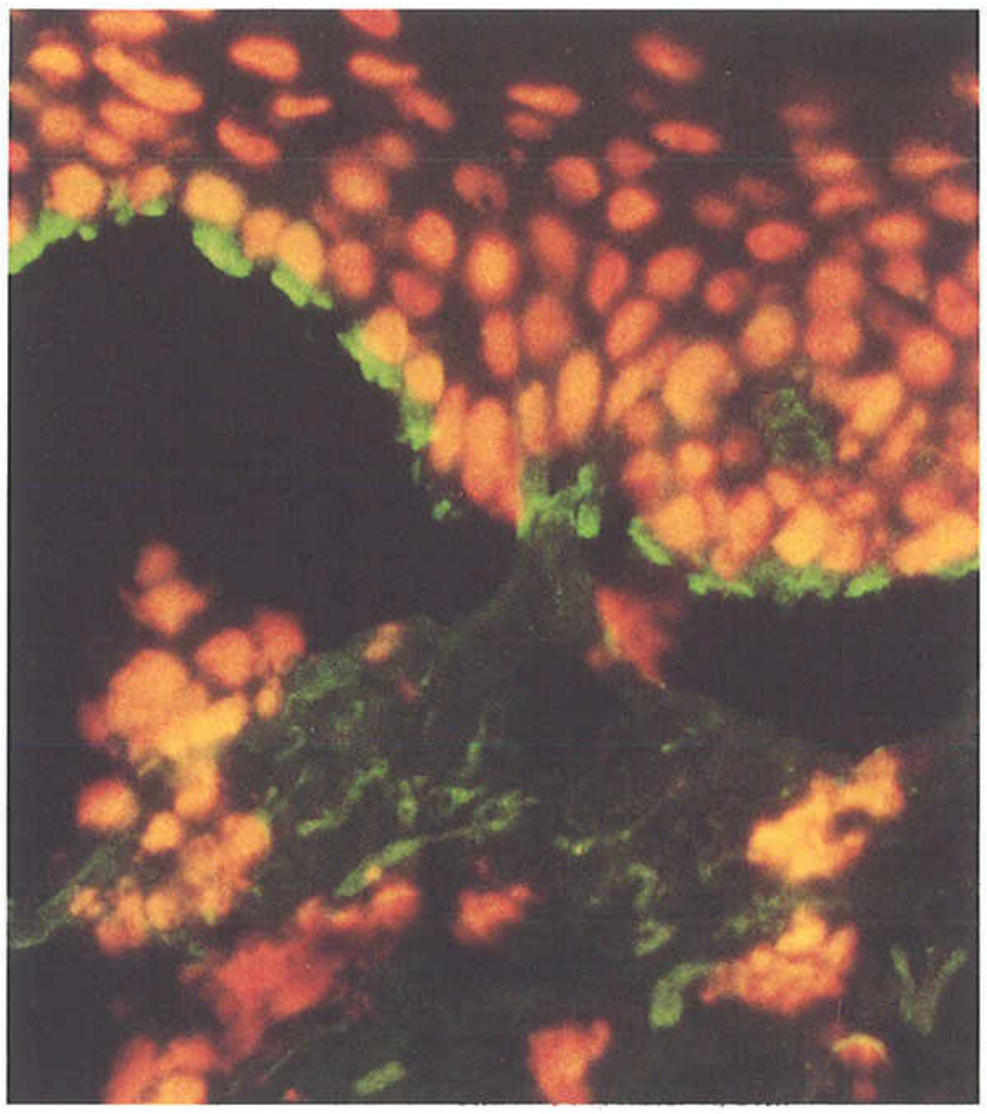

Fig.3. Indirect IF showing bullous pemphigoid antibodies binding to the epidermal side of IM NaCL-split skin.

correlation between antibody titer and bullous pemphigoid, also circulating antibodies are more often absent ealry in the disease than later.

To distinguish between Epidermolysis bullosa acquisita and bullous permphigoid, indirect IF on saline-split skin should be performed. Seventy per cent of patients with BP will have positive indirect IF on saline-split skin detected as a linear band of fluorescence on the epidermal side of the split (Fig.3), whereas EBA antibodies will bind to the dermal side of split skin.

Circulating IgG antibodies directed against the BMZ are rarely detected in patients with burns, psoriasis and leg ulcers.

