Chapter 34
Application of Direct Immunofluorescence for Skin and Mucosal Biopsies: A Practical Review

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Abstract This is a practical overview of the use of the application of direct immunofluorescence, written in telegraphic style, based on the author's personal experience and supplemented by a recommended reference reading list, conceptual diagrams and illustrative examples.

Keywords Anti-epiligrin pemphigoid • Biopsy selection • Bullous pemphigoid • Cicatricial pemphigoid • Combined use of salt split skin and n-serrated/u-serrated patterns • Dermatitis herpetiformis • Dermatomyositis • Discoid lupus • Epidermal proteins diagram • Epidermolysis bullosa acquisita • Henoch Schönlein purpura • IgA pemphigus • Lichen planus • Lichen planus pemphigoides • Linear IgA disease • n-serrated and u-serrated patterns • Nuclear reactions • Other factors • Paraneoplastic pemphigus • Pemphigoid gestationis • Pemphigus erythematosus • Pemphigus foliaceous • Pemphigus vegetans • Pemphigus vulgaris • Porphyriacutanea tarda • Pseudoporphyria • Salt split skin • Shave biopsy or a punch biopsy • Specimen transport • Subacute cutaneous lupus • Systemic lupus • Thin shave biopsies • Vasculitis

FREQUENTLY ASKED QUESTIONS

34.1. What is the difference between direct and indirect immunofluorescent testing?
34.2. Which is better a shave biopsy or punch biopsy?
34.3. What is the best biopsy site?
34.4. How should the biopsy specimen be transported?
34.5. How is the specimen processed?
34.6. What technique is useful for mounting and cutting thin shave biopsies and mucosal biopsies?
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34.27. What are the characteristic immunofluorescent findings in IgA pemphigus?
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34.38. What other factors should always be considered in any DIF testing?

34.1 What Is the Difference Between Direct and Indirect Immunofluorescent Testing?

Direct immunofluorescent examination (DIF) is performed on tissue biopsies using cryostat sections that are stained with fluorescein conjugated anti-immunoglobulin, anti-complement (C3) and anti-fibrinogen. The test detects the presence of in vivo deposits of immunoglobulin, complement and fibrinogen in the tissue sample and displays the distribution pattern of the deposits.

Indirect immunofluorescent examination tests the patients serum for the presence of circulating antibodies by incubating the serum with a laboratory tissue substrate such as human skin, monkey esophagus or rat bladder and then using a second fluorescein conjugated anti-immunoglobulin antibody to determine if and where antibody in the serum bound to the tissue substrate.

34.2 Which is Better: A Shave Biopsy or a Punch Biopsy?

Either is acceptable. A 3–4 mm punch biopsy provides a good sample and is easy to process for preparation of frozen sections. Fat attached to the deep edge of the specimen may be trimmed away to make cryostat sectioning of the skin easier.

A shave biopsy provides a larger area for evaluation but the specimen is more tedious to embed with good orientation for cryostat frozen sections.

For routine H&E histology, a deep shave biopsy, i.e. deep enough to get into the upper reticular dermis is preferable for biopsy of a vesicle or bulla because the epidermis may be fragile and easily disrupted by a the shearing force of a punch biopsy. The shave biopsy may also allow inclusion of a larger sample of intact skin close the blister edge.

34.3 What Is the Best Biopsy Site?

Table 34.1.

Table 34.1 Biopsy site selection

Suspected condition	Specimen for DIF	Specimen for routine microscopy
Pemphigus	Perilesional[a]	Lesion and intact bordering skin and mucosa to show transition zone
Bullous pemphigoid	Perilesional	Lesion and intact bordering skin and mucosa to show transition zone
Pemphigoid gestationis	Perilesional	Lesion and intact bordering skin and mucosa to show transition zone
Linear IgA disease	Perilesional	Lesion and intact bordering skin and mucosa to show transition zone
Epidermolysis bullosa acquisita	Perilesional	Lesion and intact bordering skin and mucosa to show transition zone
Dermatitis herpetiformis	Normal skin close to lesion i.e. 3 mm from lesion edge	Lesion and intact bordering skin and mucosa to show transition zone
Lichen planus	Perilesional with inclusion of portion of lesion	Lesional
Porphyria cutanea tarda	Perilesional with inclusion of portion of lesion	Lesion and intact bordering skin and mucosa to show transition zone
Pseudoporphyria	Perilesional with inclusion of portion of lesion	Lesion and intact bordering skin and mucosa to show transition zone
Lupus	Lesional	Lesional
Vasculitis	Lesional less than 24–48 h old	Lesional

Modified to table format from: Biopsy Sites: Online at http://beutnerlabs.com/request/biopsy-sites.php

[a]Intact normal skin or mucosa at lesion edge

34.4 How Should the Specimen Be Transported?

Table 34.2.

34.5 How Is the Specimen Processed?

Table 34.3.

34.6 What Technique Is Useful for Mounting and Cutting Conjunctival Specimens or Large Thin Shave Biopsies?

A button of OCT compound is placed on the cryostat chuck and briefly cooled by short immersion in liquid nitrogen to gel the button but not completely solidify it.

The thin flimsy fragment of skin, conjunctiva or other mucosa is then laid flat on the surface of the still liquid OCT

Table 34.2 Specimen transport

Michel's solution	Saline
Tried and true. Standard DIF transport solution in use for many years with consistently good result	Suitable for specimens that will be received in less than 24 h
Preferable to receive within 48–72 h	Fluorescence detection may diminish after 24 h
Stable at room temperature	Stable at room temperature up to 24 h. Use wide mouth screw cap cup like a urine collection cup, tightly closed to avoid desiccation
Commercially available in screw cap, glass vials from Zeus Scientific, The vials have a shelf life of several months	Readily available
Not suitable for routine light microscopy. A separate biopsy should be submitted in formalin for routine microscopy. I do not recommend splitting punch biopsies in the clinic. The specimens are small the tissue is fragile and it is difficult to do without good magnification and a fresh knife blade. It is preferable to have two separate specimens to avoid compromising the quality of one or both samples	Preferable to receive a separate specimen for routine microscopy that has been place in formalin at the time of the biopsy

References: [1, 2]

Table 34.3 How is the specimen processed?

In Lab Processing Steps	
Buffer wash	Specimens received in Michel's solution are washed in a buffer solution for 30 min at room temperature, after discarding the Michel's solution. The buffer solution is commercially obtained from Zeus Scientific company. It is stored at 4 °C once opened and in use
Mounting the specimen	Specimen is oriented and mounted on a cryostat microtome chuck in a button of OCT (Optimal Cutting Temperature) compound. The knife should strike the deep dermal edge of the specimen first and exit through the epidermis last to avoid folded and wrinkled sections
Freezing the specimen	Snap frozen by immersing the mounted specimen in liquid nitrogen
Sectioning the specimen	Cryostat frozen sections are cut at a thickness of 5 μm
Sections are stored at −20 °C	Slides are placed in plastic slide boxes and kept at -20C until they are stained
	We also routinely fix an extra slide for each antibody in cold acetone for 3 to 5 min. Those slides are then air dried and stained with the unfixed cryostat sections. The reactions with both sets of slides are typically congruent but sometimes one or the other is superior
Staining the sections	Sections are stained with fluorescein conjugated anti-IgA, anti-IgG, anti-IgM, anti-C3 and anti-fibrinogen using a Ventana Ultra stainer. The reagent antibodies are purchased pre-diluted from Ventana and we use the Ventana automated staining protocol template with an initial incubation time of 32 min for each antibody
Coverslipping the sections	Water soluble, nonfluorescent mounting medium is used to attach the coverslips
Slide storage	The stained slides are kept refrigerated in a slide folder until the delivery for examination
Reading the slides	The slides are examined in a darkened room using an epiluminescent fluorescence microscope – Systematic examination of intercellular space, epidermal BMZ, follicular BMZ, dermal papillae, dermal vessels, epidermal nuclei, and epidermal cytoplasm
Recording results	For each antibody, the reaction location and intensity of reaction is recorded. Once read, the fluorescent reaction will often remain visible for several days when stored in a slide folder at room temperature but the reaction does not remain visible indefinitely. Permanent record of reactions may be obtained by photomicroscopy

compound and it is stretched out flat with the skin or mucosal surface facing up.

The chuck is then immersed in liquid nitrogen to freeze the OCT and the specimen solid.

Next, the frozen button is pried from the chuck with a scalpel blade after first dribbling a small amount of tap water around the base of the frozen OCT button to allow it to be easily removed, yet remain frozen.

Once removed, the flat piece of frozen tissue and the adherent frozen OCT compound is bisected or trisected and the section fragments including the still frozen OCT compound are turned 90° and immediately re-embedded in a fresh button of OCT compound which is then snap frozen in liquid nitrogen.

This method simplifies the embedding process for this type of specimen and the tissue sections will now be perfectly vertically oriented with the epidermis or mucosa in profile when the tissue is sectioned.

34.7 What Antibodies Are Routinely Used?

Commercially prepared fluorescein conjugated Anti-IgA, Anti-IgG, Anti-IgM, Anti-C3 and Anti-fibrinogen.

34.8 What Positive Controls Are Used? How Is Positive Control Tissue Prepared and Preserved?

A positive control for each antibody is run with each batch of patient samples. Positive control slides are harvested from positive clinical cases by cutting up to 100 additional slides from the frozen block and storing the slides at −80 °C in plastic slide boxes. To store any remaining specimen, the frozen button of OCT compound containing the tissue is removed from the cryostat chuck, wrapped in aluminum foil and stored at −80 °C.

34.9 How Are Immunofluorescent Reactions Graded?

The grading of the intensity of staining is subjective but a general guideline is:

4+ = strong, glaring fluorescence
3+ = strong, bright staining, not glaring
2+ = strong reaction
1+ = dim, but definite
Trace = faint, equivocal

Generally, in positive specimens, staining intensity is in the 2+ to 4+ range.

34.10 How Is a Salt Split Skin Substrate Prepared for Direct Immunofluorescent Study and How Is It Useful?

When a biopsy of intact perilesional skin is received in the laboratory, the specimen is placed in a tube of 30–40 ml of 1 M saline that has been previously cooled to 4 °C.

If the specimen was received in Michel's solution, it should be washed in buffer prior to placing in the saline solution.

The specimen is allowed to incubate in the saline solution at 4 °C for 48 h.

