Immunofluorescence in dermatology

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The accurate diagnosis of bullous and other immune diseases of the skin requires evaluation of clinical, histologic, and immunofluorescence findings. Immunofluorescence testing is invaluable in confirming a diagnosis that is suspected by clinical or histologic examination. This is especially true in subepidermal bullous diseases that often have overlap in the clinical and histologic findings. Direct immunofluorescence is performed on perilesional skin for patients with bullous diseases and lesional skin for patients with connective tissue diseases and vasculitis. (J Am Acad Dermatol 2001;45:803-22.)

Learning objective: At the completion of this learning activity, participants should be familiar with the ideal method of obtaining immunofluorescence testing for the diagnosis of immune skin diseases and be aware of the value and limitations of immunofluorescence studies.

Immunofluorescence has been used for 4 decades, both to investigate pathophysiology of skin disorders and to help physicians in the diagnosis of various cutaneous disorders, especially bullous diseases and connective tissue diseases. This article addresses the present status of immunofluorescence in dermatology.

DIAGNOSIS AND PATHOPHYSIOLOGY OF BULLOUS DISEASES

Great progress has been made during the past 5 decades in our understanding of the biology of the skin as it relates to bullous diseases. This has led to more accurate classification and diagnosis. Understanding of the immunologic basis of bullous diseases has greatly improved. New diseases have been defined and continue to be defined. Newly defined diseases during the past 5 decades include bullous pemphigoid (BP),[1] mucosal or cicatricial pemphigoid (CP),[2] linear IgA disease (LAD), IgA pemphigus,[3] paraneoplastic pemphigus (PNP),[4] and others that do not yet have a title.[5] The main reason for the continued identification of new bullous diseases is that the diagnosis of bullous diseases at present is based on immunologic and molecular findings rather than clinical or histologic findings alone.

Abbreviations used:

BMZ:	basement membrane zone
BP:	bullous pemphigoid
CP:	cicatricial pemphigoid
DEJ:	dermoepidermal junction
DH:	dermatitis herpetiformis
DIF:	direct immunofluorescence
DLE:	discoid lupus erythematosus
EBA:	epidermolysis bullosa acquisita
HG:	herpes gestationis
HSP:	Henoch-Schönlein purpura
ICS:	intercellular space
IIF:	indirect immunofluorescence
LAD:	linear IgA disease
LCV:	leukocytoclastic vasculitis
LE:	lupus erythematosus
LP:	lichen planus
MCTD:	mixed connective tissue disease
NLE:	neonatal lupus erythematosus
PCT:	porphyria cutanea tarda
PE:	pemphigus erythematosus
PF:	pemphigus foliaceus
PNP:	paraneoplastic pemphigus
PV:	pemphigus vulgaris
SCLE:	subacute cutaneous lupus erythematosus
SLE:	systemic lupus erythematosus

The autoimmune bullous diseases result from an immune response against adhesion molecules of the epidermis and basement membrane zone (BMZ).[6] The pemphigus group of diseases is associated with antibodies to desmosomal proteins.[7-14] The antibodies in each type of pemphigus are directed against a

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Table I. Molecular classification of pemphigus

Pemphigus type	Target desmosomal protein
PV	Desmoglein 3 (and desmoglein 1)
PF	Desmoglein 1
PNP	Desmoglein 3, desmoplakin 1, desmoplakin 2, BP 230, envoplakin, periplakin, other
IgA pemphigus	Desmocollin 1

Table II. Molecular classification of subepidermal bullous diseases

Bullous disease	Targeted molecule
BP	BP 180, BP 230 (hemidesmosome and lamina lucida)
HG	BP 180, BP 230 (hemidesmosome and lamina lucida)
CP	BP 180, laminin V (hemidesmosome and lamina lucida)
EBA	Type VII collagen (anchoring fibrils)
Bullous SLE	Type VII collagen (anchoring fibrils)
LAD (adults and children)	LAD antigen (BP 180) (hemidesmosome and lamina lucida)
DH	Unknown

unique desmosomal protein or a specific combination of desmosomal proteins (Table I).[15] There is strong direct experimental evidence that antibodies in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) cause acantholysis and blister formation[7,8,16] by directly interfering with desmosomal function.[17] The subepidermal bullous diseases are associated with antibodies against one or more components of the BMZ[15,18] (Table II). Antibodies in subepidermal bullous diseases induce blisters primarily by activating the inflammatory process.[19,20]

The diagnostic specificity of the clinical findings varies among bullous diseases. There is clinical overlap among various groups of bullous diseases. For example, LAD[21,22] may mimic BP and dermatitis herpetiformis (DH). IgA pemphigus[3] mimics PF, pemphigus herpetiformis, and subcorneal pustular dermatosis. PNP[23] may mimic PV and Stevens-Johnson syndrome. Inflammatory epidermolysis bullosa acquisita (EBA) is indistinguishable from BP.[24] The noninflammatory mechanobullous form of EBA[25-31] may be indistinguishable from porphyria cutanea tarda (PCT) and pseudoporphyria. Mucosal pemphigoid[32] is clinically indistinguishable from antiepiligrin disease, mucosal EBA, mucosal LAD, and occasionally mucosal lichen planus (LP). Bullous systemic lupus erythematosus (SLE)[33-35] may be indistinguishable from EBA,[25-31] LAD,[36,37] and BP.

Histologic examination should be performed on an early vesicle and helps reveal the site of formation and the presence, intensity, and composition of the inflammatory cell infiltrate as well as other associated findings. A differential diagnosis is generated on the basis of the combination of findings in the biopsy specimen.

IMMUNOFLUORESCENCE IN BULLOUS DISEASES

Direct immunofluorescence (DIF) helps detect molecules such as immunoglobulins and complement components within biopsy specimens.[38] The ideal site for the biopsy specimen depends on the type of disorder. For bullous diseases, DIF is performed using perilesional skin, that is, normal-appearing skin immediately adjacent to a lesion (vesicle, bulla, urticarial plaque, or erythematous patch). The immune deposits are partially or completely degraded in inflamed or blistered skin, and DIF may be falsely negative.

Indirect immunofluorescence (IIF) is a test in which a patient's serum is examined for the presence of antibodies to a defined antigen.[38] This test is helpful in confirming the diagnosis of a bullous disease and is sometimes important in the differentiation among various bullous diseases. The substrates used in the detection of circulating antibodies in bullous diseases include human skin,[39,40] monkey esophagus,[41] guinea pig lip or esophagus, and salt-split human skin.[42] The sensitivity and specificity of the substrates may vary for the various bullous diseases.[43,44] For example, guinea pig lip may be especially helpful for the detection of circulating PF antibodies. Monkey esophagus is highly sensitive for PV antibodies.[45-47] The use of multiple substrates for the same serum may increase sensitivity.

Direct immunofluorescence

The differential diagnosis of a DIF test depends on 4 features: the primary site of immune deposition; the class of immunoglobulin or other type of immune deposit; the number of immune deposits and, if multiple, the identity of the most intense deposits; and deposition in other sites besides the main site. With the use of these parameters, a pattern approach can lead to an accurate diagnosis in the majority of specimens.[48]

"Intercellular space" deposition. The intercellular space (ICS) fluorescence pattern results from binding of antibodies to desmosomal proteins around the keratinocyte cell surface and is charac-

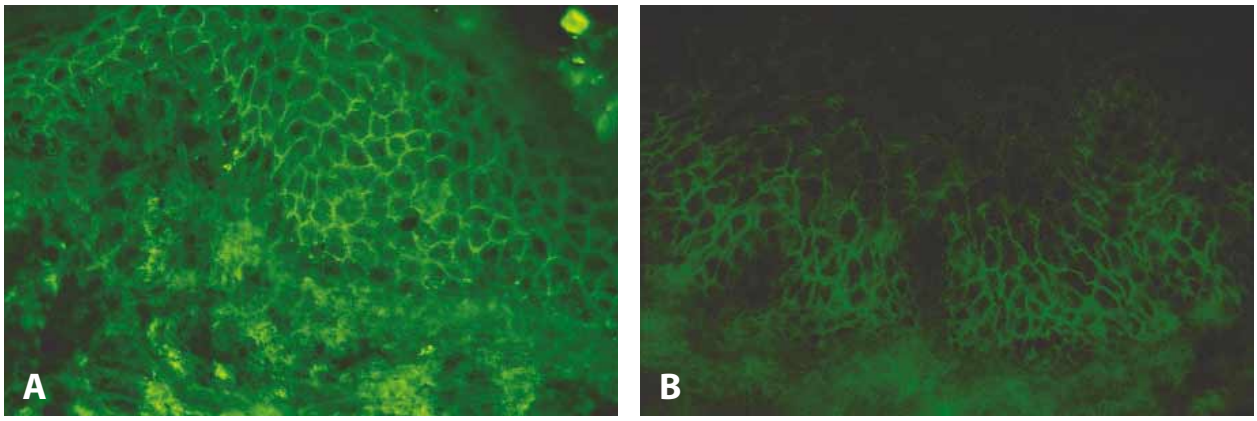

Fig 1. PV. **A,** DIF: Note deposition of IgG around epidermal cells. **B,** IIF using monkey esophagus: Note binding of IgG antibodies to the epithelial cell surface.

teristic of the pemphigus group of disorders.[48,49] Parameters that may be helpful in the diagnosis of the subtype of pemphigus include (1) the class of immunoglobulin deposited, (2) relative intensity of fluorescence in different levels of the epidermis, and (3) any other deposition besides that in the ICS. The majority of specimens with the ICS pattern have IgG antibodies in the ICS only. Deposition of IgA alone is seen occasionally. Deposition along the BMZ may also be seen.