Lichen Planus Pemphigoides

There are cases that have clinical, histological and immunological findings suggesting both LP and BP. The characteristic immunofluorescent features of lichen planus may overlap with the findings of the pemphigoid type. The presence of IgG and fibrin deposits particularly in clusters of large cytoid bodies, is characteristic of lichen planus. Also biopsies from perilesional skin show linear IgG and complement (C3) deposition at the BMZ in lichen planus pemphigoides. Circulating IgG class antibodies occur in some cases. Not all bullous eruptions in cases of lichen planus can be classified as having both lichen planus and pemphigoid37.

3. Pemphigoid Gestationis

Pemphigoid gestationis (PG) is a rare, pruritic, subepidermal blistering disease of pregnancy and the puerperium. The incidence of PG is 1 in 50,000[38] and is extremely rare in blacks. It begins in any trimester of pregnancy, usually second or third, and tends to recur in subsequent pregnancies. PG may flare with the use of oral contraceptive, also PG has been reported in association with hydatidiform mole and choriocarcinoma[39]. Typical lesions include urticarial papules and plaques with polycyclic wheals that form vesicles and bullae. Lesions start periumplically and spread ot involve abdomen, buttocks and extremities. Mucous membranes lesions are rare. The bullous lesions resolved within 3 months of delivery, but rare case have been reported to last for 12 years post partum. The IgG antibody cross the placenta and cause a blistering disease in infants which resolves spontaneously within several months once the maternal antibodies are cleared.

Direct IF shows deposits of complement (C3) in a smooth linear pattern along the BMZ of uninvolved skin in patients with pemphigoid gestationis. In addition, deposits of IgG are found in the same pattern in 30 percent of patients. Direct IF of clinically normal or lesional skin from infants of affected mothers has also showed desposits of C3 and IgG at the epidermal BMZ.

Indirect IF demonstrates circulating IgG anti-BMZ antibodies in a low percentage of patients with pemphigoid gestationis. Using

an indirect complement fixation IF technique, the majority of patient sera demonstrate complement - fixing IgG directed against a BMZ antigen (Fig.4) this antibody being known as the PG factor. This factor may be the result of humoral sensitization to foreign fetal antigen and cross reacts with antigens of the dermo-epidermal junction. PG factor activity has been found in the cord blood of infants born to mothers with PG. IgG1 has been found in both tissue and serum and is considered to be the predominant subclass of IgG[40] thus distinguishing PG from bullous pemphigoid, in which the IgG4 subclass predominates.