The incubation in cold (4°C)1 M NaCl causes a cleavage plane in the lamina lucida resulting in portions of the epidermis detaching from the dermis and forming saline induced vesicles of varying size.

After the incubation, it is removed from the saline, placed on a paper towel to allow gentle absorption of any surface saline and then it is mounted in OCT compound and frozen in liquid nitrogen for preparation of cryostat sections as usual.

The undersurface of the epidermis or mucosa forms the roof of the vesicle and the lamina densa regions of the basement membrane zone and the dermis form the floor.

Depending on where the staining reaction now localizes i.e. along the undersurface of the roof or along the floor provides additional or confirmatory diagnostic information [3].

Figures 34.1, 34.2, and 34.3.

34.11 What Are n-Serrated and u-Serrated Patterns and How Are They Useful?

Figures 34.4 and 34.5.

These are very useful patterns to look for when there is a linear band of immunoglobulin or complement along the epidermal junction because they further define the location of the immunoreactants as either above the lamina densa (n-serrated pattern) as in all forms of pemphigoid and most cases of linear IgA dermatosis or below the lamina densa (u-serrated pattern) as in epidermolysis bullosa acquisita (EBA), bullous lupus erythematosus and rarely one form of linear IgA dermatosis. The third pattern, true linear, does not aid in defining the location of the immunoreaction.

These patterns have been elegantly shown by correlation with immunoelectron microscopy and dual label immunofluorescence mapping to identify the location of the immunoreactants.

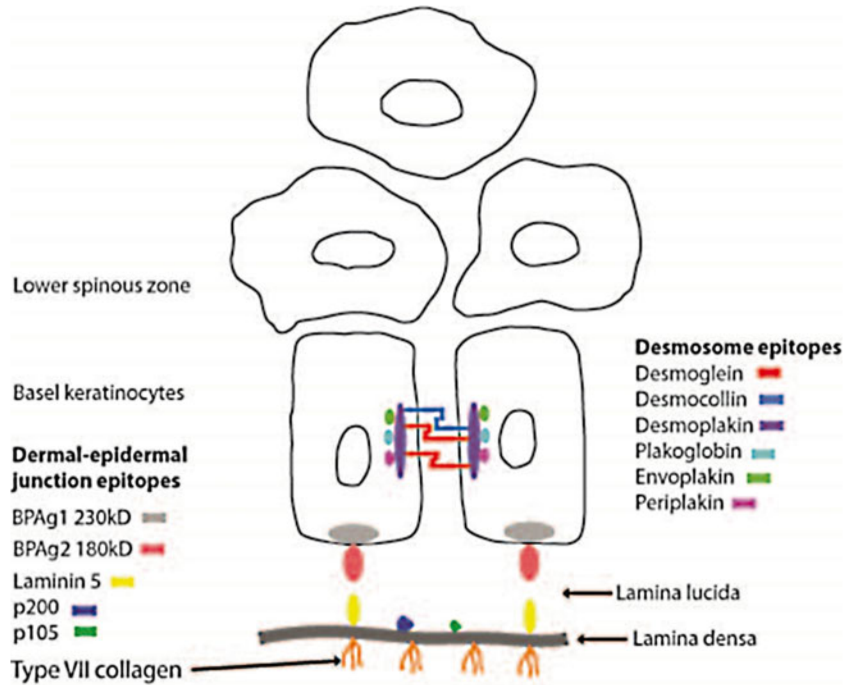

Fig. 34.1 Target epidermal proteins diagram

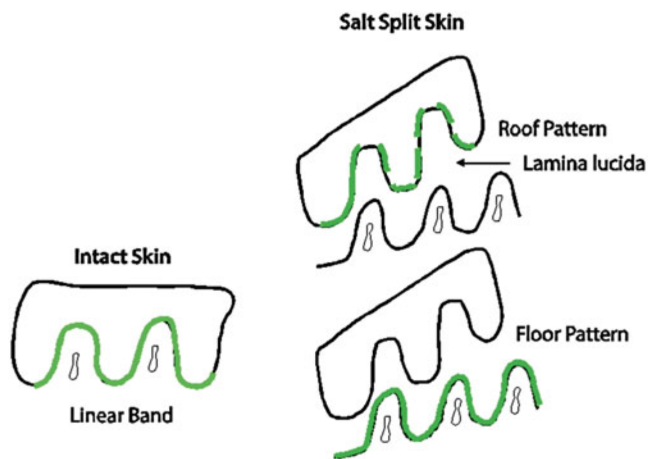

Fig. 34.2 Salt split skin diagram with roof and floor pattern

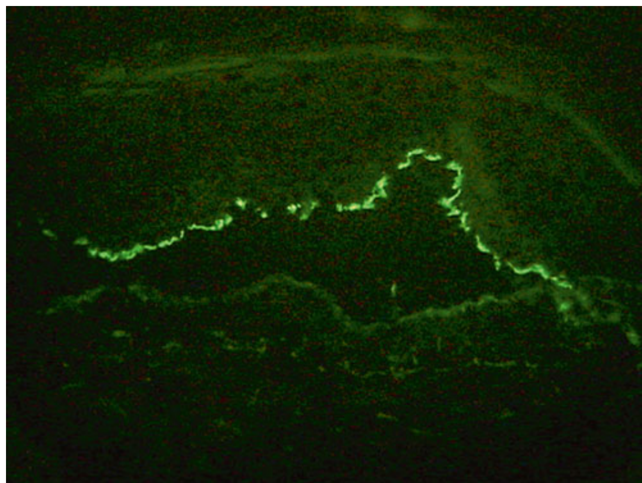

Fig. 34.3 Salt split skin DIF IgG roof pattern

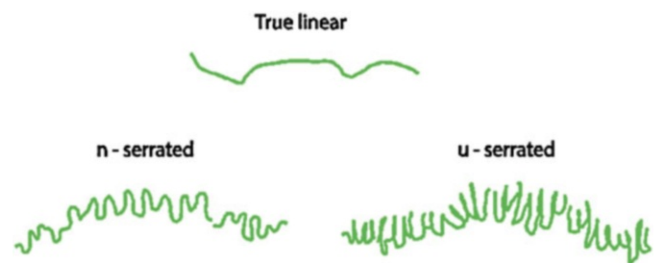

Fig. 34.4 Diagrammatic representation of the fluorescent n-serrated, u-serrated and true linear basement membrane zone patterns

Once learned, the two patterns are easily distinguished and there is good interobserver agreement.

The patterns are visible at 40× magnification but difficult to document photographically without higher magnification i.e. photograph with digital zoom or use an oil immersion objective.

Photography can be performed in black and white if the fluorescence intensity is too great at oil immersion.

Identification of either serrated pattern, which in my experience, is nearly always possible, eliminates the need for salt split skin DIF testing (see below) in the most common dilemma of differentiation of bullous pemphigoid and pemphigoid-like forms of EBA, because bullous pemphigoid can be easily distinguished from EBA and bullous lupus by its n-serrated pattern.

It may also be useful in combination with salt split skin DIF for recognizing or suspecting unusual often neutrophil rich forms of pemphigoid, such as anti-epiligrin pemphigoid, anti-p200 and anti-p105 pemphigoid, where the target epitopes are at or above the lamina densa but in the floor of salt

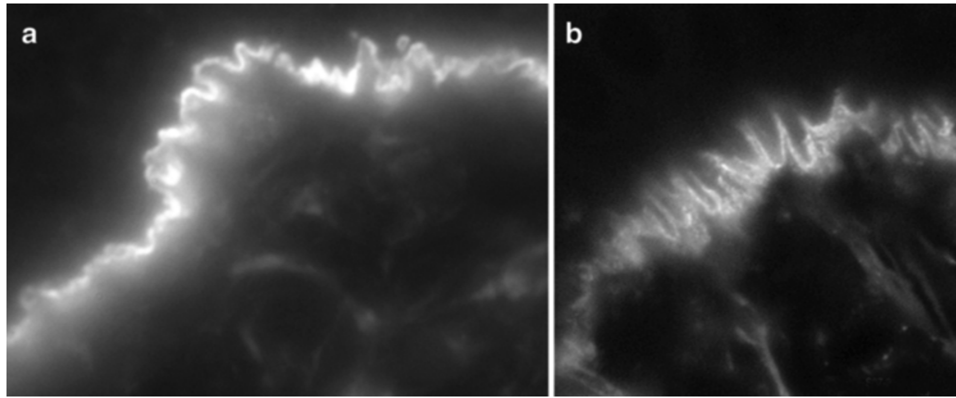

Fig. 34.5 (**a**) n-serrated, linear, IgG ×1000 black & white, (**b**) u-serrated pattern, ×1000 black & white

Table 34.4 Potential for combined use of Salt Split Skin and n-serrated/u-serrated patterns based on known locations of target epitopes

	Salt split skin roof pattern	Target epitope	Salt split skin floor pattern	Target epitope
n-serrated	Bullous pemphigoid	BPAg1 230kD, BPAg2 180kD	Anti-epiligrin cicatricial pemphigoid	Laminin 5 Serum immunoblot for confirmation
n-serrated	Pemphigoid gestationis	BPAg2 180kD	Unusual neutrophil rich pemphigoid Anti-p200	p200 Serum immunoblot for confirmation
n-serrated	Cicatricial pemphigoid—some forms	BPAg1 230kD BPAg2 180kD	Unusual pemphigoid Anti-p105	p105 Serum immunoblot for confirmation
n-serrated	Linear IgA disease (most)	Portion of BPAg2 180kd		
n-serrated			Unusual cicatricial pemphigoid other than laminin 5	Need immunoblot to detect other known epitopes laminin 6, uncein, and other incompletely characterized antigens
u-serrated	Does not occur		Epidermolysis bullosa acquisita	Type 7 collagen
u-serrated	Does not occur		Bullous systemic lupus	Type 7 collagen Look for other clinical and serologic evidence of lupus
u-serrated IgA	Does not occur		IgA Epidermolysis bullosa acquisita(uncommon)	Type 7 collagen

split skin. In such cases testing of serum by immunoblot can define the target epitope.

In my experience, one of these serrated patterns, most commonly, the n-serrated pattern is visible in variable amounts when there is a linear band of immunoglobulin or complement. The display is often multifocal and quite easily identified but sometimes it may be limited to a small area and only found on close inspection [4–8].

Table 34.4.

