THE USE OF IMMUNOFLUORESCENT TECHNIQUES IN DERMATOLOGY

P. H. McKee, J. C. Sandford and D. Ann Oakman
Department of Pathology, Royal Victoria Hospital, Belfast
and
The Queen’s University of Belfast

IN 1975 the Department of Pathology established an immunofluorescence laboratory to aid diagnosis in dermatology.

The purpose of this paper is to show (1) the use and limitations of the techniques involved in immunofluorescent procedures, (2) to describe the skin diseases in which immunofluorescence is of diagnostic or therapeutic value and (3) to review our experience over the past two years. There are many skin diseases in which diagnosis can be difficult at a histopathological level, let alone for the clinician. Immunofluorescence is particularly of value in establishing the diagnosis in the members of the bullous skin diseases, e.g. pemphigus, pemphigoid, dermatitis herpetiformis and benign mucous membrane pemphigoid. In classical pemphigus with a representative, early lesion, the typical features usually are present but not infrequently the diagnosis can be exceedingly difficult, particularly with old lesions, and especially if they have become secondarily infected. In such instances immunofluorescent studies can be crucial to establish the correct diagnosis. Bullous pemphigoid, dermatitis herpetiformis and benign mucous membrane pemphigoid have essentially similar microscopic features — they are all typified by the presence of a sub-epidermal vesicle. Although distinguishing features do exist, they are not always present and, thus, immunofluorescence can often resolve the problem. Immunofluorescent studies are essential in patients suffering from discoid and systemic lupus erythematosus. They are occasionally of value in establishing the diagnosis of a vasculitis.

TECHNICAL METHODS

Fluorescence occurs when an object is irradiated with light of a particular (shorter) wavelength and emits light of a longer wavelength. This results in a colour change. A fluorochrome is a substance which possesses the ability to fluoresce. A variety of fluorochromes are used, e.g. fluorescein and rhodamine. Thus, if one irradiates fluorescein with blue light it emits light of an apple-green colour. To perform immunofluorescence an antibody is ‘tagged’ with a fluorochrome. The fluorochrome-labelled antibody may be reactive against immunoglobulins, complement, or other components. On addition of the fluorochrome-labelled antibody to its appropriate antigen, it becomes fixed. This can be detected by observing the colour change when the specimen is viewed under light of the correct wavelength.
There are essentially two types of test used in an immunofluorescence laboratory. One of these is for skin biopsies, the other for examining serum samples. However, before describing these, it seems appropriate to discuss the method of taking biopsies, and their transportation.

Ideally, a technician from the laboratory should be present at the time of taking the biopsy. It is essential that the clinician be aware of the correct site from which to take the biopsy in each of the various diseases commonly studied (Fig. 1).

**FIG. 1**

*Suitable sites for biopsy. Stippled line represents the basement membrane region.*

It is of great importance that biopsies taken from patients with bullous pemphigoid and pemphigus include both a portion of a blister (preferably a whole blister, if this is possible) with adjacent uninvolved skin. This has the advantage that both light microscopy and immunofluorescent studies can be performed on one specimen. In dermatitis herpetiformis the biopsy should be taken from uninvolved skin adjacent to a lesion and not the lesion itself. If routine histopathological examination is also required, then the biopsy should include both blister and normal skin, the normal skin being separated and sent for immunofluorescent examination. Immediately after the specimen has been taken from the patient it should be snap-frozen in an appropriate cooling mixture. A variety of techniques are available, but we find the use of iso-pentane pre-cooled in liquid nitrogen satisfactory. Following this the specimen is transferred to a
cryostat for sectioning. Where laboratory facilities are limited, or when the technician cannot attend, the specimen may be wrapped in aluminium foil or parafilm and frozen in liquid nitrogen. The specimen can then be transported to the laboratory.

Serum samples (without anti-coagulant) can be collected in the usual manner and submitted to the laboratory.

Skin biopsies are most often examined by the direct immunofluorescent technique (Fig. 2), the purpose of this being to demonstrate the presence and location of in vivo bound substances such as immunoglobulins and complement.

**Fig. 2**

![Diagram](image)

**SKIN** + **FLUOROSCEIN-LABELLED ANTIBODY** -> **ANTIGEN-ANTIBODY COMPLEX**

*The direct immunofluorescent test.*

Fluorescein-labelled anti-human immunoglobulin, etc., is added to a suitably prepared tissue section and the specimen is examined under blue light. If the anti-human immunoglobulin has been 'fixed', its presence will be identified by fluorescence.