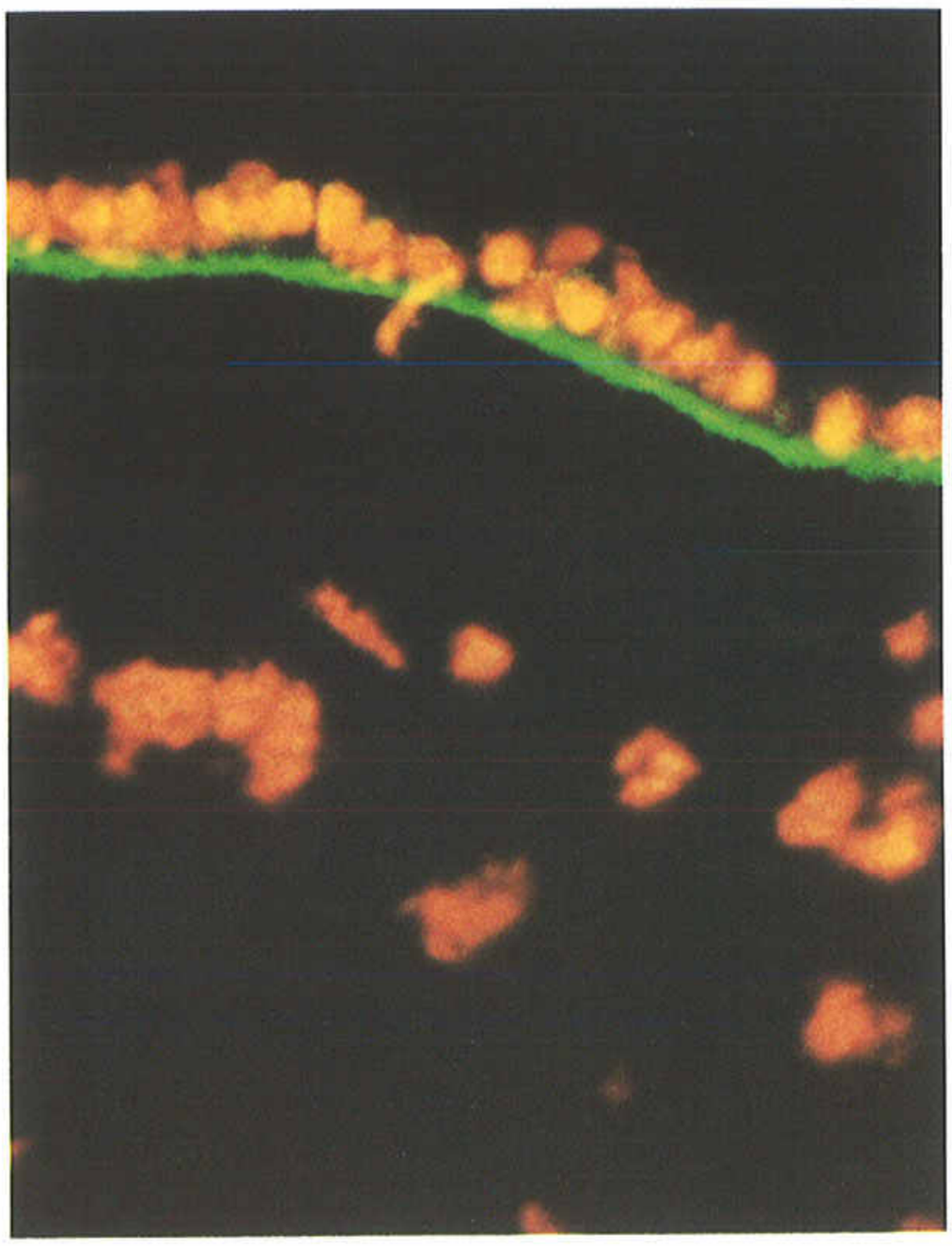

Fig.4. Indirect C3 IF method showing PG antibodies binding to the BMZ of Amnion.

4. Epidermolysis Bullosa Acquisita (EBA)

EBA is an autoimmune subepidermal bullous disease that presents in the fourth to sixth decade. It is common in females and blacks and has been reported in children[41]. Roenigk and Associates[42] established the first criteria for the disease : adult onset with negative family history, trauma induced bullae which resemble dystrophic epidermolysis bullosa. Classic EBA is a non inflammatory disease that presents with skin fragility, blisters and erosions at site of trauma and heals with scarring and milia formation. These lesions tend to develop on the dorsa of the hands, feet, elbows and knees. Alopecia and nail dystrophy may occur. Approximately one half of patients with EBA have a widespread inflammatory vesiculobullous disease that resembles bullous pemphigoid, in which healing may occur without scarring or milia formation. Another group of patients may have disease that resembles cicatricial pemphigoid. Lesions may be haemorrhagic and resemble bullous amyloidosis or may be grouped and resemble dermatitis herpetiformis.