34.12 What Are the Characteristic Immunofluorescent Findings in Bullous Pemphigoid?

C3and/or IgG, linear, epidermal basement membrane, usually 2-3+ intensity.

May see concurrent weaker identical reactions with IgM and IgA in some cases but IgG and C3 are the dominant immunoreactants.

n-serrated pattern

On salt split skin, the roof of the split (undersurface of the detached epidermis) is stained with a continuous or interrupted dash-like (hemi-desmosomal)) pattern (Fig. 34.6).

Clinicopathologic Correlation: Histopathology: typically an eosinophil rich inflammatory infiltrate with a subepidermal vesicle or bulla. Intact epidermal junction, if urticarial phase. May see eosinophilic spongiosis or eosinophils aligned along the epidermal junction. May be sparsely cellular in some cases. May be neutrophil rich infiltrate with unusual variants such as Anti-p200.

Clinical: The most common immunobullous disorder. Typically elderly, age 70 or greater, tense vesicles and bullae or erythematous patches and plaques or urticarial plaques without blister formation. May occur at any age but uncommon except

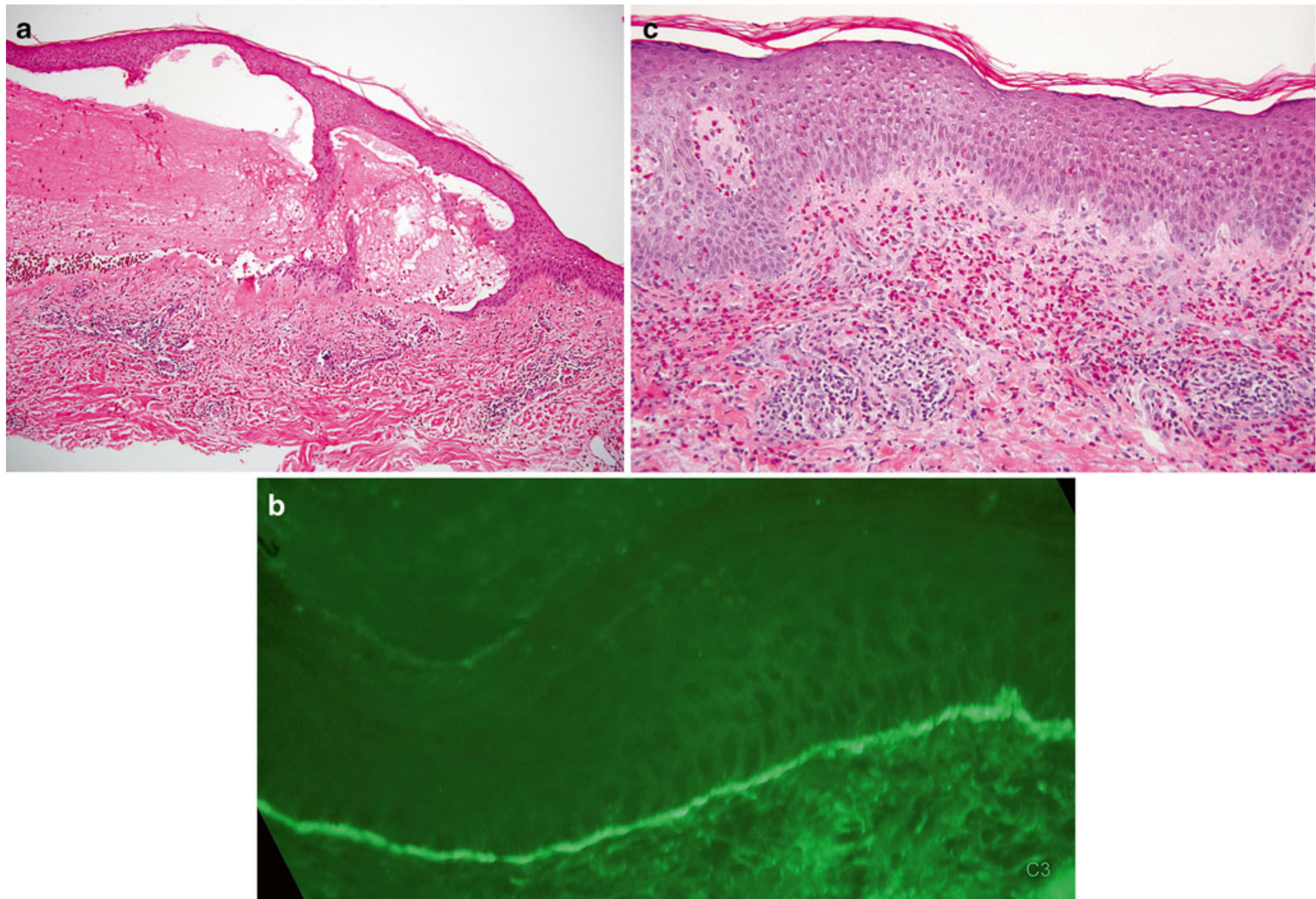

Fig. 34.6 (**a**) Subepidermal vesicle of bullous pemphigoid, (**b**) Linear band of C3 along the epidermal basement membrane zone, (**c**) Urticarial phase bullous pemphigoid. Numerous interstitial eosinophils

in older age group. There are occasionally unusual clinical presentations [9, 10].

34.13 What Are the Characteristic Immunofluorescent Findings in Mucous Membrane Pemphigoid (Cicatricial Pemphigoid)?

2-3+, Linear band with C3, and/or IgG and sometimes IgA. Mucosal involvement: oral and/or conjunctival most commonly. May involve laryngeal, esophageal, and anogenital regions. Skin may also be involved with identical reactions.

n-serrated as are all forms of pemphigoid,

Salt split skin by direct or indirect methods: roof or floor. If floor pattern, suspect anti-epiligrin pemphigoid. Lack of u-serrated pattern excludes epidermolysis bullosa acquisita.

Clinicopathologic correlation: Mixed inflammatory infiltrate that may include eosinophils, plasma cells, neutrophils and lymphoid cells. May see fibrosis depending on the duration of the lesion that is biopsied.

Oral ulceration and erosion. Risk of blindness with ocular involvement due to scarring, risk of stricture with laryngeal, esophageal, and anogenital involvement. May have concurrent skin involvement.

Relatively uncommon. Investigate for possible associated malignancy in anti-epiligrin pemphigoid [5, 11].

34.14 What Are the Characteristic Immunofluorescent Findings in Pemphigoid Gestationis (Herpes Gestationis)?

Linear band of C3 with or without IgG. n-serrated pattern. Staining of roof of salt split skin (not usually necessary). Identical to bullous pemphigoid.

Clinicopathologic correlation: Histopathology is essentially identical to bullous pemphigoid. May have a less dense

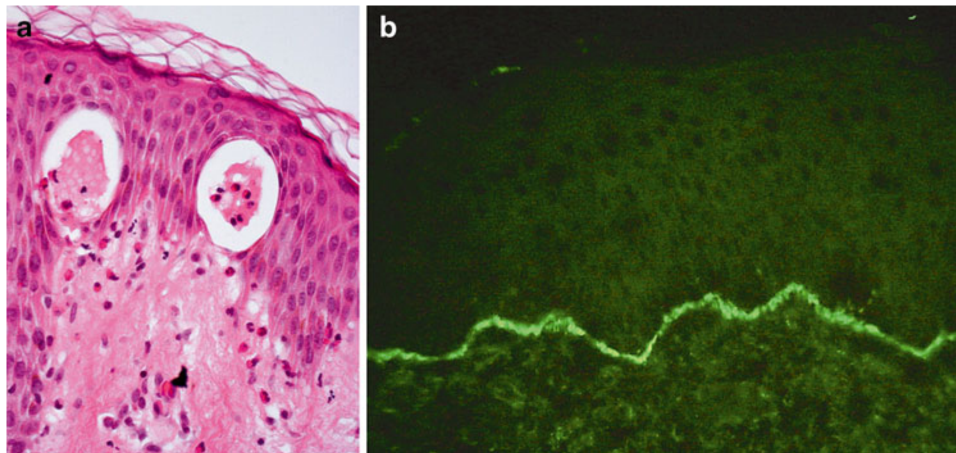

Fig. 34.7 (**a**) Pemphigoid gestationis. Eosinophils papillary dermal edema and small foci of microvesicle formation. (**b**) Pemphigoid gestationis. Strong linear band of C3 at the epidermal basement membrane zone [11]

eosinophilic infiltrate. May not be distinguishable clinically from pruritic urticarial papules and plaques, insect bite reaction or drug induced inflammatory infiltrate except but immunofluorescent study which is the gold standard for diagnosis. ELISA exists for BPAg2 180 kDa antigen, the target epitope. Onset usually in 2nd or 3rd trimester, urticarial papules and plaques often beginning the peri-umbilical area and developing tense vesicles and bullae. Usually resolves with delivery. Occasionally onset is peri-partum or postpartum. Distinguished from other pregnancy related dermatoses by the immunofluorescent findings. Skin involvement in newborn is rare. Onset early in pregnancy and development of blisters reported to be a risk for reduced and low birth weight [12–15].

Figure 34.7.

34.15 What Are the Characteristic Immunofluorescent Findings in Anti-Epiligrin Pemphigoid?

Mucous membrane involvement most commonly oral but may be ocular or other mucosae.

IgG, C3 with or without IgA, linear basement membrane band. N-serrated pattern.

Floor pattern on salt split skin with n-serrated pattern on intact skin Serum for immunoblot for confirmation.

Investigate or keep under close surveillance for risk of associated development of carcinoma, typically adenocarcinoma, lung, stomach, uterine [16–19].

34.16 What Are the Characteristic Immunofluorescent Findings in Epidermolysis Bullosa Acquisita?

Strong 2-3+, linear band of IgG and/or C3 at the epidermal basement membrane zone.

Histopathologically, it may be indistinguishable from pemphigoid on H&E or may be cell poor or neutrophil rich. The antigenic target is type VII collagen below the lamina densa and a u-serrated pattern distinguishes it from pemphigoid. Distinction from bullous lupus erythematosus is mainly by clinicopathologic correlation with other features serologic and clinical features of lupus in the latter. Both have type VII collagen as the target epitope.

Clinically, blisters heal with scarring and milia. They tend to occur more prominently in areas of trauma. Milia may form. Mucosal involvement may occur. Serum testing for antibodies to type VII collagen is possible by an ELISA method is possible [20–22].