IgG deposition in the ICS only. This pattern is characteristic of all types of pemphigus except IgA pemphigus. DIF is positive in 90% to 100% of patients with active disease if an appropriate biopsy specimen has been obtained. The pattern of fluorescence appears continuous around individual keratinocytes on scanning magnification (Fig 1, *A*). A punctate or granular fluorescence may be appreciated at higher magnification. The latter pattern reflects binding of antibodies to desmosome-associated proteins. The fluorescence pattern seen in PV and PF, as well as their variants pemphigus vegetans and pemphigus erythematosus (PE),[50] respectively, is similar. Occasionally the fluorescence may be limited to or most intense in the level of the epidermis that is involved with blister formation, that is, lower epidermal layer for PV and superficial epidermal layers for PF.[51] This variation in the intensity of fluorescence at the various layers of the epidermis may be caused by differences in the relative amounts of the target desmosomal proteins for each of the two diseases, namely desmoglein 1 for PF and desmoglein 3 for PV.[17,52] Complement component C3 may be seen in a pattern similar to that of IgG.[48,53] The frequency and, usually, the intensity of C3 deposition are lower than those of IgG.[53,54]

IgG deposition in the ICS and BMZ. The combination of ICS and BMZ deposition may be seen in two settings. The first is PE in which the immunopathology of PF and that of LE exist together.[55] There is confusion in the literature regarding criteria for the diagnosis of PE. The diagnosis of PE has been given to various groups of patients including those with definite PF and LE, patients with PF and a positive antinuclear antibody test, patients with PF and concomitant BMZ fluorescence, and patients with PF who have skin lesions that clinically mimic LE in distribution or morphology.

Deposition of immunoreactants in the ICS and BMZ is also seen in PNP[23] (Fig 2). Patients with PNP have antibodies to BMZ proteins in addition to antibodies to desmosomal proteins (see IIF and Table I). The pattern of fluorescence at the BMZ is similar to that seen in BP. In the absence of clinical or histologic information, it is difficult to distinguish between some cases of PE and PNP. Frequently the ICS deposition in PNP is weak or diffuse and nonspecific.

Drug-induced pemphigus is somewhat heterogeneous.[56,57] The majority of patients have clinical, histologic, and immunofluorescence findings identical to those of the idiopathic form of the disorder. Approximately two thirds of patients have drug-induced PF and have antibodies to desmoglein 1.[58] One third of the patients have antibodies to desmoglein 3 and clinical and histologic findings similar to those of PV.[58] A small minority of patients with drug-induced pemphigus have no detectable antibodies by DIF and IIF. It is hypothesized that in such patients, the offending drug may directly induce acantholysis and blister formation without the participation of an immune response.[59]

IgA deposition in the ICS. IgA deposition in the ICS is characteristic of IgA pemphigus.[3,60] This condition has been published under various terms, such as "subcorneal pustular dermatosis with inter-

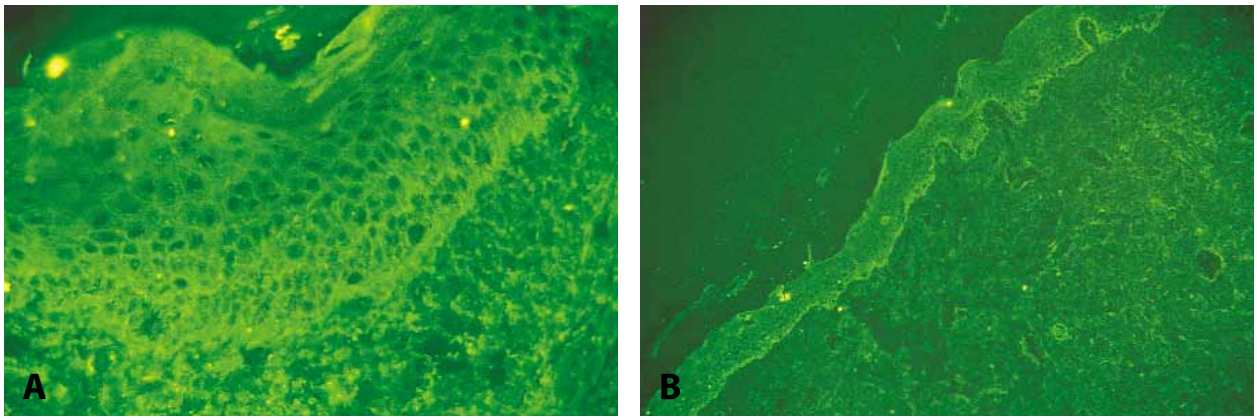

Fig 2. PNP. **A,** DIF: Note faint deposition of IgG around epidermal cells. **B,** DIF: Note faint deposition of C3 along the BMZ. It is not unusual for deposition in PNP to be faint or appear nonspecific.

cellular IgA deposition" and "intraepidermal neutrophilic dermatosis with intercellular IgA deposition." Because antibodies are directed against desmosomal proteins, the term *pemphigus* is appropriate for the condition. The clinical and histologic findings of IgA pemphigus may be similar to those of PF and subcorneal pustular dermatosis.

Predictive value of DIF in bullous diseases. False-negative DIF in pemphigus occurs in approximately 10% of specimens[61] and may result from technical error (eg, by using wrong or weak antisera), the presence of clinical or subclinical inflammation and early blister formation within the biopsy specimen (this is especially true in cases with PNP), or the use of a limited panel of antisera that does not include IgA antisera (for cases with IgA pemphigus). DIF may be "truly" negative in a rare case with drug-induced pemphigus.[57]

An important parameter to evaluate by the practicing physician is the predictive value of DIF. Positive predictive value refers to the likelihood that a patient with a positive test has disease. Negative predictive value refers to the likelihood that a person with a negative test does not have the disease. There are no studies that critically address these parameters in the diagnosis of pemphigus. The positive predictive value of DIF in the diagnosis of pemphigus is extremely high and approaches 100%. The negative predictive value is 85% to 90%. The negative predictive value is not 100% because of the occasional false-negative results. It is highly likely that most false-negative results are seen in biopsy specimens of inflamed or blistered skin. This is especially true in PNP. In cases in which DIF is negative or nonspecific when the histopathology supports the diagnosis of pemphigus, the physician should consider repeating the test and/or obtaining IIF to confirm the diagnosis.

BMZ deposition. The detection of immune deposits at the BMZ by DIF is characteristic of the subepidermal bullous diseases. There are several parameters to evaluate for the accurate interpretation of BMZ deposition. These include (1) the type of immune deposit (including class of immunoglobulin); (2) the number of immune deposits, namely, whether the deposition is of one immunoreactant versus multiple immunoreactants; (3) the morphology of the fluorescence at the BMZ (there are various patterns of deposition at the BMZ including continuous, discontinuous, linear, granular, and homogeneous[62]); and (4) evaluation for fluorescence in any other site besides the BMZ, such as dermal blood vessels.

Exclusive BMZ deposition. This pattern of deposition may be further subdivided into 3 subgroups: deposition of IgG and/or C3, deposition of multiple immunoreactants, and deposition of IgA.