EBA has been associated with other systemic disease. The most frequent

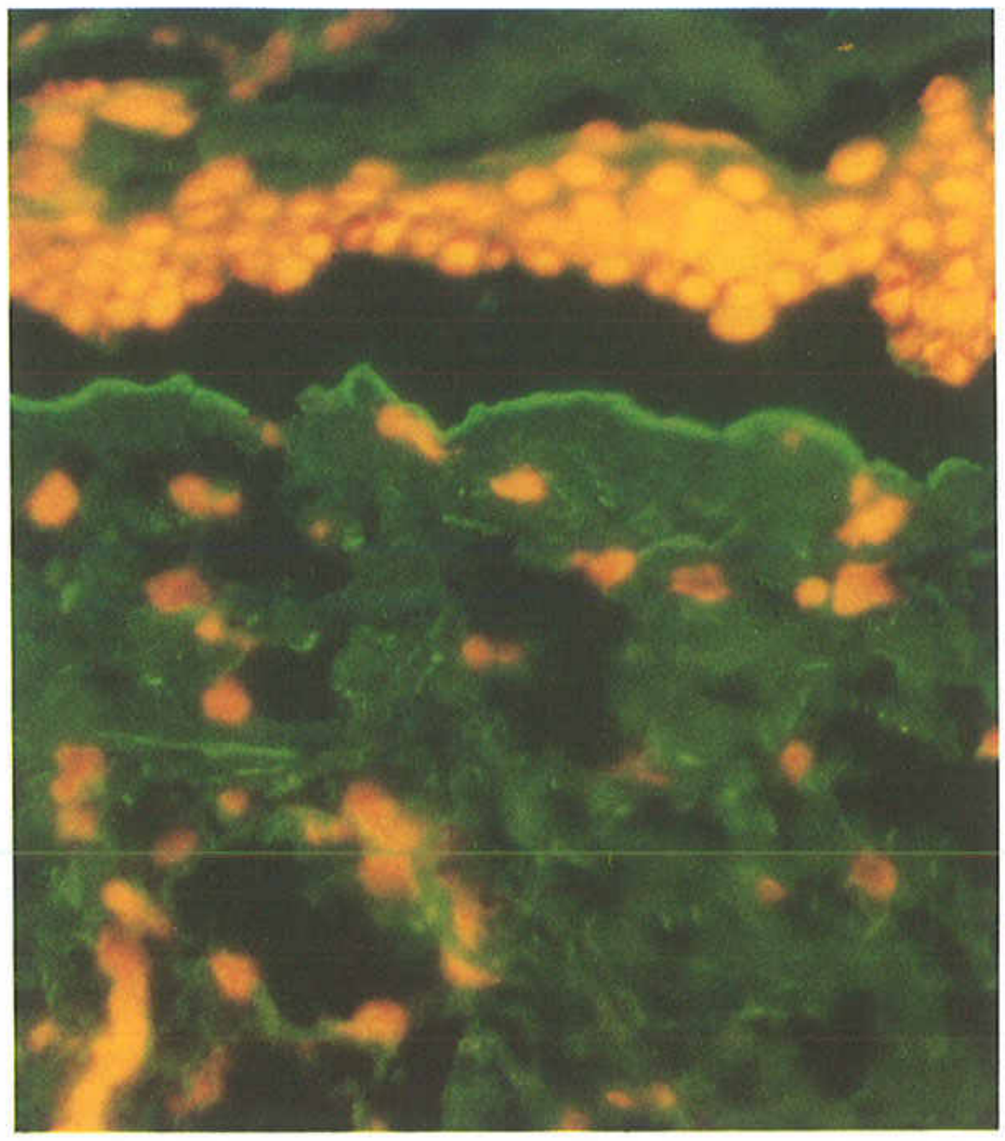

Fig.5. Direct split skin technique in a typical case of EBA showing linear BMZ fluorescence exclusively to the dermal side. (IgG).