34.17 What Are the Characteristic Immunofluorescent Findings in Dermatitis Herpetiformis?

Granular deposition of IgA in dermal papillae. Deposition is usually strong 2-3+ and present repetitively in multiple dermal papillae. The deposition may also extend along the epidermal basement membrane zone in some areas. IgA is the

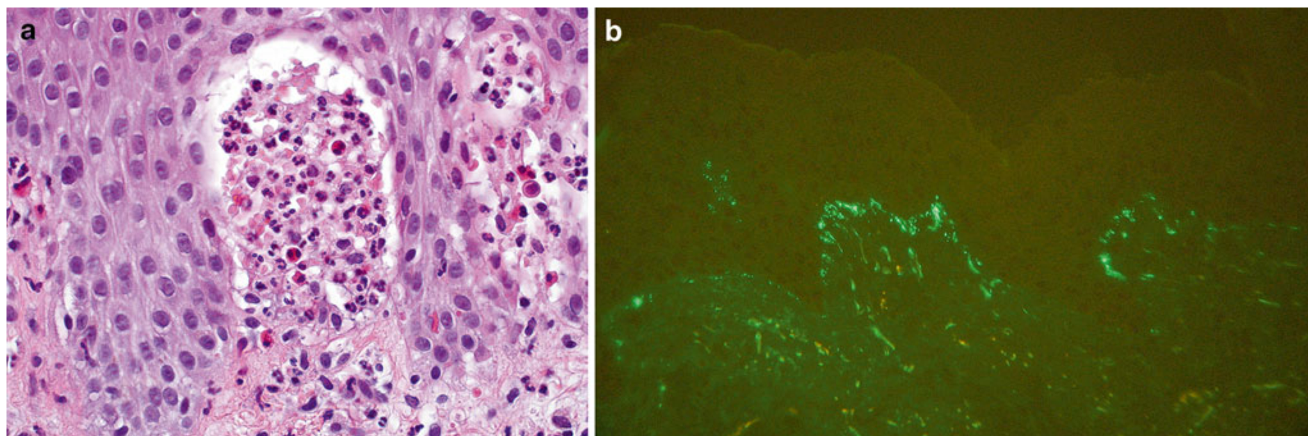

Fig. 34.8 (**a**) Papillary dermal microabscess of dermatitis herpetiformis. (**b**) Granular deposition of IgA in dermal papillae. Dermatitis herpetiformis

dominant immunoreactant. May sometimes see a similar less intense pattern of staining with other conjugates including fibrinogen, C3, IgM and seldom IgG.

Figure 34.8.

Clinicopathologic correlation: Classically, neutrophil accumulation with papillary dermal microabscess formation if an early, juicy papule or early undisturbed small vesicle is biopsied. Many times because of intense pruritus, primary lesions with the classic histology are not found and the H&E morphology may just show erosion and excoriation with a mixed inflammatory infiltrate. Look for clues of neutrophils aligning along the dermal epidermal junction or accumulating in the papillary dermis at the intact skin edge of eroded and ulcerated portions of the epidermis. Characteristic clinical distribution of elbows, knees, upper back and shoulder region, sacral region and buttocks, grouped vesicles on an erythematous base in early onset.

H&E morphology with papillary dermal microabscess formation may also be seen with linear IgA dermatosis, bullous systemic lupus, epidermolysis bullosa acquisita, and some forms of bullous pemphigoid. The distinction is made by the direct immunofluorescent study in conjunction with clinicopathologic correlation [9, 20].

34.18 What Are the Characteristic Immunofluorescent Findings in Linear IgA Disease?

Strong, linear band of IgA, 2-3+ along the epidermal basement membrane zone. IgA is the dominant immunoreactant. Most commonly an n-serrated pattern would be anticipated. There is a variant of epidermolysis bullosa acquisita that is recognized by a u-serrated pattern.

Figure 34.9.

Clinicopathologic correlation: Neutrophil predominant inflammatory reaction often with papillary dermal microabscess formation indistinguishable from dermatitis herpetiformis. Clinical clues to the diagnosis are the formation of a ring of blisters at the edge of an erythematous plaque. Childhood form is identical, histopathologically, to the adult form. The adult form may be more commonly drug induced. The identification of a drug induced etiology is made, clinically. It cannot be distinguished from idiopathic forms histopathologically. The drug induced form has also been reported to present in some cases with a clinical pattern mimicking toxic epidermal necrolysis. Since the target epitope is usually above the lamina densa, an n-serrated pattern would be expected most commonly. In the event that a u-serrated pattern is found the possibility of bullous lupus should also be investigated, clinically; however, an IgA form of EBA has been reported [9, 20, 23, 24].

34.19 What Are the Characteristic Immunofluorescent Findings in Discoid Lupus?

A granular deposition of immunoglobulin and complement along the epidermal basement membrane zone with or without an identical deposition along the follicular basement membrane zone. IgM is commonly present but the reaction should be relatively strong, at least 2+, if it is the sole immunoreactant in order to avoid false positive weak reactions on sun damaged skin. The specificity of the reaction is increased with the finding of a similar pattern with IgG and/or IgA.

Figure 34.10.

Clinicopathologic correlation: The immunofluorescent findings are reinforced by finding typical H&E morphology of hyperkeratosis, follicular hyperkeratosis, focal epidermal

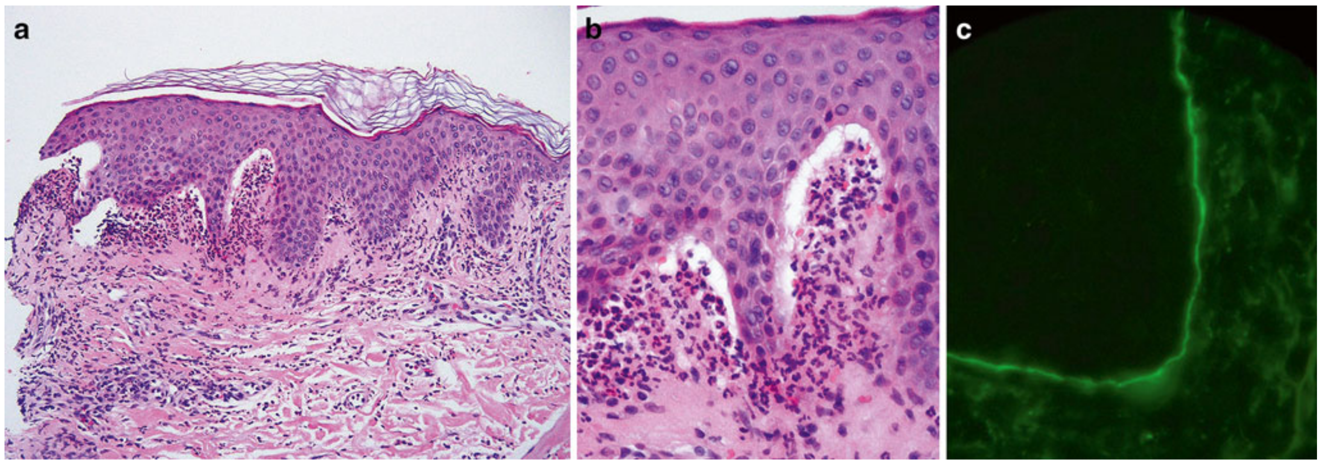

Fig. 34.9 (**a**) Papillary dermal microabscessses in linear IgA disease. (**b**) Papillary dermal microabscesses. Linear IgA disease. (**c**) Linear IgA, epidermal basement membrane zone

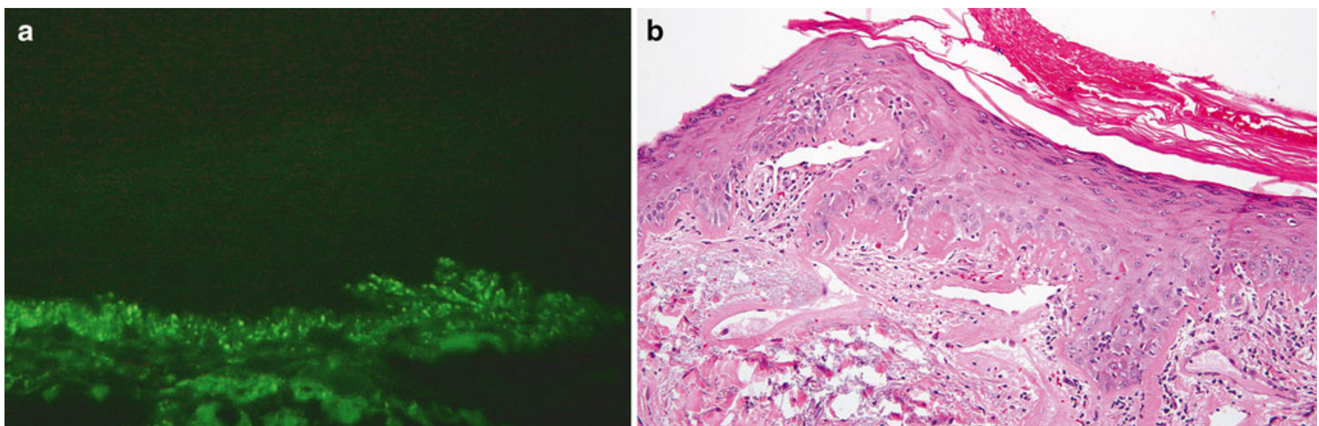

Fig. 34.10 (**a**) Granular, IgG band along the epidermal basement membrane zone in discoid lupus. (**b**) Discoid lupus. Thick, glassy epidermal basement membrane, hypergranulosis, hyperkeratosis and telangiectasia with thickened vessel basement membranes

or infundibular epithelial atrophy with epidermal and or follicular infundibular basement membrane thickening, telangiectasia and a superficial and deep perivascular and perifollicular lymphohistiocytic inflammatory reaction that also may focally involve the epidermal interface where there may also be vacuolar change in the basal keratinocyte cytoplasm. There may be increase dermal mucin. Although most commonly it is cutaneous disease only, it cannot be distinguished, histologically, from systemic lupus with discoid lupus lesions. The findings must be integrated, clinically with the clinical morphology, serologic investigation and other clinical data [25–27].

34.20 What Are the Characteristic Immunofluorescent Findings in Systemic Lupus?

A strong granular band of immunoglobulin often with all three immunoglobulins and usually complement along the epidermal. and often the follicular infundibular basement membrane zone. There may be staining of epidermal nuclei with immunoglobulin.