Serum samples are examined by the indirect immunofluorescent technique (Fig. 3), the purpose of this test being the detection of circulating antibodies.

To perform the test, the patient’s serum is added to a variety of substrates (tissues); if the appropriate antibody is present it will ‘fix’ to its corresponding antigen in the substrate. Fluorescein-labelled anti-human immunoglobulin is then added and the section is examined under blue light.
The indirect immunofluorescent test.

**Diseases Studied**

*(Table 1)*

**Immunofluorescent findings in the more commonly investigated skin diseases.**

| Disease                        | Skin                                      | Serum                                |
|--------------------------------|-------------------------------------------|--------------------------------------|
| PEMPHIGUS (variants)           | Intercellular staining of squamous epithelium | Anti-intercellular substance antibody |
| BULLOUS PEMPHIGOID             | Linear basement membrane staining         | Anti-basement membrane antibody      |
| DERMATITIS HERPETIFORMIS       | IgA in dermal papillae                    | ? anti-reticulin antibody             |
| BENIGN MUCOUS MEMBRANE PEMPHIGOID | Linear basement membrane staining         | Low titre anti-basement membrane antibody |
| SYSTEMIC LUPUS ERYTHEMATOSUS   | Granular staining at basement membrane (lesion and normal skin) | Anti-nuclear factor, etc. |
| DISCOID LUPUS ERYTHEMATOSUS    | Granular staining at basement membrane (lesion only) | May contain anti-nuclear factor or |
| SYMPTOMS                        | FINDINGS                                      | 1999 |
|--------------------------------|-----------------------------------------------|------|
| SYSTEMIC SCLEROSIS             | Negative                                     | Anti-nuclear factor |
| MIXED CONNECTIVE TISSUE DISEASE| IgM, rarely IgG at basement membrane or within blood vessels | Anti-ribonucleoprotein, antibody to 'extractable' nuclear antigen |
| VASCULITIDES                   | May find immunoglobulin and complement        | Negative |

**Figs. 4 to 7**

**Fig. 4 (Top left).** Pemphigus vulgaris. 'Fish-net' staining around prickle cells x 250.

**Fig. 5 (Top right).** Bullous pemphigoid. Linear staining at basement membrane region x 100.

**Fig. 6 (Lower left).** Dermatitis herpetiformis. Positive staining in the dermal papillae x 100.

**Fig. 7 (Lower right).** Systemic lupus erythematosus. Positive granular staining at basement membrane region x 250.
Pemphigus

Pemphigus vulgaris has long been known to be an immunologically mediated disease (Beutner and Jordon, 1964). Immunoglobulins and complement are found in the inter-cellular region of the squamous epithelium (Fig. 4). The much rarer pemphigus erythematosus is characterised by immunoglobulins and complement in a granular distribution at the epidermo-dermal junction in addition to the changes mentioned above. Pemphigus foliaceus shows features similar to pemphigus vulgaris.

Examination of the serum from a patient with pemphigus can virtually always be shown to contain an antibody (usually IgG), which reacts with the intercellular region of squamous epithelium. Circulating antibodies can sometimes be difficult to detect and repeat samples may be necessary. A source of possible confusion may arise with the pemphigus-like antibodies that are known to develop on occasion in people with severe burns (Thivolet and Beyvin, 1968).

The presence and titre of anti-intercellular antibody have been shown to be of prognostic value in patients with pemphigus (Beutner, Chorzelski and Jordon, 1970). An individual change in titre is not sufficient for adjustment of steroid dosage, but an increasing titre is certainly suggestive of impending relapse and, thus, possibly an indication for altering treatment dosages. Repeat tests should be performed perhaps weekly whilst the patient is in relapse, and every three or six months when in remission.

Bullous Pemphigoid

This interesting disease can be a problem both clinically and histologically. It is, therefore, fortunate that pemphigoid is characterised by the presence of a specific (as far as is known) antibody (Fig. 5) active against the basement membrane region of squamous epithelium (Beutner, Jordon and Chorzelski, 1968). Unfortunately, there does not seem to be much correlation between titres of basement membrane antibody and the severity of the disease process.

Benign Mucous Membrane Pemphigoid

Recent studies (Hudson and Black, 1975) have shown that this disease has features similar to bullous pemphigoid. IgG and complement (more rarely IgA) are found at the basement membrane region of the biopsy. Although usually in low titre, circulating antibodies have been detected against the basement membrane zone.