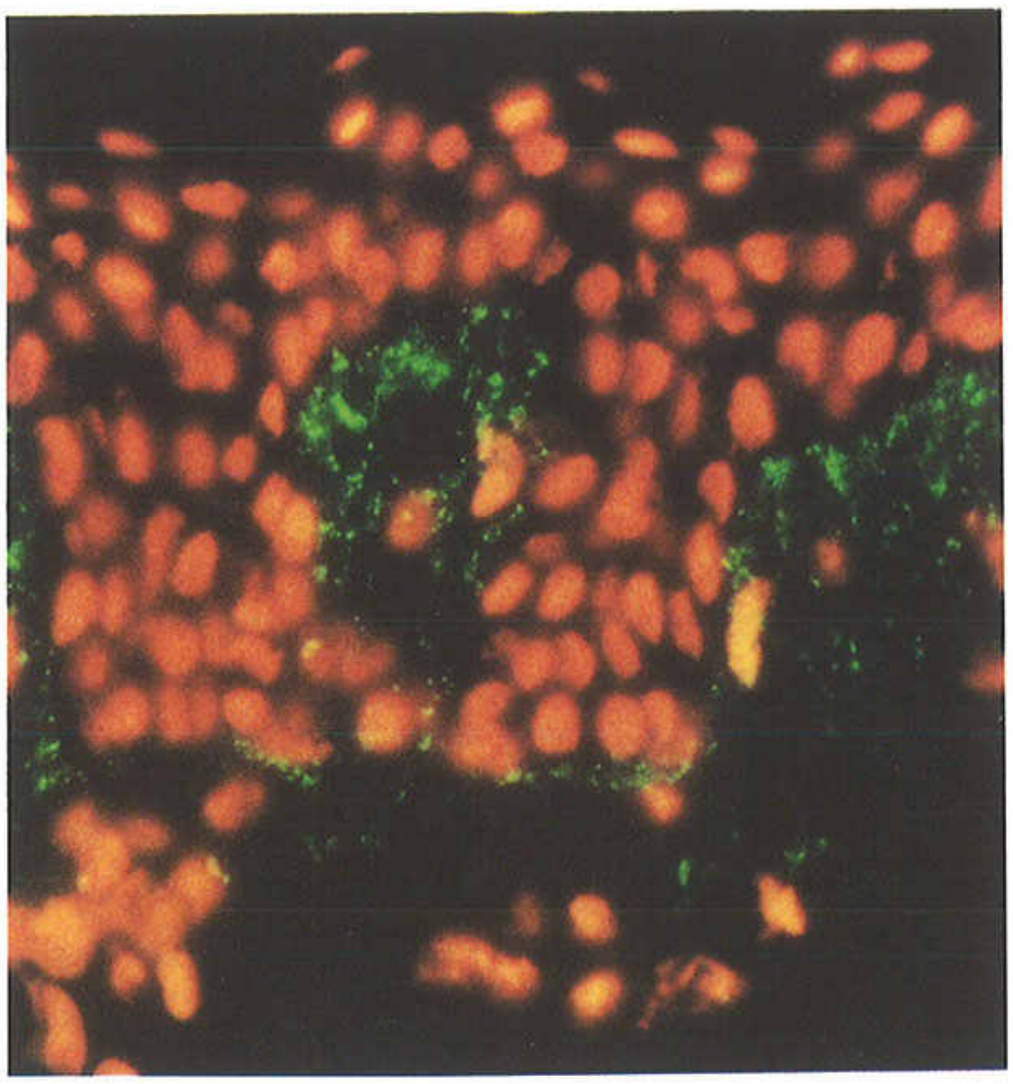

Fig.6. Direct IF showing granular IgA deposits in dermal papillae in DH.

association is with inflammatory bowel disease. Other associations are SLE[43]. thyroiditis, diabetes mellitus, rheumatoid arthritis and amyloidosis.

Direct IMF from perilesional skin shows broad linear deposits of IgG and C3 at the BMZ. In addition, linear deposits of IgM, IgA or fibrinogen have been reported. The presence of three or more immunoreactants at the BMZ may be more indicative of EBA than of bullous pemphigoid which usually has only two IgG and C3.

Earlier studies have shown that 25 to 60 per cent of the sera taken from EBA patients will have circulating IgG antibodies which produce continuous linear pattern of fluorescence staining at the BMZ of human skin.

The diagnosis of EBA depends on the salin-split skin technique, where normal skin is split by saline and the patient's serum is then added. Anti-BMZ antibodies present in the serum will bind to the dermal side of the junction and leave the epidermal side unstained.

5. Dermatitis Herpetiformis

Dermatitis herpetiformis (DH) is a chronic papulovesicular disease usually developing in young adults. Males are more affected than females in a ratio of 3:2. The primary lesion is either an urticaria-like plaque or a vesicle. Intact blisters are rarely found because of extreme pruritus[44] thus patients commonly present with crusts or erosions. Sites of involvement are characteristically the extensor surfaces including the knees, elbows, saerum, buttocks and shoulders. Mucous membranes are rarely affected. Two-thirds of DH patients have gluten-sensitive enteropathy, and they will therefore respond to dietary restriction of gluten-containing products. The clinical course of the disease is characterized by recurrent exacerbations and remission.

Dermatitis herpertiformis is associated with a number of autoimmune diseases such as pernicious anaemia, rheumatoid arthritis, dermatomyositis and thyroid dysfunction[45]. There is also a strong HLA association, with 70-80 per cent of patients being HLA B8 positive[46] and greater than 90 per cent HLA-DR3 positive.