The H&E morphology may show an interstitial neutrophilic inflammatory reaction with some leukocytoclasis. Some cases may have associated leukocytoclastic urticarial

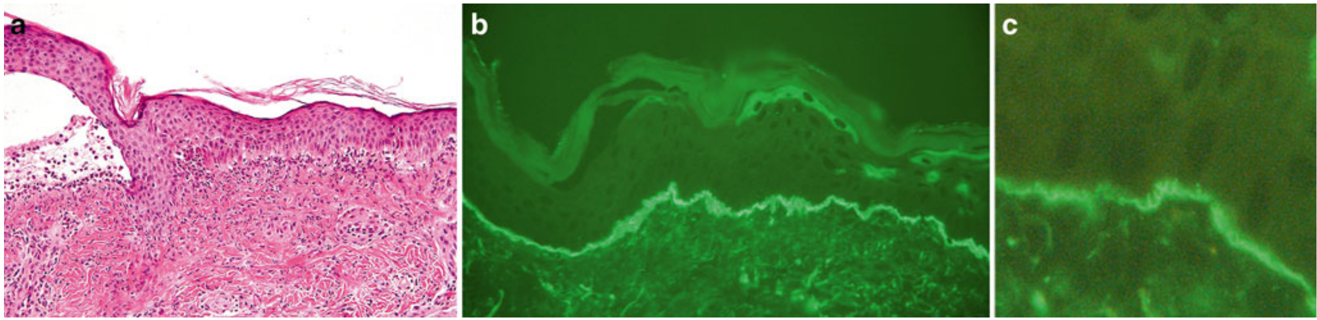

Fig. 34.11 (**a**) Bullous systemic lupus. Interstitial neutrophilic infiltrateand alignment of neutrophils along the epidermal junction at the edge of the bulla. (**b**) Broad linear band of IgG along the epidermal basement membrane zone. (**c**) U-serrated pattern indicative of deposition below the lamina densa

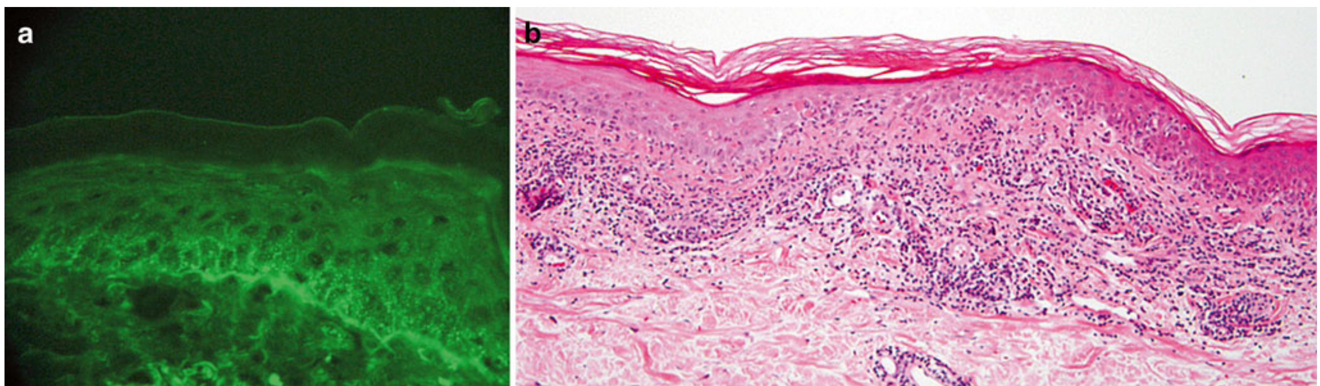

Fig. 34.12 (**a**) "Dusty" granular staining of basal keratinocyte cytoplasm with IgG in subacute cutaneous lupus. (**b**) Perivascular, interstitial and focal patchy lichenoid interface lymphohistiocytic inflammation with hyperkeratosis. Subacute cutaneous lupus

vasculitis. There may also be relatively sparse lymphocytic inflammation with epidermal atrophy, basal vacuolar change, and increased dermal mucin. Correlation, clinically, with the clinical morphology and serologic tests for lupus are necessary for definitive diagnosis.

In bullous systemic lupus, a linear band of IgG with or without IgA and IgM may be seen along the epidermal basement membrane zone. An u-serrated pattern may be evident since the target epitope is type VII collagen.

Figure 34.11.

Clinicopathologic correlation: In bullous systemic lupus, there is a neutrophil rich inflammatory infiltrate with vesicle and bulla formation. Neutrophils aligned along the epidermal junction. Papillary dermal microabscess formation may be seen. It must be distinguished from neutrophil rich EBA and linear IgA dermatosis and neutrophil rich forms of pemphigoid by correlation clinically, with clinical morphology and other clinical data including serologic tests for lupus [27, 28].

34.21 What Are the Characteristic Immunofluorescent Findings in Subacute Cutaneous Lupus?

Deposition of "dust-like" particles in the cytoplasm of lower epidermal keratinocytes with or without speckled staining of epidermal nuclei. This correlates with the antibodies to the Ro cytoplasmic antigen. It is easily distinguished from any non-specific granular stain precipitate because it is localized to the epidermis in a repetitive, non-random, pattern across the width of the specimen and it is not found in the underlying dermis.

Figure 34.12.

Clinicopathologic correlation: H&E morphology is a patchy or widespread lichenoid inflammatory infiltrate without conspicuous basement membrane thickening. There may be hyperkeratosis and focal vacuolar change with occasional apoptotic keratinocytes. There may be increased dermal

mucin on colloidal iron stain. The most distinctive finding is the direct immunofluorescent pattern in conjunction with the clinical morphology of scaly, annular, polycyclic or serpiginous plaques usually on the trunk and upper extremities and clinical serologic investigation. Some cases of subacute lupus may be drug induced. These may be suspected histologically, if eosinophils are also evident in the dermal inflammatory infiltrate.

The histopathologic and immunofluorescent findings must be integrated clinically with the clinical morphology, serologic investigation and other clinical data to exclude the possibility of subacute cutaneous lupus occurring in association with systemic lupus and to aid in recognition of a drug induced etiology [29].

34.22 What Are the Characteristic Immunofluorescent Findings in Porphyria Cutanea Tarda?

A thick smudgy band of IgG along the epidermal basement membrane zone and a similar prominent thick, smudgy basement membrane of multiple superficial dermal vessels.

Figure 34.13.

Clinicopathologic correlation: The H&E biopsy is typically from acral skin and shows a non-inflammatory or at most pauci-inflammatory subepidermal vesicle or bulla with dermal papillae that protrude from the floor of the vesicle into the blister space, also known as festooning of dermal papillae. The vessels may have visibly thickened basement membranes on H&E and PAS stains. Caterpillar bodies may be seen along the undersurface of the detached epidermis.

Clinically, there is some skin fragility with blisters typically on acral sun-exposed sites that heal with scarring and milia. There may be excess facial hair and sclerodermoid changes may develop in some lesions. There is an elevated level of serum and urine uroporphyrins due to a deficiency of the enzyme uroporphyrinogen decarboxylase. This is also associated with hepatic iron overload and there may be associated with hemochromatosis, hepatitis C or alcoholic liver disease [30, 31].

34.23 What Are the Characteristic Immunofluorescent Findings in Pseudoporphyria?

The immunofluorescent findings and the H&E morphology of pseudoporphyria are indistinguishable histologically from porphyria cutanea tarda.

The distinction is made clinically by correlation with the clinical history, drug history and clinical investigation of serum and urine porphyrin levels. Pseudoporphyria occurs in young women who are frequent tanning bed users and it has been causally linked to a variety of drugs, most notably the nonsteroidal anti-inflammatory drug naproxen and other similar compounds as well as furosemide, chlorthiazide-triamterine and others. It may be seen in children, particularly in association with nonsteroidal inflammatory drug use in juvenile rheumatoid arthritis. It is not associated with liver disease or iron overload [32].

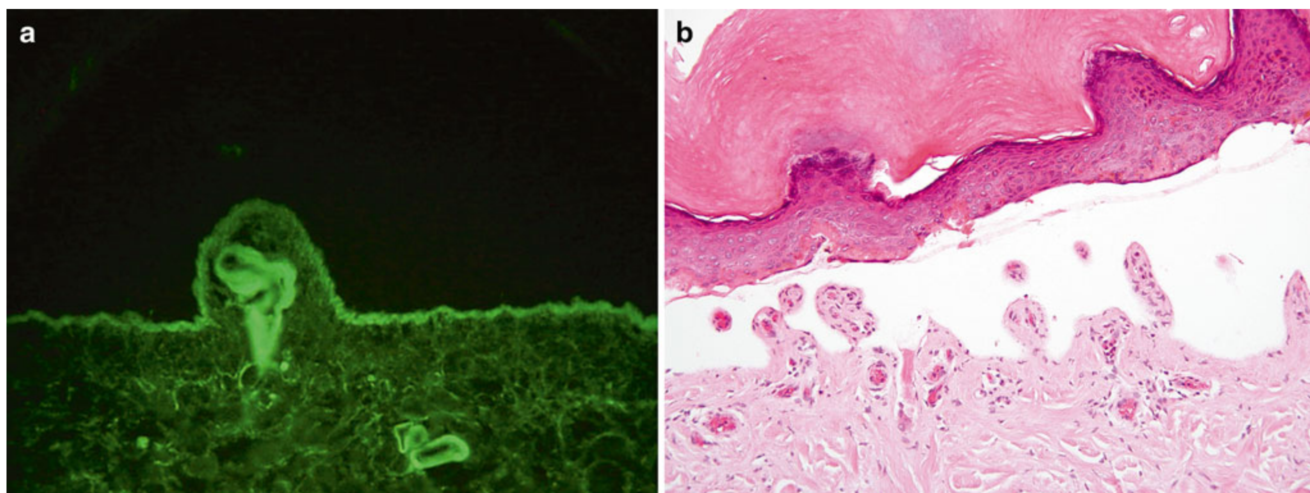

Fig. 34.13 (**a**) Thick band of IgG along epidermal basement membrane zone and smudgy staining of the walls of superficial vessels in porphyria cutanea tarda. (**b**) Non inflammatory subepidermal bulla with festooning of dermal papillae and caterpillar bodies along a portion of the under surface of the detached epidermis in porphyria cutanea tarda

34.24 What Are the Characteristic Immunofluorescent Findings in Pemphigus Vulgaris?

A delicate smooth staining of the intercellular space in the epidermis with IgG with or without C3. Weaker reactions with IgA and IgM may also be seen but the dominant immunoreactant is IgG. The reaction may be noted throughout the epidermis or it may be concentrated in the lower portions of the epidermis.