Deposition of IgG and/or C3 at the basement membrane zone. Deposition of IgG, C3, or both at the BMZ is seen in BP[63] (Fig 3, *A*), mucosal pemphigoid,[32,64-72] herpes gestationis (HG),[73-76] EBA[77] (Fig 4, *A*) and bullous SLE.[33,78-80] There are clues that are helpful in the differential diagnosis. Deposition of C3 with significantly higher intensity than IgG strongly favors the pemphigoid group of diseases (BP, mucosal pemphigoid, and HG). It is not unusual for C3 to be the exclusive immunoreactant at the BMZ in patients with HG and occasionally BP. The pattern of deposition in BP and HG has been described as linear, wavy, tubular, and granular. The variation in pattern may result from variations in the angle at which the cryosections are made, the intensity of deposition,

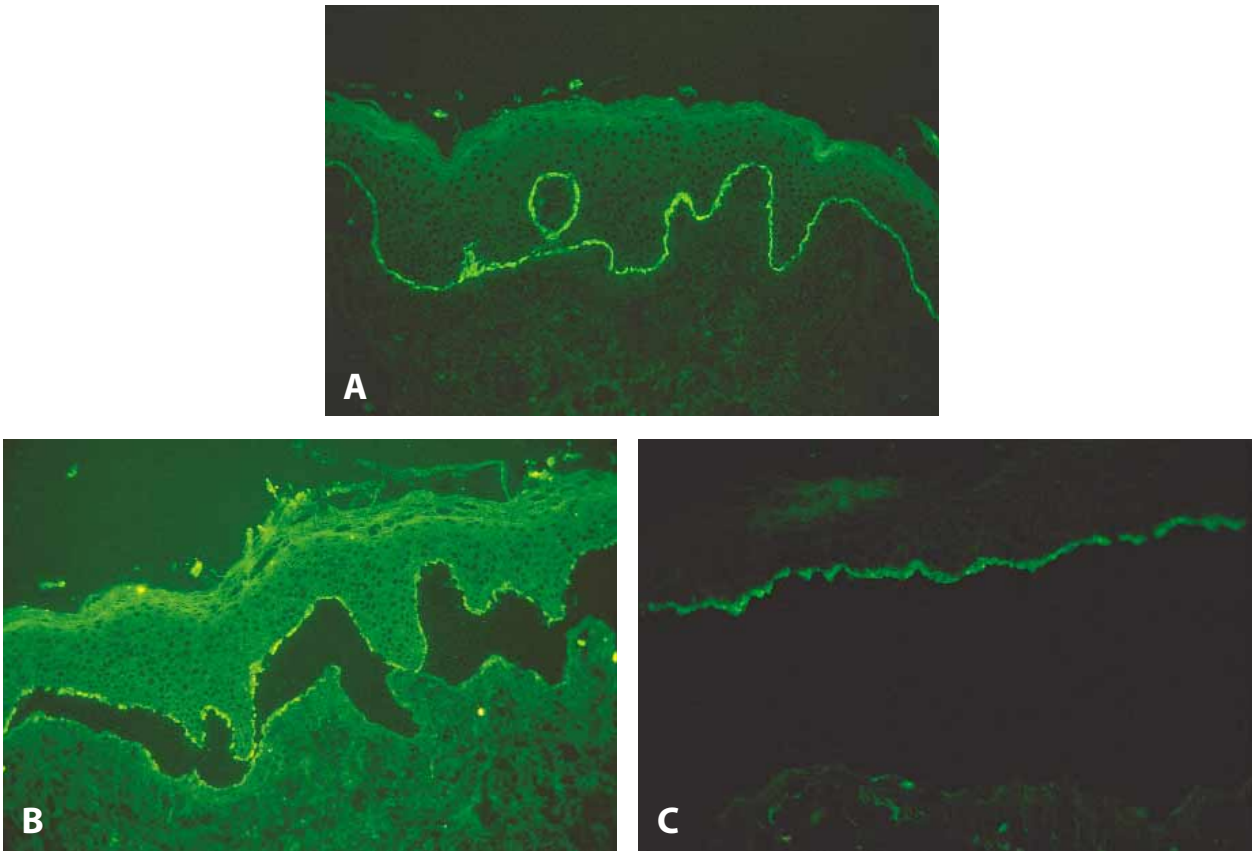

Fig 3. BP. **A,** DIF: Note continuous deposition of C3 along the epidermal basement membrane. **B,** DIF following salt-split processing: Note deposition of IgG on the epidermal side, and faintly on the dermal side. **C,** IIF using human salt-split skin: Note deposition of IgG antibodies limited to the epidermal side.

and the site of biopsy.[62] In specimens that contain adnexal structures, a similar deposition may be seen along the BMZ of follicular and sweat gland epithelium. Differentiation between BP and HG is not possible by immunfluorescence or histopathology. There is ample evidence confirming that HG is a variant of BP induced by pregnancy.[73] The HG factor (discussed later) is present in both HG and BP. If the intensity of IgG deposition at the BMZ is significantly higher than that of C3, EBA and bullous SLE are more likely than pemphigoid. The differential expression of intensity between IgG and C3 among the above disorders is not understood.[81-83]

Multiple deposits at the BMZ. This pattern of deposition strongly favors EBA (Fig 4, *A*) and bullous SLE over the pemphigoid group of diseases. In EBA, intense IgG deposition is almost consistently present.[77] The intensity of C3 deposition is usually less than that of IgG. Deposition of IgA is present in approximately two thirds of cases and deposition of IgM in approximately one half of cases.[34,77] The morphologic pattern of deposition in the above two disorders is usually homogeneous, thick, and broad.[34] The BMZ of adnexal epithelia reveals similar deposition. In bullous SLE, approximately 60% of cases reveal BMZ deposition indistinguishable from that of EBA.[78] In the remaining cases, the deposition is granular and mimics that seen in cases with nonbullous SLE. Compared with nonbullous SLE, bullous SLE is more frequently associated with deposition of IgA.[78] In the absence of clinical history, it is not possible to distinguish EBA, bullous SLE, and nonbullous SLE with certainty. This is not surprising since most patients with bullous SLE have detectable antibodies to type VII collagen that is also the target of EBA antibodies.[84] Differentiation between bullous SLE and EBA is based on an underlying diagnosis of SLE by clinical and serologic criteria.

Deposition of IgA at the BMZ. Linear deposition of IgA at the BMZ is characteristic of LAD[36,37,85,86] (Fig 5). The so-called chronic bullous disease of childhood reveals identical findings and represents the childhood form of LAD.[22,87,88] Deposition of C3 is present less frequently and with lower intensity com-

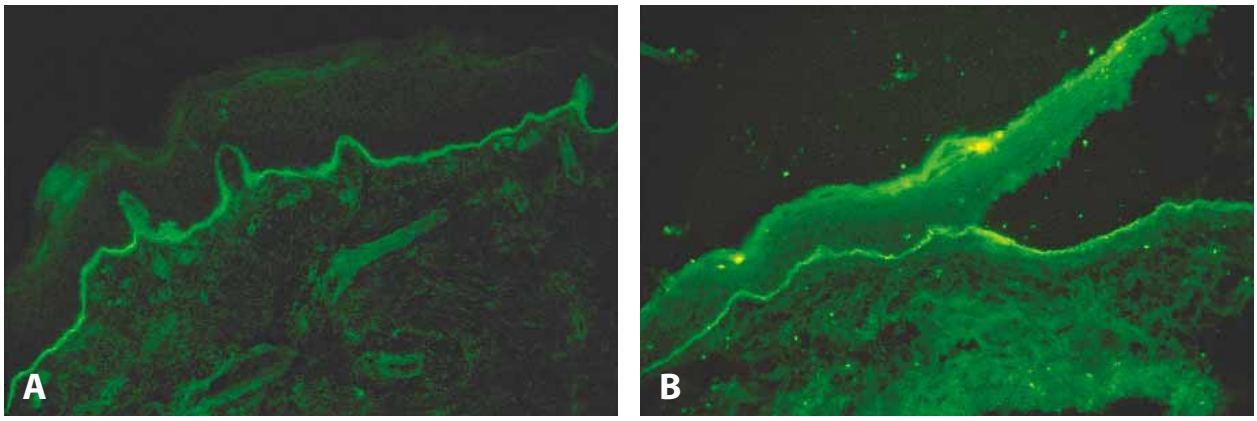

Fig 4. EBA. **A,** DIF: Note continuous homogeneous deposition of C3 along the BMZ. **B,** DIF after salt-split processing: Note deposition of IgM limited to the dermal side.

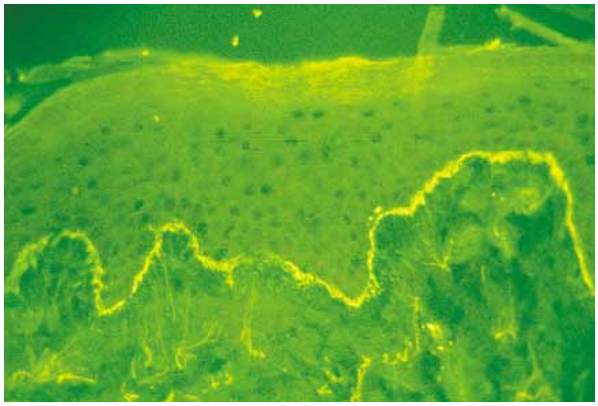

Fig 5. LAD. DIF: Note deposition of IgA along the BMZ.

pared with IgA. The morphology of IgA deposition in LAD is similar to deposition of other immunoreactants along the BMZ in other subepidermal bullous diseases such as BP and EBA.