The majority of cases of DH demonstrate granular deposits of IgA that is focussed in the dermal papillae of lesional and perilesional skin (Fig.6). Occasionally patients show continuous deposition of granules along the basement membrane zone. Atypical cases of DH have been reported to have "fibrillar" deposition of IgA. Approximately 10 to 15 per cent of patients may have IgG or IgM with IgA deposition.

Indirect IF has been unsuccessful to demonstrate and IgA class antibody that binds to skin in DH patients, although 30 to 40 percent of patients have IgA-circulating immune complexes in their sera. The nature of the antigen to which IgA is bound is unknown. Neither indirect immunofluorescence nor immunoblotting has shown the component of normal skin to which it binds.

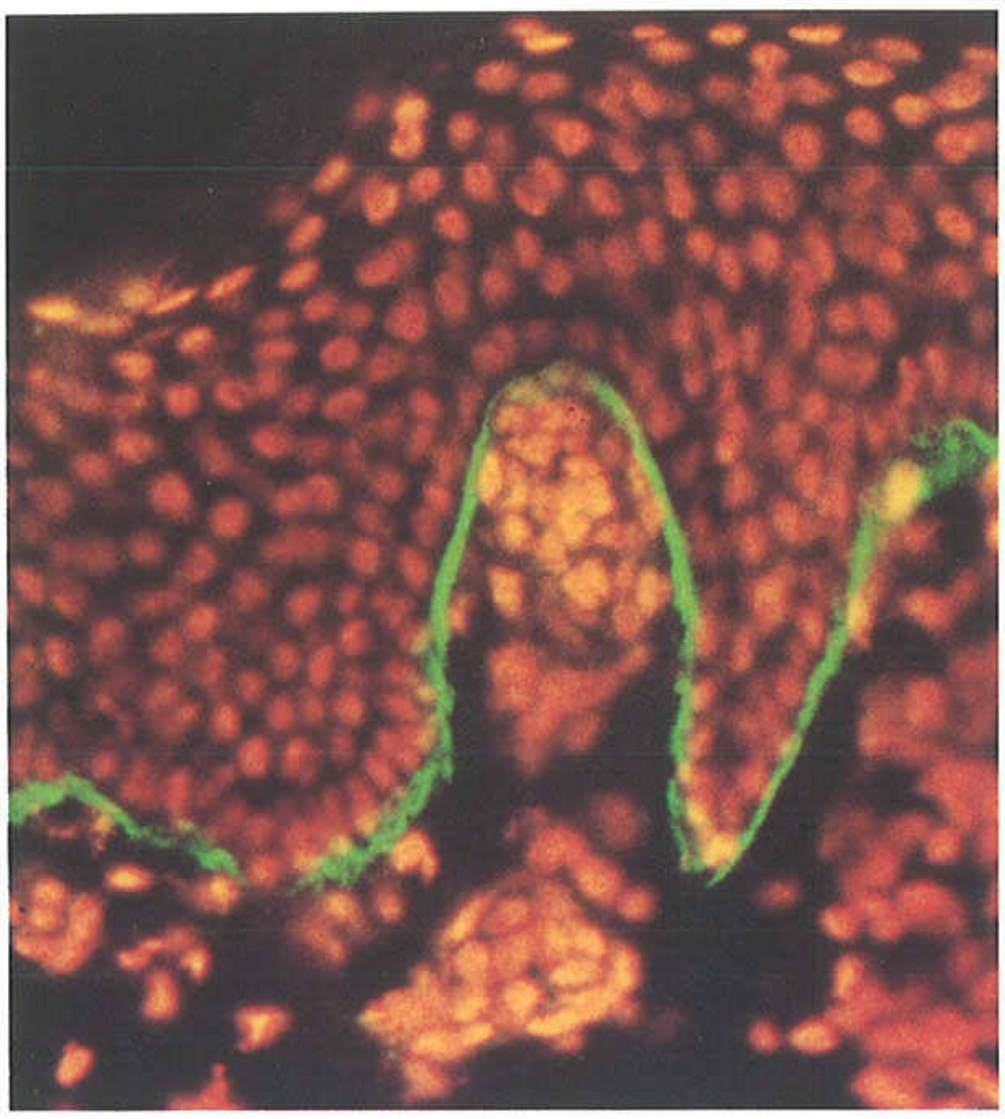

Fig. 7. Direct IF showing linear band of IgA in LAB

6. Linear IgA Bullous Dermatosis

Linear IgA bullous dermatosis (LABD) is a term used to describe a group of subepidermal vesiculobullous disorders which have the direct IF findings if IgA deposition at the BMZ in a linear pattern. The disease occurs in both adults and children, and there are some clinical differences between the two. LABD is divided into LABD of adults and LABD of childhood that is more commonly known as chronic bullous disease of childhood (CBDC).