Figure 34.14.

Clinicopathologic correlation: There is suprabasal acantholysis in the epidermis and at times in the basal infundibular epithelium of hair follicles. The blisters are fragile and must be handled carefully during gross examination and embedding. Oral mucosal involvement is common and the disease at times may first be found in an oral mucosal biopsy during an investigation of oral mucosal ulcers and erosions. Skin fragility with positive Nikolsky sign and flaccid vesicles and bullae are clinical clues to the diagnosis. Occasionally, limited forms of the disease may occur in areas of prior trauma. Supplemental investigations include indirect immunofluorescent study for determination of antibody titer and sometimes for further verification of the diagnosis. An ELISA is also available for the main target epitope, desmoglein 3 and it is also available for desmoglein 1 [33, 34].

34.25 What Are the Characteristic Immunofluorescent Findings in Pemphigus Foliaceus?

Staining of the intercellular space with IgG, particularly in the upper half of the epidermis. There may also be staining with C3 and the staining may extend to involve the entire spinous zone.

Figure 34.15.

Clinicopathologic correlation: The cleavage plane in the H&E sections is in the region of the granular cell layer and upper portion o f the spinous zone. The findings may be subtle or obvious. Because the blisters are superficial and fragile, the specimens must be handled carefully during gross examination and embedding to preserve optimal morphology. It may be best to avoid sectioning the specimen, until the time of embedding, if possible. In some cases, a clue to the diagnosis may be the absence of a granular cell layer and stratum corneum due to prior detachment of those structures. An identical cleavage plane occurs with staphylococcal scalded skin syndrome but the immunofluorescent study is negative in that condition. Likewise, a similar pattern of cleavage may be seen in bullous impetigo but in that condition not only is the direct immunofluorescent study negative but staphylococci can often be found in abundance in the blister space. Indirect immunofluorescent study may also be used as an adjunctive diagnostic tool and for titer determination. An ELISA is available for desmoglein 1, the target epitope. Clinically, there are scaly crusted plaques with or without visible superficial vesicles and bullae involving the upper trunk but the disease may become widespread. A characteristic corn flake-like scale is sometimes found. The oral mucosa is not involved [35, 36].

34.26 What Are the Characteristic Immunofluorescent Findings in Pemphigus Erythematosus?

Staining of the intercellular space with IgG and granular staining of the epidermal basement membrane zone with IgG.

Clinicopathologic correlation: This is a rare condition with scaly plaques involving the face and upper trunk in a seborrheic distribution. It may be associated with internal malignancy, including thymoma and Castleman's disease. The cleavage plane is also in the region of the granular cell layer and superficial spinous zone of the epidermis. The granular immunoglobulin band at the epidermal junction is lupus-like [37].

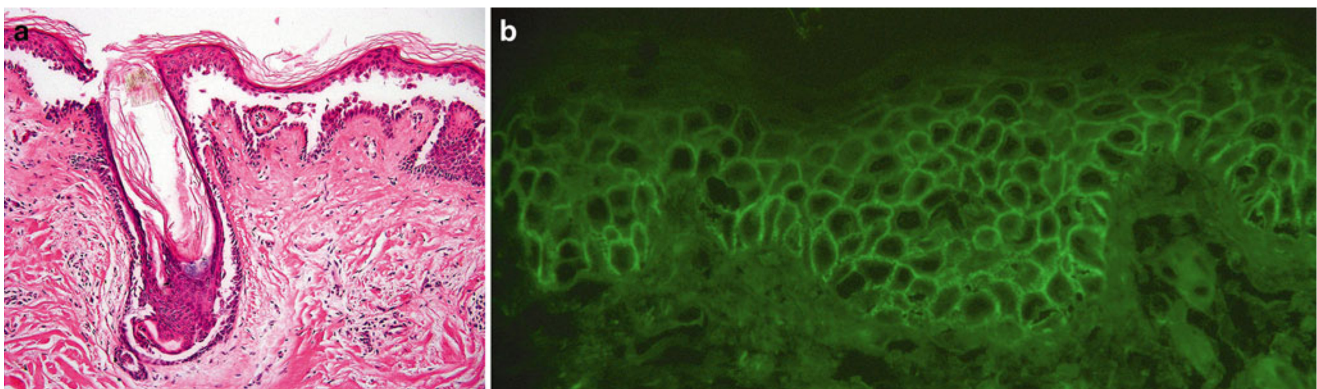

Fig. 34.14 (**a**) Suprabasal acantholysis of epidermis and follicular infundibular epithelium in pemphigus vulgaris. (**b**) IgG in the intercellular space of the epidermis in pemphigus vulgaris

34.27 What Are the Characteristic Immunofluorescent Findings in IgA Pemphigus?

Staining of the intercellular space of the epidermis with IgA in the appropriate histopathologic setting.

Figure 34.16.

Clinicopathologic correlation: An intraepidermal neutrophilic and a subcorneal pustular pattern are the two characteristic histopathologic forms of this disease in conjunction with the pemphigus pattern on DIF. Indirect immunofluorescent study may also demonstrate an elevated titer of IgA antibodies with an intercellular reaction pattern. This is an uncommon condition [38–40].

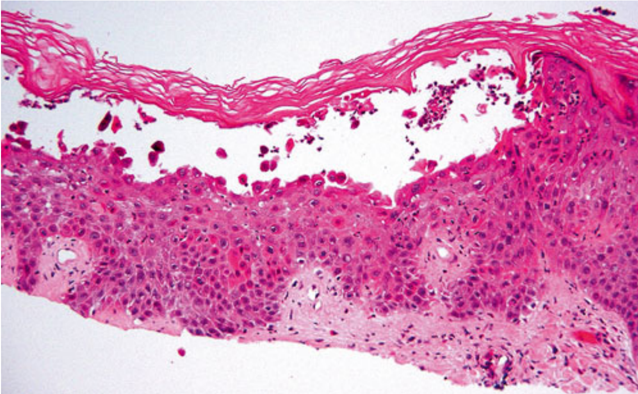

Fig. 34.15 Superficial epidermal acantholytic cleavage plane in pemphigus foliaceous

34.28 What Are the Characteristic Immunofluorescent Findings in Paraneoplastic Pemphigus?

Staining of the intercellular space with IgG and also staining of the epidermal basement membrane zone.

Figure 34.17.

Clinicopathologic correlation: The predominant morphology may be an inflammatory reaction along the epidermal interface with focal apoptotic keratinocytes, mimicking erythema multiforme. Acantholysis may be inconspicuous or subtle. Clinically, there is severe oral mucosal involvement leading to biopsy which reveals the characteristic findings. Indirect immunofluorescent study using rat bladder

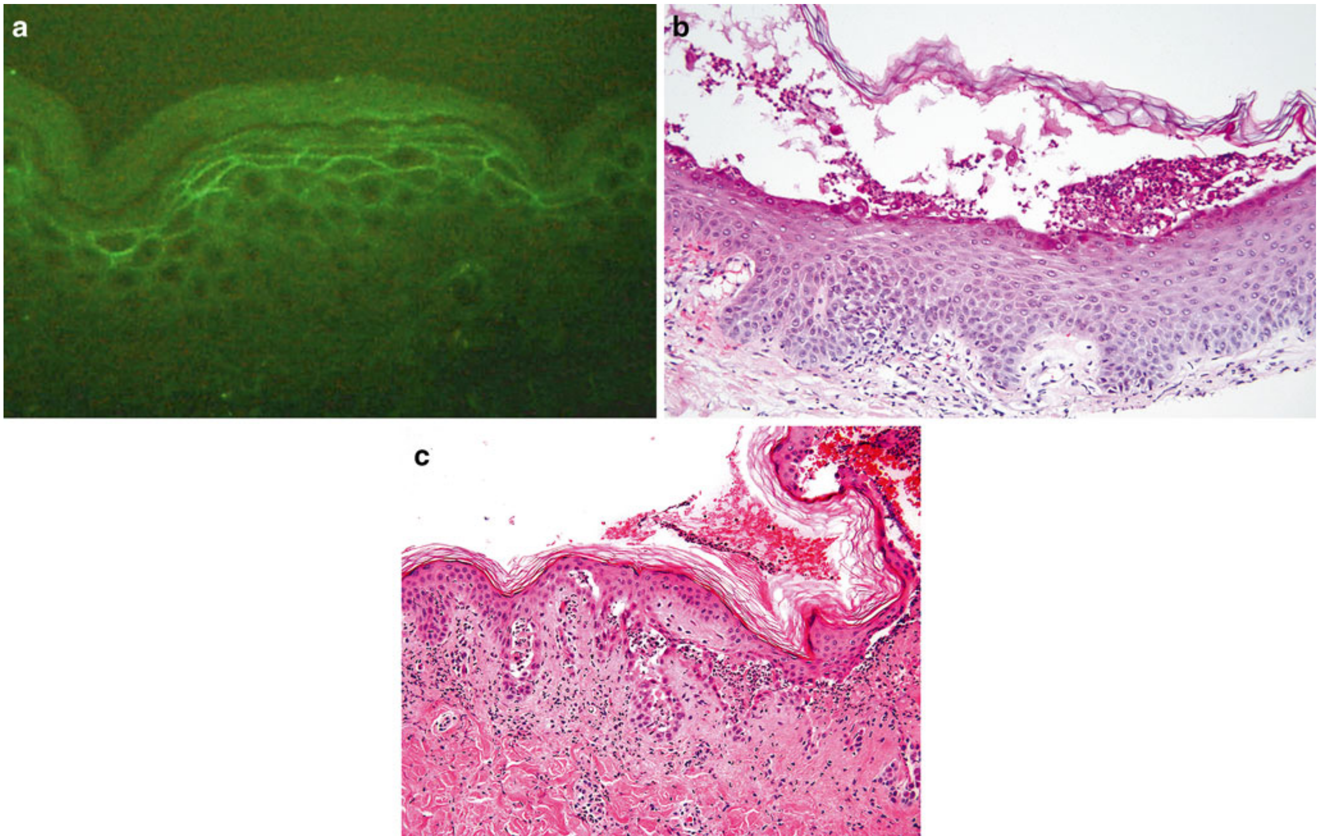

Fig. 34.16 (**a**) Intercellular staining in the superficial epidermis with IgA. (**b**) Subcorneal pustular form of IgA pemphigus. (**c**) Intraepidermal neutrophilic form of IgA pemphigus