DIF using salt-split specimens. The combination of clinical, histologic, and immunofluorescence findings may occasionally be inconclusive in the differentiation between the pemphigoid group of diseases on the one hand and EBA and bullous SLE on the other. DIF using salt-split biopsy specimens is a simple method to differentiate between BP and EBA.[77,89-91] Deposition in BP (and the other pemphigoid disorders) is within the lamina lucida (especially the outer portion close to the basal cell membrane).[92] This site of deposition corresponds to the location of the extracellular domain of BP180 antigen that contains the dominant epitopes recognized by the pathogenic BP antibodies.[93,94] In contrast, deposition of antibodies in EBA is in the sublamina densa area where the target antigen, type VII collagen within the anchoring fibrils, is present.[95] Incubation of a biopsy specimen in 1 mol/L sodium chloride results in a split in the lower lamina lucida.[42] Accordingly, immune deposits in pemphigoid would be present on the epidermal side of the split (see Fig 3, *B*) whereas the immune deposits in EBA (and bullous SLE) would be present on the dermal side[90] (see Fig 4, *B*). Exclusive deposition on the dermal side may also be seen in antiepiligrin disease (also referred to as antiepiligrin CP[96]) and rarely in BP.[97,98] It is not unusual for specimens of BP to reveal slight dermal fluorescence in addition to the primary fluorescence on the epidermal side. This observation may be caused by the fact that certain BP antibodies may recognize epitopes within the BP180 molecule that are close to or within the lamina densa.[98,99] The specimen originally used for DIF may be thawed and used for the salt-split DIF technique unless the original DIF findings have been nonspecific because of the presence of inflammation or early blister formation. DIF on salt-split biopsy specimens is usually performed only if IIF on salt-split normal human skin (see later) is negative. The latter test is easier, more routinely available, and similar in accuracy.

Mucosal disease. Several subepidermal disorders may have primary or exclusive involvement of mucosal surfaces. These include mucosal pemphigoid,[2,67,100,101] EBA,[77,102] antiepiligrin disease,[96] and LAD.[21,87] Exclusive deposition of IgA alone is extremely helpful in the diagnosis of LAD.[85] IgG and C3 are the predominant immune deposits in mucosal pemphigoid and antiepiligrin disease. Multiple immune deposits may be seen in EBA.[77] Mucosal LP may be distinguished by the presence of cytoid bodies and a characteristic thick band of fibrinogen. If the patient with mucosal bullous and erosive disease has skin lesions in addition to mucosal lesions, a skin

biopsy may be helpful by being easier to obtain and easier to use for salt-split DIF testing. The latter test would be helpful in distinguishing EBA and antiepiligrin disease on the one hand and mucosal pemphigoid on the other.

Deposition at the BMZ and blood vessel walls. Homogeneous deposition of immunoreactants (usually multiple) within superficial dermal blood vessel walls, in addition to BMZ deposition, is characteristic of PCT, pseudo-PCT, and erythropoietic protoporphyria.[103] The most frequent immunoreactants are IgG and IgA. Deposition of C3 is somewhat less frequent and is often granular. The deposition in erythropoietic protoporphyria is usually more extensive and extends from the blood vessel walls into the surrounding dermis.

Papillary dermal deposition. Granular deposition of IgA and C3 in the papillary dermis and along the BMZ is diagnostic of DH[104-109] (Fig 6). Deposition of IgA is present in 100% of patients when the biopsy specimen is obtained from normal-appearing perilesional skin. Deposition of C3 is seen in approximately half of cases.[104] Deposition of IgG or IgM, or both, is less frequent and less intense.[104]

Predictive value of DIF in subepidermal bullous diseases. In patients with subepidermal bullous diseases, the positive and negative predictive values of DIF approach 100%. False-negative results may occur secondary to technical error (extremely rare) or poor sampling (biopsy specimen from inflamed or bullous lesions).

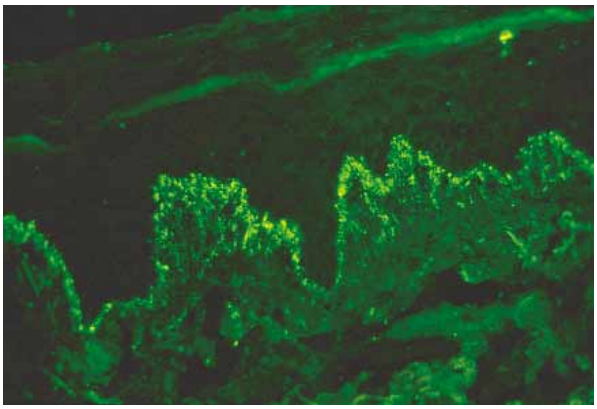

Fig 6. DH. DIF: Note deposition of IgA in a granular pattern along the BMZ and dermal papillae.

Indirect immunofluorescence

IIF is helpful in confirming a suspected diagnosis as well as in differentiating among closely related bullous diseases. Both the class of circulating immunoglobulin and the site of its binding within the skin are important for diagnosis. The circulating antibodies in most bullous diseases belong to the IgG class. IgA is characteristic of LAD and IgA pemphigus. The binding site of the antibodies is either the ICS or the BMZ.

IgG anti-ICS antibodies

These antibodies are present in PV (Fig 1, *B*), PF, PE, PNP, and drug-induced pemphigus. The intensity of fluorescence may be higher in the superficial epidermal layers in PF compared with PV[51] because of the abundance of target antigen molecules (desmoglein 1) in the superficial epidermis for PF antibodies compared with the lower epidermis.[17] However, this observation should be interpreted with caution. The exact subtype of pemphigus may be determined with certainty only by histologic examination of an early vesicle. Pemphigus-like antibodies have been reported in patients with burns,[61] penicillin drug eruption,[110] skin grafts,[61] BP,[111] and mucosal pemphigoid.[112,113]

Antibodies in PNP are directed against both desmosomal proteins and hemidesmosomal proteins and may produce BMZ fluorescence in addition to ICS fluorescence. In addition, PNP antibodies bind the desmosomes of simple and transitional epithelia in addition to stratified squamous epithelia. Antibodies in other pemphigus subtypes bind only stratified squamous epithelia. The best screening test for PNP is IIF on rat bladder epithelium. It is 75% sensitive and 83% specific in the diagnosis of PNP.[114] Although the technique of the latter test is similar to IIF on other substrates, it is less widely available.

In addition to its diagnostic value, IIF titers correlate directly with clinical disease activity and may be used to follow progress of disease and response to therapy.[115-117]

IgA anti-ICS antibodies. IgA anti-ICS antibodies are characteristic of IgA pemphigus and are present in approximately 50% of patients.[60,118-130]

IgG anti-BMZ antibodies. Antibodies to the BMZ are present in the sera of patients with BP,[40,131,132] mucosal pemphigoid,[2] HG,[133] EBA,[134] and bullous SLE.[84] The prevalence of anti-BMZ antibodies in these disorders differs. Therefore the results of the test may be indirectly helpful in suggesting the diagnosis. For example, IIF is positive in only 10% of patients with HG and 25% of patients with mucosal pemphigoid. The test is positive in approximately 75% of patients with BP and 50% of patients with EBA[77] using intact human skin or monkey esophagus as substrate. Anti-BMZ antibodies are not detectable on intact substrates in bullous SLE but may be detected on salt-split human skin substrate. The pattern of fluorescence is not helpful in differential diagnosis among the aforementioned disorders. Fluorescence may be thicker and more homo-

geneous in EBA compared with pemphigoid. The differentiation between EBA and bullous SLE on the one hand and pemphigoid on the other depends on IIF on salt-split skin (see later).

IgA anti-BMZ antibodies. IgA anti-BMZ antibodies are characteristic of the adult and childhood form of LAD.[22,85] They are present in one third to one half of patients.

Other antibodies. Several antibodies against nonskin components have been reported in DH. These include antiendomysial, antireticular, and antigliadin antibodies.[104,135-138] They are not diagnostic of DH.

Predictive value of IIF in bullous diseases. The positive predictive value of IIF varies among the bullous diseases and depends on the frequency of positivity. For example, IIF is positive in approximately 90% of patients with active PV resulting in a high predictive value. The positive predictive value is much lower in EBA, LAD, and IgA pemphigus in which IIF is positive in only 50% of cases. The negative predictive value of IIF also varies. In general, negative predictive value for all bullous diseases is low because many patients may have negative IIF. False-negative results may occur secondary to substrate sensitivity, technical error, and rarely, the prozone phenomenon.