The adult type occurs between 14 to 83 years of age[47]. It is more common in females. The clinical presentation is variable, some patients present with grouped, small, tense vesicles distributed on elbow, knees, back, scalp and buttocks, that are typical of DH. Other patients present with non grouped, large, tense bullae on normal or erythematous plaques that are typical of BP. Unlike DH, mucous membranes may be affected in LABD. Ocular and oral lesions occur in 50 per cent of patients. When mucosal disease involves the conjunctivae it may leave scarring[48]. Pruritus may be significantly less severe than that experienced by patients with DH or may be absent. These patients do not share the gluten-sensitive enteropathy and do not respond to a gluten-free diet.

7. Chronic Bullous Disease of Childhood (CBDC)

CBDC is an acquired subepidermal bullous disease, occuring most commonly in preschool age children. In these patients the distribution of the lesions is different from LABD in adult. The lesions most commonly involve the perineal area, external genitalia, thighs and flexor surfaces. The face and particularly the perioral area, eyelids and scalp may be affected. Patients present with pruritic vesicles and bullae. The bullae sometimes haemorrhagic, usually arise on normal skin. The most typical lesions are annular or circinate with blisters at the periphery "the string of peals sign".

Direct IF shows linear deposition of IgA at the BMZ. Patients with IgG in a linear pattern at the BMZ as well as IgA have been reported as having LABD. IgG staining is weak and it is thought to be a secondary immunoglobulin[49]. In contrast to bullous pemphigoid, a small percentage of patients with LABD have C3 at the BMZ.

Circulating IgA antibodies directed against the human epithelial basement membrane zone have been detected in low titers (in only 10 to 30 per cent of the adult type). Positive indirect IF has been reported more often in LABD of childhood than in adulthod (75 per cent in children). One study found a positive indirect IF using monkey esophagus in 60 per cent but when saline-split skin was using the rate was 90 per cent.

8. Bullous Systemic Lupus Erythematosus

There have been reports of a bullous form of SLE, and others of coexisting SLE and pemphigoid, pemphigus, dermatitis herpetiformis[50] and erythema multiforme. The bullous lesions of lupus have been classified

into LE-specific and LE-non specific manifestations. To be classified as having LE-specific lesions, patients are required to have a histological pattern consistent with that of lupus, and some reports showed widespread vesiculobullous eruption during the exacerbation of SLE. The clinical features of bullous SLE are vesicles or bullae which may be single or grouped and may arise on erythematous or normal appearing skin. Bullous SLE is usually associated with flares of lupus. In most cases the age of onset is in twenties or thirties but cases have also been reported in children.

Direct IF of perilesional skin shows linear or granular deposition of IgG at the dermoepidermal junction in 95 per cent of cases and C2, IgM and IgA are found in approximately 70 per cent of cases.
Indirect IF has revealed circulating anti-BMZ antibodies in 30 per cent of cases. By saline-split skin testing, these antibodies bind to the dermal side of the separated skin.

Conslusion

In summary the immunofluorescence methods discussed in this paper have proved useful in advancing our understanding of the pathogenesis of the autoimmune bullous dermatoses. Recently more advanced immunological methods have become available. These new techniques depend on objective biochemical parameters and have helped us to understand the distinction between diseases that may appear clinically similar though biochemically different.

The immunoelectron microscopy method is one of the important technique used to localize precisely the immune deposits in the patient's skin or to localize the structure within normal skin to which autoantibodies in the patient's serum bind. Thus antigenic targets for various autoantibodies have been defined and their biochemical nature determined.

The last point of importance is that in many of these diseases we still do not know exactly which step plays a critical role in the initiation and generation of these antigen-antibody reactions.

From our knowledge of the factors that predispose to these autoimmune dermatoses we could postulate the importance of the role of hormones, sex, infection, malignancy and drugs in their aetiology.

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