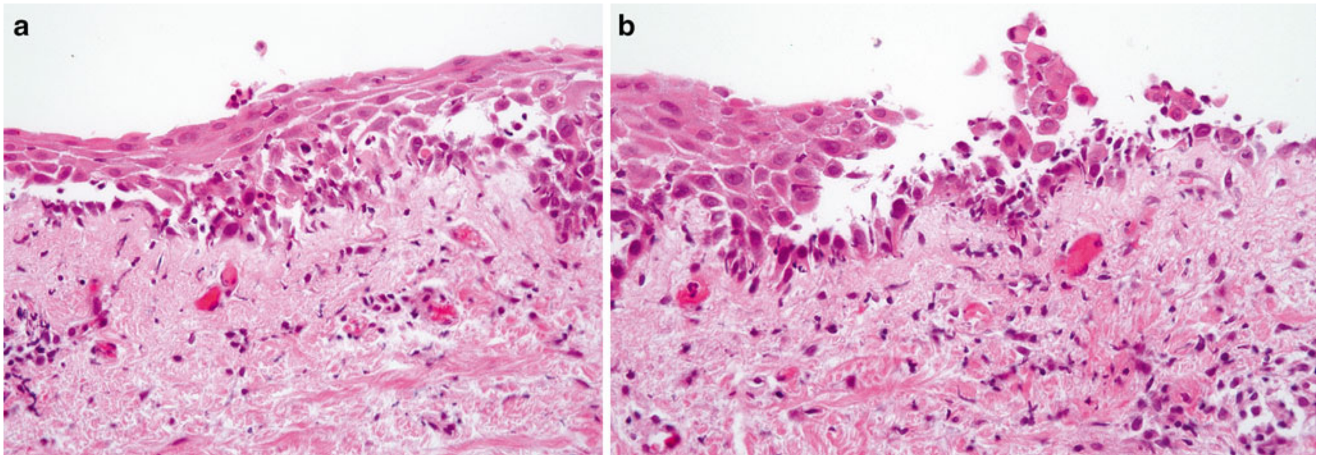

Fig. 34.17 (**a**) Paraneoplastic pemphigus with sparse interface lymphocytic inflammation, occasional apoptotic keratinocytes and subtle acantholysis. (**b**) The same specimen with a more obvious focus of lower epidermal acantholysis

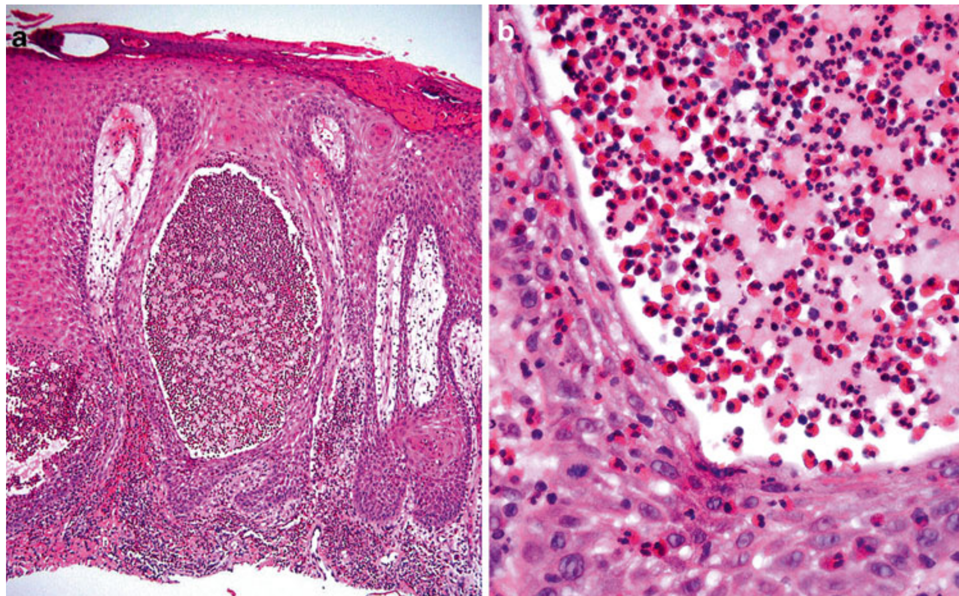

Fig. 34.18 (**a**) Prurigo-like epidermal hyperplasia with a large intraepidermal abcess that is composed mostly of eosinophils and focal basal epidermal acantholytic change in pemphigus vegetans. (**b**) Portion of the intraepidermal abscess with numerous eosinophils and some admixed neutrophils

mucosa is a confirmatory diagnostic test for detection of desmoplakin antibodies. Immunoblotting using nitrocellulose strips containing separated epidermal proteins may also be performed in a research laboratory with that capability to define the full spectrum of reactivity which includes BPAg 1–230 kDa, desmoglein 1 and 3, desmoplakins, and plakoglobin. Clinical investigation for leukemia, lymphoma and other malignancy should be pursued, if not already evident [41–43].

34.29 What Are the Characteristic Immunofluorescent Findings in Pemphigus Vegetans?

Staining of the intercellular space with IgG.

Figure 34.18.

Clinicopathologic correlation: Histologically, there is prominent prurigo-like epidermal hyperplasia with elongated

and thickened rete ridges. A hallmark finding is the presence of intraepidermal eosinophilic abscesses and foci of suprabasal acantholysis. This is an uncommon condition that is considered a form of pemphigus vulgaris. It is largely confined to the intertriginous areas as vegetant plaques [44, 45].

34.30 What Are the Characteristic Immunofluorescent Findings in Lichen Planus [46]?

A bright (3-4+), broad, shaggy band of fibrinogen along the basement membrane zone, usually in biopsies from oral mucosa. There may be some focal weak granular staining with C3 seen.

Figure 34.19.

Clinicopathologic correlation: Biopsies of lichen planus are not usually performed for direct immunofluorescent study except in the investigation of oral mucosal disease. The fibrinogen band is a characteristic, repetitive finding but a definitive diagnosis and distinction from drug induced lichenoid mucositis requires careful examination of the lichenoid inflammatory infiltrate for the presence of eosinophils or abundant plasma cells. In the presence of either of the latter findings, the possibility of a lichen planus-like drug induced mucositis should be considered [46, 47].

34.31 What Are the Characteristic Immunofluorescent Findings in Lichen Planus Pemphigoides?

A linear band of C3 at the epidermal or mucosal basement membrane zone with or without IgG and an n-serrated pattern.

Figure 34.20.

Clinicopathologic correlation: This condition is suspected or identified when a patient with a known established history of lichen planus develops vesicles or bullae involving either established lesions of lichen planus or previously uninvolved skin. It may also be seen in the oral mucosa of patients with lichen planus. It is an uncommon condition [46, 48, 49].

34.32 What Are the Characteristic Immunofluorescent Findings in Henoch Schönlein Purpura?

A granular deposition of IgA in superficial dermal small vessels. There also is frequently strong staining of dermal vessels with fibrinogen and there may be a granular deposition of C3 and sometimes IgM.

Figure 34.21.

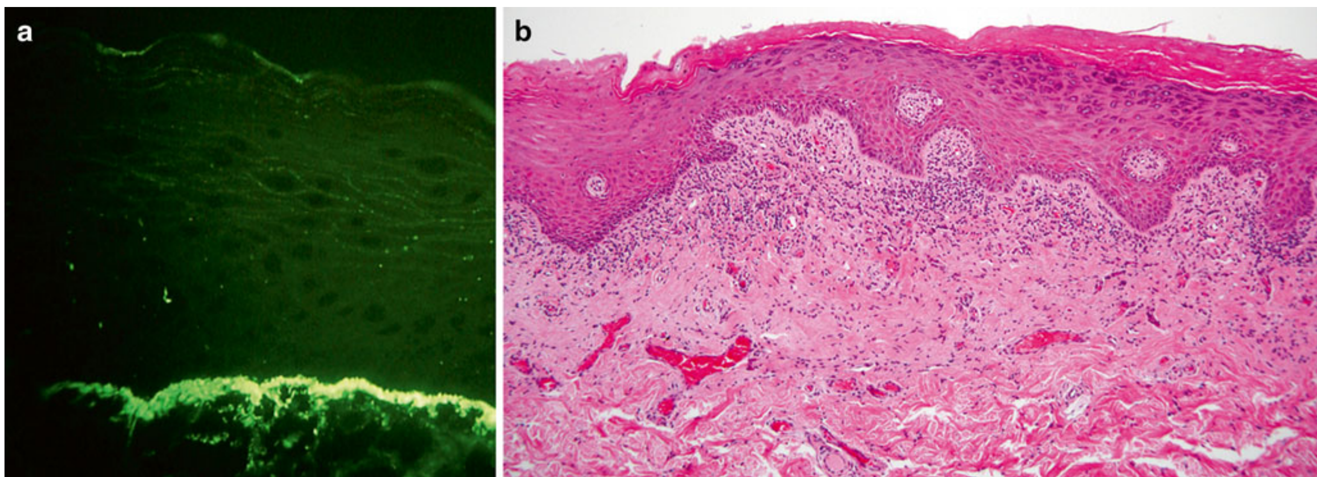

Fig. 34.19 (**a**) Broad, shaggy, strong, fibrinogen band along the basement membrane zone of this oral mucosal biopsy of lichen planus. (**b**) Oral mucosal biopsy of lichen planus with a transition from squamous mucosa to hyperkeratotic mucosa with a granular cell layer and band-like lymphohistiocytic infiltrate in the superficial lamina propria that focally involves the mucosal interface