Differentiation between BP and EBA. IIF on salt-split human skin[90,91] is very helpful in differentiating BP from EBA. A lamina lucida split is induced in normal human skin by incubation with 1 mol/L sodium chloride for 24 to 72 hours at 4°C. Cryosections of the substrate are incubated with the serum.[42] Fluorescence exclusive to the dermal side of the split is characteristic of EBA and results from binding of antibodies to collagen VII in anchoring fibrils. Fluorescence limited to the epidermal side or, occasionally, the epidermal and dermal sides of the split is characteristic of pemphigoid disorders[90] (see Fig 3, *C*) and results from binding of antibodies to the extracellular domain of BP180 antigen. If IIF on intact substrates is negative, IIF on salt-split human skin should be performed since the latter test is more sensitive than the former and may be positive.[91] In cases in which both tests are negative, the salt-split technique may be performed on a perilesional biopsy specimen, as discussed earlier.

Herpes gestationis factor

The HG factor is an amplified IIF procedure in which the presence of a small amount of circulating antibodies (undetectable by standard IIF) is amplified and detected by the complement-fixing properties of the antibodies.[133,139,140] The test is positive in approximately 50% of patients with HG. The test is positive in other diseases with circulating complement-fixing anti-BMZ antibodies, such as BP.[141,142] Human skin substrate (preferably salt-split human skin) is incubated with the patient's serum followed by a source of active complement (fresh human serum), and finally fluorescein-labeled antisera against human complement.[140] Complement-fixing antibodies in the serum bind the BMZ and then fix a much larger number of complement molecules to the site of deposition. The large number of complement molecules at the BMZ bind the anticomplement antisera and produce visible fluorescence. Because the HG factor test is a multiple-step procedure with stricter conditions than routine IIF, it is frequently negative. The test has low diagnostic value in the case of a pregnant woman with blisters that reveal the histologic and DIF findings of HG. However, the test may be helpful if histopathology and DIF results are not diagnostic.

Summary

There is overlap in the clinical and histologic features of the various autoimmune bullous diseases. A diagnosis based solely on clinical or histologic findings may not be accurate. DIF is extremely helpful in confirming a suspected diagnosis and in distinguishing among closely related diseases. IIF is helpful in cases in which the DIF is negative or nonspecific. IIF is also helpful in differentiating BP and EBA. Special substrates may be required for the diagnosis of specific bullous diseases such as PNP. Table III shows an algorithm for the laboratory diagnosis of a bullous disease by histopathology and immunofluorescence. An accurate diagnosis is helpful in the choice of therapy and the successful management of patients with bullous diseases.

CONNECTIVE TISSUE DISEASES

DIF is helpful in the diagnosis of connective tissue diseases, especially various subsets of LE and vasculitis.

Lupus erythematosus

LE comprises a group of disorders that share clinical, histologic, and immunologic features. The immunologic features include circulating autoantibodies as detected by serologic testing and cutaneous immune deposits as detected by DIF. The serologic testing has been reviewed recently.[143] There is clinical as well as immunologic overlap between the various subsets of LE. For example, patients with SLE may have the characteristic cutaneous lesions of subacute cutaneous LE (SCLE) or discoid lupus erythematosus (DLE). Patients who present with SCLE may develop systemic involve-

Table III. Algorithm for the diagnosis of autoimmune bullous diseases

Histopathology	DIF	IIF	Diagnosis
Suprabasal	1. IgG ± C3 at ICS	IgG at ICS, monkey esophagus	PV > PNP
	2. IgG ± C3 at ICS + BMZ	IgG at ICS, rat bladder	PNP
Subcorneal	1. IgA at ICS	IgA at ICS	IgA pemphigus
	2. IgG ± C3 at ICS	IgG at ICS	PF
	3. IgG ± C3 at ICS, Ig ± C3 at BMZ	IgG at ICS + ANA	PE
Subepidermal noninflammatory	1. IgG, C3 ± IgM, IgA at BMZ	1. Dermal side of SSS	EBA
		2. Epidermal side of SSS	BP
	2. IgG, IgA ± IgM, C3 in blood vessel walls	Negative	PCT, pseudo-PCT
Subepidermal with eosinophil-rich infiltrate	C3, IgG at BMZ	Epidermal side of SSS	BP, HG, mucosal pemphigoid
Subepidermal with neutrophil-rich infiltrate	1. Granular IgA in dermal papillae and BMZ	Negative on epithelium (+antiendomysial antibodies)	DH
	2. Linear IgA ± C3, BMZ	IgA at BMZ	LAD
	3. IgG, IgM, C3, IgA, fibrinogen	1. Dermal side of SSS	EBA, rare antiepiligrin disease
		2. Dermal side of SSS and positive lupus serology	Bullous SLE

±, With or without; >, more likely than; *SSS,* salt-split skin. For other abbreviations, see abbreviation box at beginning of article.

Table IV. DIF in LE in various biopsy sites

	Lesional	Nonlesional, sun-exposed	Nonlesional, non–sun-exposed
SLE	50%-100%[148,165,175]	73%-90%[178,179,181]	26%-92%[170,179,180]
SCLE	54%-100%[155,156,160]	18%-100%[155,156,160,161]	0-100%[155,156,162,163]
DLE	60%-94%[146,165,170]	0-10%[148,221]	0-10%[146,221]

ment. A small proportion of patients who present with DLE may progress to SLE. The immunologic overlap is manifested by the similarity between the cutaneous immune deposits among the various subsets of LE, as detected by DIF.

DIF is helpful in confirming the diagnosis of LE when suspected clinically, histologically, or both. DIF may be helpful in distinguishing among the various subsets of LE since the frequency of deposition, its morphology, and site of deposition vary among the various subsets of LE. DIF may help differentiate LE from disorders that may have similar clinical findings and overlapping histologic findings, such as polymorphous light eruption and benign lymphocytic infiltration of the skin.

DLE. Immune deposits in DLE are characteristically found along the dermoepidermal junction (DEJ).[144-147] Cytoid bodies may also be seen.[144,147] These represent degenerated basal keratinocytes that "dropped" into the papillary dermis and adhered to circulating immunoglobulin, and complement. The immune deposits most frequently present along the DEJ are IgG and IgM.[144-147] The immunoglobulins most frequently present in cytoid bodies are IgM and IgA.[144] Complement and IgG are less frequently seen.[144] Several patterns of fluorescence along the DEJ have been described and include linear, granular, and shaggy (Fig 7). Linear bands are continuous and may be thick or thin. Granular deposits are not continuous and can be coarse or fine. Shaggy deposits represent thick bands along the DEJ.[144]

The frequency of immune deposits in DLE depends on the biopsy site, past treatment, and duration of the lesion. Immune deposits are present in 60% to 94% of biopsy specimens from lesional skin[145,146,148,149] and are usually absent in nonlesional skin[144,150] (Table IV). In one study DIF was positive in 96% of biopsy specimens of the face, 65% of other sun-exposed specimens, and 30% of non–sun-exposed specimens.[145] The frequency of positive DIF in presently treated lesions (61%) is lower than in

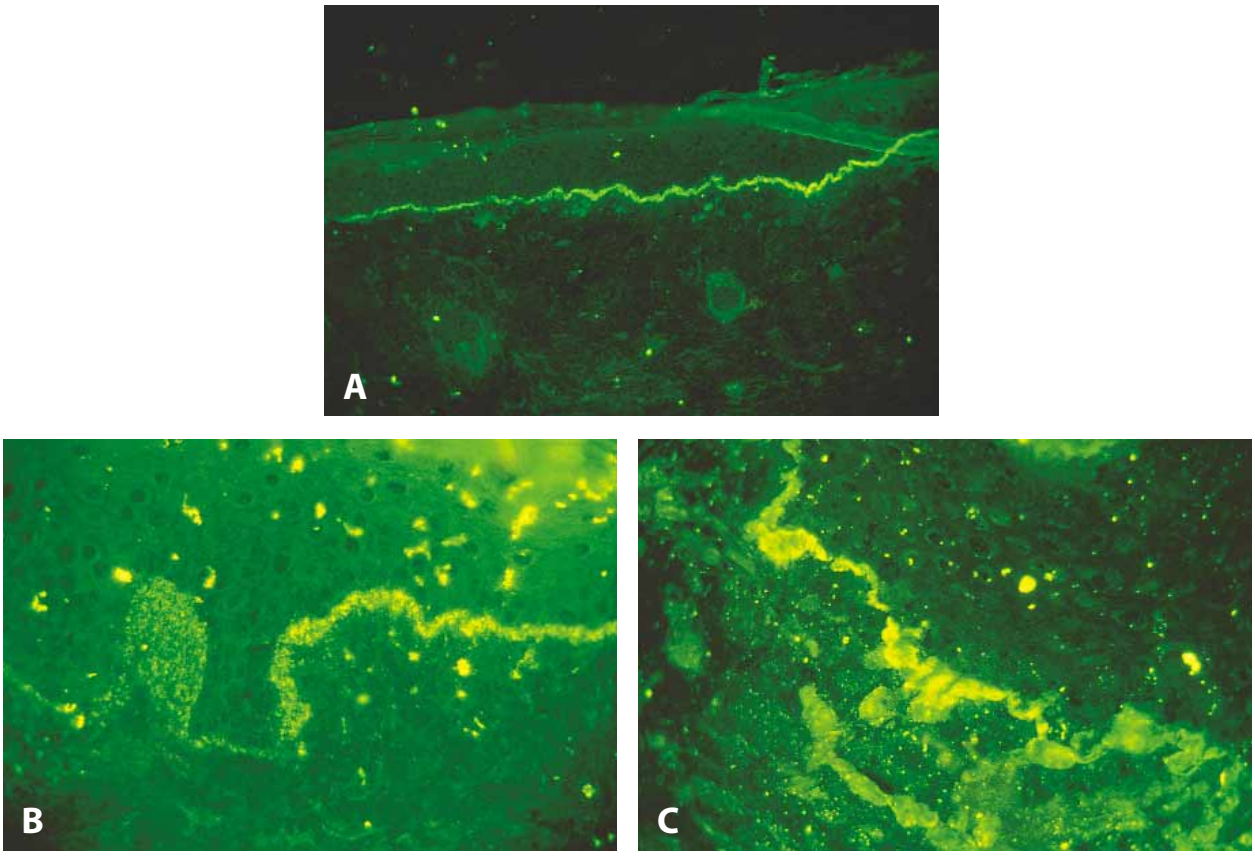

Fig 7. LE. DIF: Note continuous linear deposition of C3 along the BMZ **(A)**. **B,** Note deposition of C3 in a granular pattern along the BMZ. **C,** Note deposition of IgG in a homogeneous and shaggy pattern along the BMZ.

untreated lesions.[145] Prior treatment, up to 3 weeks before biopsy, may also decrease the frequency of positive DIF.[145] The duration of the lesion also influences the frequency of positive DIF.[145] One study noted that only one third of lesions less than 1 month old have a positive DIF. As the age of the lesion increases, the frequency with which the DIF is positive increases to 82% in lesions older than 1 year.[145]

Immune deposits at the DEJ may be seen in many other disorders, including rosacea, LP, and primary biliary cirrhosis (Table V).[144,151,152] The class of immunoglobulin may be helpful in distinguishing DLE from the other disorders. IgG deposits are more specific for DLE.[150,153] The combination of IgG and IgM favors the diagnosis of DLE.[144] In summary, to confirm the diagnosis of DLE, the most appropriate biopsy site for IF is the oldest, untreated lesion, preferably from an area that is not habitually exposed to the sun.

SCLE. Immune deposits in SCLE may be present along the DEJ and basal keratinocytes.[144,154] Cytoid bodies may also be seen.[144,150,155] The immune deposits most frequently found along the DEJ are IgG and IgM. The classes of immunoglobulins in cytoid bodies are IgM and IgA. Complement and IgG are less frequently present.[144,150] The patterns of DEJ immunofluorescence are similar to those seen in DLE.[144,150,155] Another pattern that is unique to SCLE consists of granular fluorescence throughout the cytoplasm and nucleus of basal keratinocytes.[156,157] This pattern is believed to reflect binding of anti-Ro(SS-A) or anti-La(SS-B) antibodies (or both) to the Ro(SS-A) or La(SS-B) antigens (or both) within the keratinocytes.[156,158,159] However, this pattern has also been reported in the skin of patients with anti-Ro(SS-A) antibodies who do not have SCLE.[157] It is believed that this pattern correlates with the presence of anti-Ro(SS-A) or anti-La(SS-B)antibodies rather than SCLE lesions and may be seen in biopsy specimens of patients with these antibodies who do not have SCLE. DIF is positive in 54% to 100% of SCLE lesions.[154-156,160-162] Nonlesional skin is positive in 18% to 100% of the cases.[155,156,160,161] The prevalence of positive DIF in nonlesional, non–sun-exposed skin varies from 0% to 100%[155,156,162,163] (see Table IV).

Table V. Diseases with immune deposits along the dermoepidermal junction[144,145,164,222]

LE
Dermatomyositis
Systemic sclerosis
LCV
Rheumatoid arthritis
BP
HG
EBA
DH
Linear IgA bullous dermatosis
PCT
Pseudoporphyria
LP
Rosacea
Chronic active hepatitis
Primary biliary cirrhosis

For abbreviations, see abbreviation box at the beginning of the article.

Table VI. Sites of immune deposits in various disorders

	Dermo-epidermal junction	Epidermal cell nuclei	Papillary dermis (cytoid bodies)	Peri-vascular
SLE[144,150,164,168]	+	+	+	+
MCTD[172]	+	+	–	–
SS[172,196,198]	+	+	–	+
DM[200]	+	–	+	–
HSP[208,209]	–	–	–	+
LP[211]	+	–	+	–
EM[219,220,223]	+	–	+	+
PG[224]	+	–	+	+

+, Present; –, absent; *DM,* dermatomyositis; *EM,* erythema multiforme; *PG,* pyoderma gangrenosum; *SS,* systemic sclerosis. For all other abbreviations, see the abbreviation box at the beginning of the article.

SLE. Immune deposits in SLE may be present in 4 sites. First, the characteristic site of deposition is at the DEJ.[144,150,164-169] The use of this finding as a diagnostic test for SLE has been termed the "lupus band test."[144,150,168-170] Some authors use the term *lupus band test* to refer to immunoglobulins and complement present along the DEJ in nonlesional skin, whereas others use the term to indicate deposits at the DEJ in lesional or nonlesional skin.[144,150] Second, cytoid bodies may be seen in the papillary dermis.[144,150] Third, immune deposits may be located in the superficial dermal blood vessel walls similar to vasculitis.[150] Finally, and much less commonly, epidermal keratinocyte nuclei may show positive fluorescence[150,171,172] (Table VI). This latter finding is usually seen in patients with antibodies to U_1RNP,[150] but has also been seen in patients with other antinuclear antibodies and was first reported in patients with mixed connective tissue disease (MCTD).

The immune deposits most frequently found along the DEJ are IgG, IgM, IgA, and C3.[153,164] These immune deposits are characteristically found in combination.[144,164] Eighty-five percent of patients have multiple immune deposits along the DEJ and nearly 45% of patients demonstrate IgG and IgM with or without C3.[164] Other immune deposits include IgD, IgE, fibrin, and other complement factors.[144,173] Several patterns of fluorescence along the DEJ have been described and include linear, granular, and shaggy. The intensity of the DEJ fluorescence has been shown to correlate with double-stranded DNA antibody levels and hence disease activity.[174] The classes of immunoglobulins in cytoid bodies are frequently IgM and IgA.[144] Complement and IgG are less commonly present. The main immunoglobulin found in epidermal nuclei is IgG.[144,150]

The prevalence of immunoglobulins and complement deposition in SLE depends on several factors including the clinical morphology of the lesions, biopsy site, past treatment, and disease activity.[144] Patients with SLE may have skin lesions that are identical to DLE or SCLE or have lesions that are specific for SLE. The latter include the malar butterfly rash, diffuse photosensitive eruption, and nonspecific erythematous edematous plaques. The characteristics of DIF in biopsy specimens obtained from lesions of DLE or SCLE are similar to those of patients with DLE and SCLE. DIF is positive in 50% to 100% of specimens from SLE-specific lesional skin.[148,165,170,175,176] The frequency of DIF is lower in nonlesional skin and varies between sun-exposed and non–sun-exposed areas[153,170,177-181] (see Table IV). The frequency of positive DIF in nonlesional sun-exposed skin is 73% to 90%.[179] In nonlesional non–sun-exposed skin of the forearm, positive DIF ranges from 68% to 92%.[180,182] Nonlesional skin from the buttock reveals positive DIF in 26% to 92% of cases[153,179] (see Table IV). Frequency of positive DIF also varies in different anatomic locations.[150] Facial skin is more often positive than truncal skin.[144]

Systemic immunosuppressive treatment is associated with a lower frequency of positive DIF.[144,183] Furthermore, patients with active SLE are more likely to have positive DIF findings than those with inactive disease.[145,153] A recent study investigated the predictive value of lesional DIF and found that the positive predictive value for the diagnosis of SLE was 64% and the negative predictive value was 98%.[184] Thus a neg-

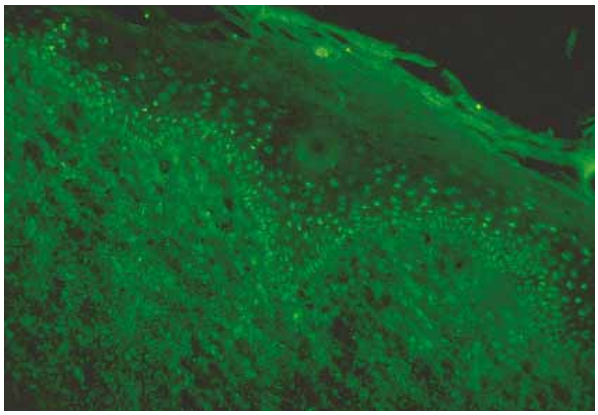

Fig 8. DIF reveals IgG deposition within epidermal keratinocyte nuclei. This pattern is characteristic of MCTD as well as SLE.

ative DIF virtually excludes SLE, whereas approximately one third of patients with positive DIF do not have SLE. Serologic testing is more reliable than DIF in the diagnosis of SLE. The presence of high-titer antinuclear antibodies by immunofluorescence and specific antibodies to various nuclear antigens such as U_1RNP and Sm is highly characteristic of SLE.