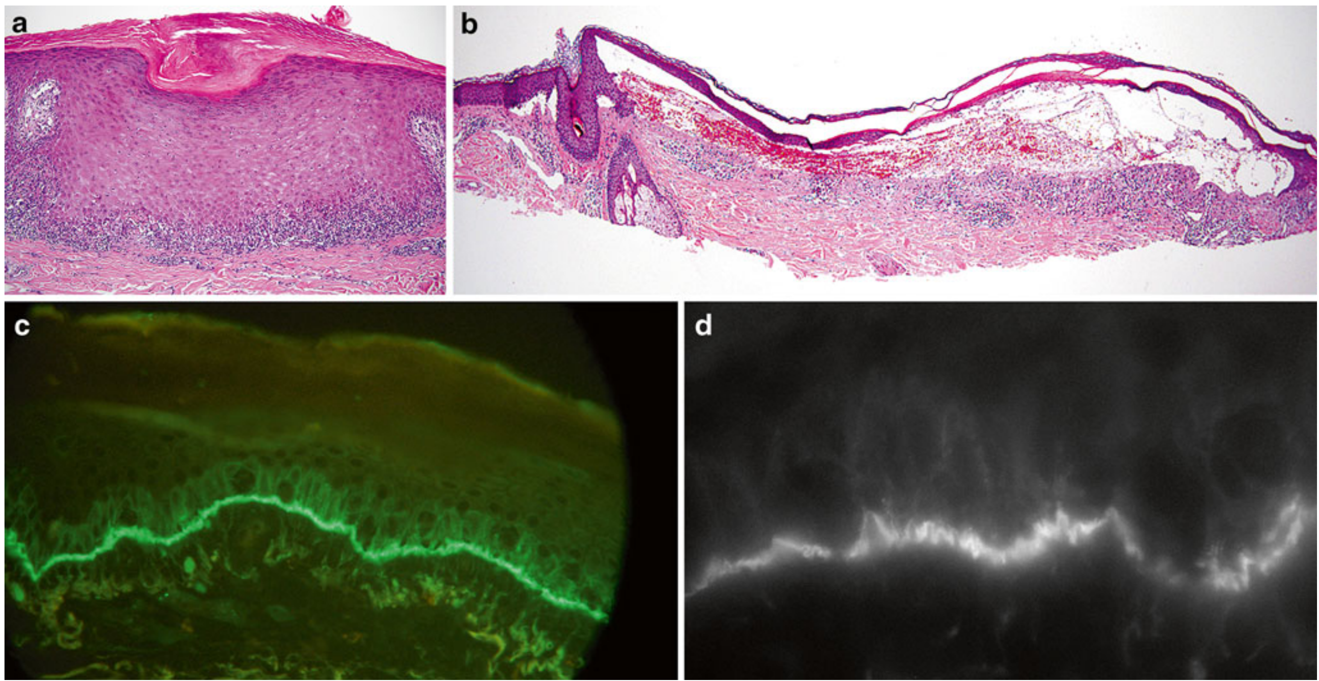

Fig. 34.20 (**a**) Lichen planus. (**b**) Subepidermal vesicle. (**c**) Strong, linear band of C3 along the epidermal basement membrane zone. (**d**) Black and white photo of n-serrated pattern visible just to the left of center in this photo. Lichen planus pemphigoides (oil immersion magnification ×1,000)

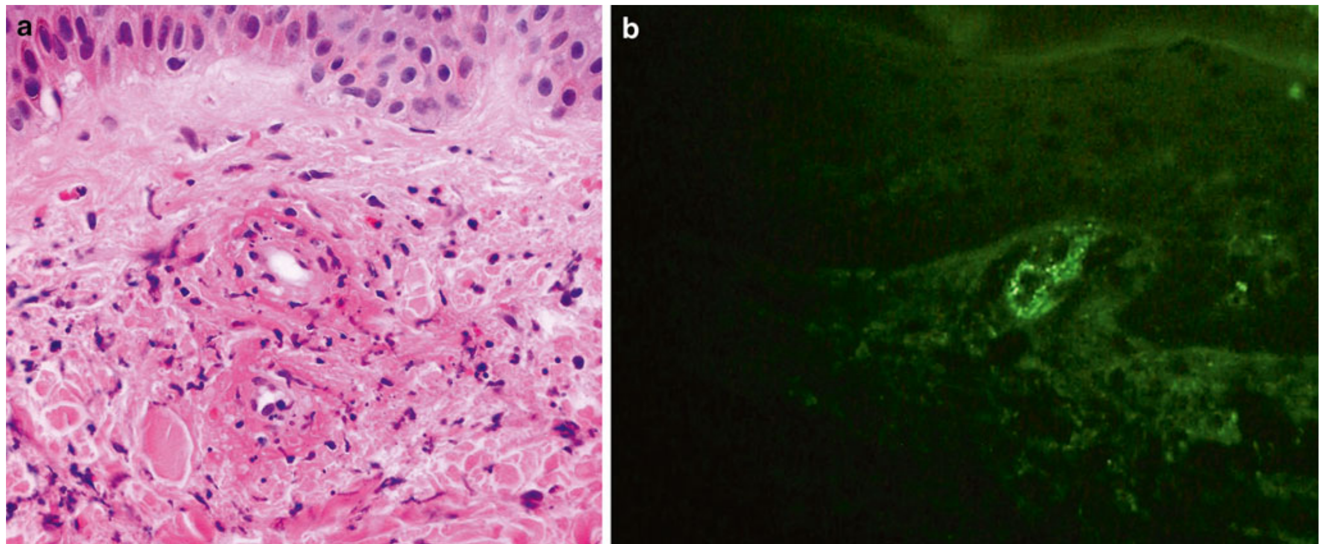

Fig. 34.21 (**a**) Leukocytoclastic vasculitis with prominent fibrin deposition in and around the walls of small vessels and neutrophils and nuclear dust in the surrounding interstitium. (**b**) Granular deposition of IgA in a papillary dermal capillary loop

Clinicopathologic correlation: Biopsies are frequently received with a note to "rule out HSP". The presence of IgA in dermal vessels supports a clinical diagnosis of HSP but it is not by itself sufficient for a diagnosis of HSP. A definitive diagnosis should be based on the findings of a characteristic clinical syndrome of HSP because IgA deposition in dermal vessels may occur in cases of leukocytoclastic vasculitis that are not HSP. In summary, the biopsy finding is supportive of a clinical diagnosis of HSP but it is not, by itself, pathognomonic of the disorder [50].

34.33 What Is the Role of Direct Immunofluorescence in Vasculitis?

In my opinion, vasculitis is a diagnosis made by characteristic findings in H&E stained sections. It is not a diagnosis that is made solely by direct immunofluorescent study. It is not uncommon to find staining of some dermal vessels with fibrinogen and sometimes with C3 when there is no clinical or histologic evidence or suspicion of vasculitis. Therefore, a diagnosis of vasculitis should never be based solely on immunofluorescent findings, in my opinion. It may be helpful to look for IgA deposition as support for a clinical diagnosis of HSP. It may be helpful in recognizing urticarial vasculitis, in cases with an urticarial reaction with leukocytoclasis but without overt fibrinoid change in small dermal vessels in the H&E stained sections. In that scenario, I would consider the presence of fibrinogen, and a granular pattern of C3, IgM or other immunoglobulin in multiple superficial small vessels potentially helpful in supporting a diagnosis of urticarial vasculitis [51].

34.34 What Antibody May Be Useful in Cases of Suspected Dermatomyositis?

Dermatomyositis may mimic lupus histopathologically but it does not show evidence of a granular immunoglobulin band. Magro has described a pattern of staining with an antibody to the C5-9 membrane attack complex that may be useful in recognizing dermatomyositis. However, practically speaking, biopsies are seldom submitted for direct immunofluorescent study for dermatomyositis. Consequently, this antibody has not been stocked for routine use [52].

34.35 What Do Cytoid Bodies Mean?

Cytoid bodies are a marker of some prior epidermal injury. They are immunoglobulin coated dead keratinocytes. They do not have any specific diagnositic value. They are often seen in any condition where there is a component of interface lichenoid inflammatory reaction.

Figure 34.22.

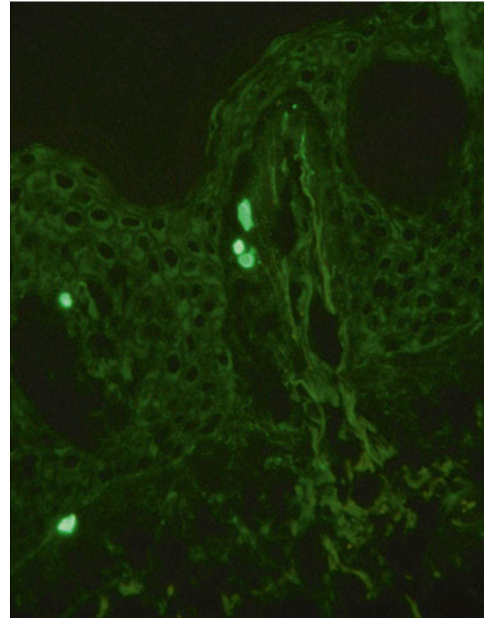

Fig. 34.22 Cytoid bodies in the papillary dermis near the epidermal junction

34.36 How Should Anti-Nuclear Reactions Be Interpreted?

Antinuclear staining is sometimes seen in the DIF biopsy specimen. If the staining pattern is moderately strong i.e. at least 2+ and present throughout the specimen, it will likely correlate well with other clinical data. Weak intensity staining of epidermal nuclei may sometimes occur as a spurious finding that I suspect is reagent related. For that reason, in my opinion, the presence or absence of an antinuclear antibody is best determined by conventional serologic testing.

34.37 Are Immunoperoxidase Stains of Any Use?

An immunoperoxidase stain for type IV collagen on a cryostat section of a biopsy processed for DIF on salt split skin is useful to verify that the separation plane is correct by showing that the basement membrane type IV collagen localized to the floor of the salt split skin. Helm et al. reported use of immunoperoxidase staining for dermatitis herpetiformis but in general, cryostat sections for DIF are the gold standard for diagnosis, in my opinion. Recently, the use of C3d and C4d immunoperoxidase stains have been described, in formalin-fixed, paraffin embedded tissue sections. I have no personal experience using either of these antibodies. In general, direct and indirect immunofluorescence are the gold standards for evaluation of these disorders, in my opinion [53–55].

34.38 What Other Factors Should Always Be Considered in Any Dif Testing?

Correlation with the H&E morphology and clinical differential diagnosis are always important. The testing should not be performed blindly in a vacuum without reliable clinical data

provided by an experienced clinical dermatologist, in my opinion. Additionally, the findings must be integrated clinically, with all relevant clinical and laboratory data, by an experienced clinician.

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