Neonatal lupus erythematosus. The diagnosis of neonatal lupus erythematosus (NLE) is based on the characteristic cutaneous lesions with or without evidence of heart block, as well as serologic markers for the disease in the infants, their mothers, or both. DIF is not routinely performed on lesions of NLE. Immune deposits in NLE are found along the DEJ.[185-187] The immune deposits most frequently found are IgG, IgM, and C3.[185,186] In a clinical and histopathologic analysis of 10 infants with NLE, the authors noted that only two had DIF performed. DIF was positive in one.[185] Other authors have noted that approximately 50% had positive DIF.[186]

Mixed connective tissue disease

MCTD shares cutaneous and systemic findings with SLE and systemic sclerosis. The disease was defined approximately 3 decades ago in patients who had systemic connective tissue disease manifestations and the presence of high-titer antibodies to the ribonuclease-sensitive component of extractable nuclear antigen, presently referred to as U_1RNP.[188] The presence of this antibody in patients with MCTD is to the exclusion of other antinuclear antibodies, unlike patients with SLE who may have U_1RNP antibodies in addition to other antinuclear antibodies. DIF is frequently positive in patients with MCTD and has characteristic morphology.[172,189] Immune deposits are detected within epidermal cell nuclei and rarely along the DEJ[172] (see Table VI). The immunoglobulin most frequently present in the epidermal cell nuclei is IgG.[190] The binding of the antibody results in a speckled pattern within the epidermis[189,191] (Fig 8). It is believed that this pattern results from the binding of circulating antibodies to U_1RNP in the nucleus of epidermal cells.

The frequency of epidermal fluorescence ranges from 46% to 100%.[172,189] DIF may be positive in approximately 15% of biopsy specimens from nonlesional skin in patients with MCTD. Fluorescence along the DEJ is observed in only 15% of cases.[172,192] Epidermal nuclear fluorescence is characteristic but not diagnostic of MCTD.[172,190,191] Similar fluorescence may be seen in about 10% to 15% of patients with SLE[171,172,193] in association with U_1RNP antibodies as well as other antinuclear antibodies. Approximately 20% of patients with systemic sclerosis may also demonstrate this fluorescence pattern.[172,194]

Systemic sclerosis

DIF in systemic sclerosis is either negative or nonspecific.[148,189,194-197] Patients with positive DIF findings are likely to have overlapping features with SLE and dermatomyositis.[196] One study reported granular deposition of IgM along the DEJ in sun-exposed skin in 60% of the patients.[194] Approximately 15% had perivascular deposits. These are usually seen in patients who have associated vasculitis. Epidermal nuclear fluorescence similar to MCTD and SLE (see Table VI) may occasionally be seen.[172,190,194,198] DIF is of little or no value in the diagnosis of systemic sclerosis.

Localized scleroderma/morphea

DIF is usually negative and has little or no value in the diagnosis of morphea. Deposition of IgM has been rarely reported along the DEJ.[172]

Dermatomyositis

The clinical features of dermatomyositis are usually characteristic and consist of heliotrope rash over the face, especially the eyelids; Gottron's papules over the extensor aspects of the distal joints; erythematous patches; and poikiloderma. Occasionally the clinical findings are not characteristic and may be difficult to distinguish from SLE. The histologic findings in dermatomyositis vary with the clinical morphology of the lesions and are generally similar to those of SCLE and SLE. In cases in which both the clinical and histologic findings are not diagnostic, differentiation between dermatomyositis and LE may be made by the use of muscle enzyme chemistry findings as well as serologic antibody testing.[143]

DIF may occasionally be helpful in differentiating between dermatomyositis and SLE. The prevalence

of positive DIF in dermatomyositis has not been studied as extensively as that in SLE. In one study, the prevalence of positive DIF in dermatomyositis was approximately 50%.[199] The site of immune deposits is similar to that in LE, namely along the DEJ, and occasionally within cytoid bodies in the superficial dermis[199] (see Table VI). Perinuclear fluorescence may be seen, particularly in biopsy specimens obtained from the periungual area.[200] The immune deposits most frequently present are IgM, IgG, and C3.[199,200] Although the immunofluorescence pattern and composition of immune deposits are similar to those in LE, the intensity of fluorescence is usually lower in dermatomyositis compared with LE. This observation, along with a lower frequency of positive DIF in dermatomyositis compared with LE, may be helpful in the differentiation between dermatomyositis and LE. A negative or weak fluorescence may favor dermatomyositis, whereas an intense fluorescence favors SLE. In cases in which the differential diagnosis includes SCLE and dermatomyositis, the presence of granular fluorescence within basal keratinocytes, in addition to the detection of anti-Ro(SS-A) and anti-La(SS-B) antibodies, strongly favors SCLE.

Vasculitis

Vasculitis is a term that applies to several conditions that are characterized by inflammation and usually destruction of blood vessel walls.[201] The 3 main pathogenetic mechanisms that result in "vasculitis" are allergic (leukocytoclastic, hypersensitivity, immune complex), infectious (bacterial, fungal, rickettsial), and primary occlusive (coagulopathies, emboli, idiopathic). Each of the 3 groups of vasculitis tends to have characteristic findings. Occasionally there is some overlap. Depending on the clinical setting, the diagnostic evaluation includes biopsy specimen examination, tissue cultures, and systemic evaluation for intravascular occlusion. DIF may be helpful in confirming the diagnosis of leukocytoclastic vasculitis (LCV) and, more specifically, to confirm the diagnosis of Henoch-Schönlein purpura (HSP).

The site of immune deposits in LCV is within the walls of postcapillary venules in the superficial dermis.[202,203] This is the same site of the neutrophilic infiltrate. The most frequent deposit is C3, followed by IgG, IgM, and fibrinogen.[203] The deposition is usually granular or fibrillar and is seen in blood vessel walls extending into both the extravascular and the intravascular space.[203] Deposition of fibrinogen is frequently diffuse throughout the dermis (Fig 9). DIF is positive in the majority of cases of LCV.[203,204] The sensitivity of the test is influenced by the duration of the lesion.[202,203,205] Lesions less than 24 hours old yield the most frequently positive results. Lesions older than 24 hours may have negative DIF because immune deposits are degraded rapidly.[202,203,205] Vessel wall deposition is not diagnostic of LCV and may be seen in biopsy specimens from the lower legs in patients without vasculitis. If a patient with suspected vasculitis has lesions at sites other than the lower legs, it is preferred that the biopsy specimen be obtained from such lesions. A diagnosis of LCV should not be made solely on the presence of positive DIF findings, nor should the diagnosis be excluded with a negative DIF test. The findings of DIF should be interpreted along with clinical, histologic, and other laboratory findings.

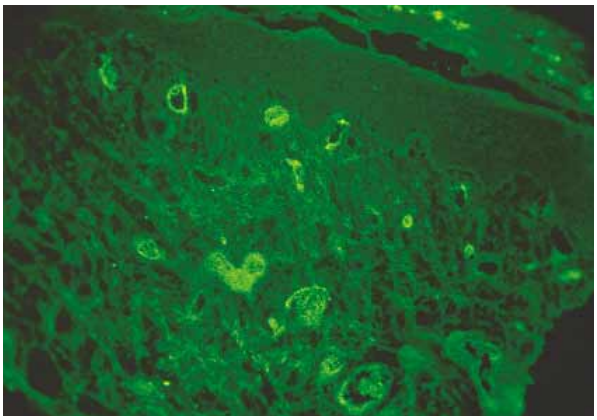

Fig 9. Leukocytoclastic vasculitis. DIF: Note deposition of C3 within superficial dermal blood vessel walls.

HSP is a form of LCV described in children who have systemic involvement (gastrointestinal, renal, and articular) in addition to cutaneous lesions[206] (see Table VI). Unlike adult cases of LCV, the primary immunoglobulin involved in HSP in both the skin and the kidney is IgA.[203,206-209] IgM and IgG are rarely observed.[210] The prevalence rate of positive DIF in HSP is variable and likely reflects variation in the duration of the lesion. Several studies have investigated the frequency of IgA deposits in cutaneous blood vessel walls in both lesional and clinically normal skin.[206,208,209] The frequency of perivascular deposits of IgA in lesional skin ranges from 75% to 100%.[206,208,209] The frequency of IgA deposits in normal skin, however, varies and ranges from zero to 100%.[206,208,209]

Lichen planus

Most patients with LP present with a characteristic clinical eruption and diagnostic histologic findings. DIF is not usually required for the diagnosis. In certain cases in which the clinical and histologic findings are not characteristic, DIF may be helpful. In

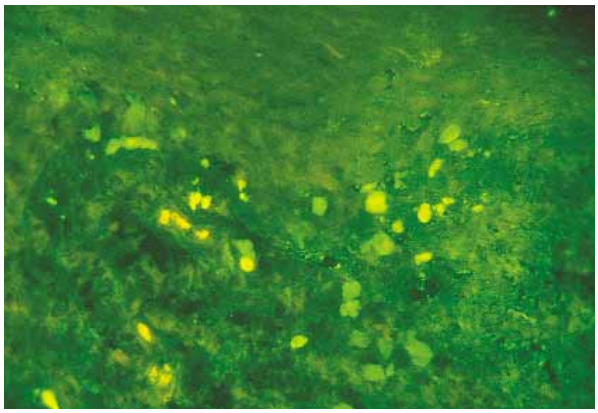

Fig 10. LP. DIF: Note deposition of IgM within scattered cytoid bodies in the papillary dermis.

addition, DIF may be helpful in cases that reveal clinical or histologic findings (or both) intermediate between LP and LE. DIF is helpful in differentiating mucosal LP from other mucosal erosive and bullous diseases such as mucosal pemphigoid.

DIF is positive in the vast majority of patients with LP. Immune deposits are present within cytoid bodies in the superficial dermis, as well as along the DEJ[211] (see Table VI). The most frequently present immune deposits are IgM and fibrinogen.[211,212] Deposition of IgG, IgA, and C3 is less frequently present.[213-215] Deposition at the DEJ is usually granular. Deposition within cytoid bodies is similar to that seen in LE[211] (Fig 10). Cytoid bodies are not characteristic of LP and may be seen in 35% to 50%[211,216] of biopsy specimens from normal skin of persons without LP. They may also be seen in many other conditions including graft-versus-host disease and lichen sclerosus.[211,215,217,218] Some findings that may help favor LP include the tendency for cytoid bodies in LP to cluster in groups, to be present in high number, to be larger, to have higher fluorescence intensity, and to contain multiple immune deposits.[211]

Erythema multiforme

DIF is helpful in differentiating bullous erythema multiforme from other primary autoimmune bullous disorders. DIF may reveal immunoglobulin deposition in superficial vessel walls, DEJ, and cytoid bodies.[211,219,220]

Summary

Immunofluorescence is helpful in the diagnosis of connective tissue diseases, vasculitis, and other cutaneous disorders. False-negative and false-positive results exist. The results of DIF testing are evaluated in the context of clinical and histologic findings.

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Answers to CME examination

Identification No. 801-112

December 2001 issue of the Journal of the American Academy of Dermatology

Questions 1-29, Mutasim DF, Adams BB. J Am Acad Dermatol 2001;45:803-22.

1. c
2. a
3. c
4. d
5. c
6. e
7. d
8. c
9. d
10. a
11. b
12. e
13. a
14. d
15. d
16. d
17. a
18. a
19. c
20. a
21. c
22. d
23. b
24. a
25. b
26. a
27. b
28. c
29. d

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CME examination

Identification No. 801-112

Instructions for Category I CME credit appear in the front advertising section. See last page of Contents for page number.

Questions 1-29, Mutasim DF, Adams BB. J Am Acad Dermatol 2001;45:803-22.

Directions for questions 1-10: Give single best response.

1. In a patient with a bullous eruption, histologic examination should be performed on
   a. the oldest vesicle
   b. perilesional skin
   c. an early vesicle
   d. a ruptured vesicle
   e. erythematous skin adjacent to vesicle
2. The most appropriate biopsy site for direct immunofluorescence (DIF) in the work-up of a bullous disorder is
   a. normal skin adjacent to a lesion
   b. urticarial skin
   c. an old vesicle
   d. an early vesicle
   e. normal skin distant from a lesion
3. IgA pemphigus may be clinically and histologically similar to
   a. paraneoplastic pemphigus
   b. pemphigus vulgaris
   c. pemphigus foliaceus
   d. bullous pemphigoid
   e. bullous systemic lupus erythematosus
4. The most appropriate substrate for indirect immunofluorescence (IIF) in pemphigus vulgaris is
   a. human skin
   b. guinea pig lip
   c. rat bladder
   d. monkey esophagus
   e. guinea pig esophagus
5. Pemphigus vulgaris results from antibodies to
   a. hemidesmosomes
   b. lamina lucida
   c. desmosomes
   d. anchoring fibrils
   e. lamina densa
6. Deposition of IgG, C3, or both at the basement membrane zone is seen in each of the following *except*
   a. bullous pemphigoid
   b. cicatricial pemphigoid
   c. herpes gestationis
   d. bullous systemic lupus erythematosus
   e. pemphigus vulgaris
7. The target antigen in epidermolysis bullosa acquisita is collagen
   a. I
   b. II
   c. IV
   d. VII
   e. XVII
8. What percentage of patients with herpes gestationis have a positive herpes gestationis factor?
   a. 0%
   b. 25%
   c. 50%
   d. 75%
   e. 100%
9. Sodium chloride (1 mol/L) induces a split in human skin at the level of the
   a. granular layer
   b. spinous layer
   c. basal layer
   d. lamina lucida
   e. lamina densa
10. Linear deposition of IgA along the basement membrane zone is seen in
    a. chronic bullous disease of childhood
    b. IgA pemphigus
    c. dermatitis herpetiformis
    d. epidermolysis bullosa acquisita
    e. bullous pemphigoid

Directions for questions 11-15: Match the disease (numbered items) with the immunofluorescence finding (lettered items). Each letter may be used more than once or not at all.

   a. IgG deposition in the intercellular space and basement membrane zone
   b. IgG in the intercellular space
   c. IgA in the intercellular space

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d. Multiple deposits at the basement membrane zone
e. IgA at the basement membrane zone

11. Pemphigus foliaceus
12. Chronic bullous disease of childhood
13. Paraneoplastic pemphigus
14. Epidermolysis bullosa acquisita
15. Bullous systemic lupus erythematosus

Directions for questions 16-19: Give single best response.

16. DIF plays a role in the diagnosis of each of the following *except*
    a. leukocytoclastic vasculitis
    b. discoid lupus erythematosus
    c. mixed connective tissue disease
    d. lichen sclerosus
    e. systemic lupus erythematosus
17. With the use of DIF, which of the following sites is most likely to be positive in systemic lupus erythematosus?
    a. Sun-exposed lesional skin
    b. Sun-exposed nonlesional skin
    c. Sun-protected lesional skin
    d. Sun-protected nonlesional skin
18. The highest frequency of positive DIF in discoid lupus erythematosus is in
    a. sun-exposed oldest lesional skin
    b. sun-exposed earliest lesional skin
    c. non–sun-exposed earliest lesional skin
    d. non–sun-exposed oldest lesional skin
    e. non–sun-exposed nonlesional skin
19. DIF is generally negative in
    a. subacute cutaneous lupus erythematosus
    b. mixed connective tissue disease
    c. systemic sclerosis
    d. lichen planus
    e. Henoch-Schönlein purpura

Directions for questions 20-24: Match each disease (numbered items) with the most characteristically associated immune deposit (lettered items). Each letter may be used once, more than once, or not at all.

a. IgG
b. IgM
c. IgA
d. C3
e. IgE

20. Mixed connective tissue disease
21. Henoch-Schönlein purpura
22. Leukocytoclastic vasculitis
23. Lichen planus
24. Subacute cutaneous lupus erythematosus

Directions for questions 25-29: Match the disease (numbered items) with its most specific location of immunofluorescence (lettered items). Each letter may be used once, more than once, or not at all.

a. Dermoepidermal junction
b. Vascular
c. Epidermal keratinocyte nuclei
d. Cytoid bodies

25. Henoch-Schönlein purpura
26. Systemic lupus erythematosus
27. Pyoderma gangrenosum
28. Mixed connective tissue disease
29. Lichen planus