Dermatitis Herpetiformis

Immunofluorescent examination of the biopsy typically shows the presence of granular IgA in the dermal papillae (Fig. 6). Complement may also be demonstrated in a similar location. Rarely they may be found at the basement membrane region, and are occasionally linear. It is to be emphasised that the IgA deposits may be patchy in distribution and, therefore, repeat biopsies occasionally are necessary to establish the diagnosis.
Examination of the serum in patients with dermatitis herpetiformis may reveal a variety of antibodies. These include anti-reticulin antibody, anti-gastric parietal cell antibody, anti-nuclear factor and thyroid microsomal antibody. Of these, anti-reticulin antibody is of most significance.

**Systemic and Discoid Lupus Erythematosus**

Immunological investigations are obviously of great importance in both the above conditions.

Examination of a skin biopsy can be of diagnostic value in both discoid and systemic lupus. It shows granular deposits of immunoglobulins (IgG and/or IgM) and complement at the epidermodermal junction (Fig. 7). This is of particular importance in the diagnosis of systemic lupus, in that these deposits are found in clinically normal (sun-exposed) skin as well as in the lesion itself. Cases of so-called transitional lupus may show deposits in normal skin. It is worthwhile remembering that false negative results can occur in patients on steroid therapy. Some workers (Dantzig, Mauro, Rayhanzadeh and Rudofsky, 1975) have shown that there is a high correlation between positive cutaneous immunofluorescence and the more severe forms of lupus nephritis.

**Scleroderma**

Examination of skin biopsies from patients with scleroderma and morphea are disappointingly negative. Examination of the serum in cases of scleroderma frequently reveals an anti-nuclear antibody, often of the speckled or nucleolar type. In morphea the serum is usually free from such antibodies.

However, in the less common mixed connective tissue disease (mesenchymal, inflammatory scleroderma) recent studies (Winkelmann, Carapeto and Jordon, 1977) have shown that immunofluorescence may have some part to play in diagnosis. Immunoglobulins (IgM, rarely IgG) and complement were described at the basement membrane or within blood vessels. Serological investigation is said to reveal the presence of antibodies to ribonucleoprotein and 'extractable' nuclear antigen.

**Vasculitides**

A variety of primary vascular inflammatory diseases exist and many of these involve cutaneous vessels, e.g. allergic vasculitis, polyarteritis nodosa. If immunofluorescent tests are to be performed, the biopsy should be taken from an early lesion. Direct immunofluorescence may reveal immunoglobulin and complement in and around blood vessels. However, interpretation of these biopsies is often exceedingly difficult.

**Results**

During the period May 1975 to May 1977 specimens were received from 170 patients. These included 337 skin biopsies and 379 serum samples. The submitted material included a wide range of conditions (Table 2).
Dermatitis herpetiformis was the most commonly encountered disease, material being examined from a total of 34 patients (Tables 3 and 4). Skin biopsies were shown to contain IgA with or without complement in the dermal papillae, or less commonly along the basement membrane in 23 (67%) patients. Examination

(DIRECT IMMUNOFLUORESCENCE OF SKIN BIOPSY)

- IgA in DP*
- IgA + C** in DP
- Granular IgA at BM†
- Linear IgA at BM
- Negative
- Total

*DP — dermal papillae; **C — complement
†BM — basement membrane

(DIRECT IMMUNOFLUORESCENT EXAMINATION)

- Anti-reticulin antibody
- Anti-gastric parietal cell antibody
- Anti-nuclear factor
- Anti-intercellular substance antibody
- No antibody
- Total

(DIRECT IMMUNOFLUORESCENT EXAMINATION)

- Anti-reticulin antibody
- Anti-gastric parietal cell antibody
- Anti-nuclear factor
- Anti-intercellular substance antibody
- No antibody
- Total

202
of the serum disclosed a variety of antibodies, including anti-reticulin and anti-gastric parietal cell antibodies.

Specimens were received from 15 patients with bullous pemphigoid (Table 5). In all cases in which immunofluorescent studies were performed on skin biopsies, immunoglobulins and/or complement were found deposited in a linear fashion along the basement membrane. The serum in all cases contained an anti-basement membrane antibody of the IgG sub-class.

Oral mucosa and serum were examined from two patients with cicatricial pemphigoid. In each case IgG and complement were demonstrated as a linear deposition along the basement membrane of the mucosa. Antibodies were not detected in their sera.

Pemphigus is an exceedingly rare disease and we were fortunate in being able to study seven cases. Of these, six patients had skin biopsies performed (Table 6).

| No. of cases | Direct immunofluorescence of skin biopsy |
|--------------|----------------------------------------|
| 3            | IgG in ICR††                          |
| 1            | IgA in ICR                             |
| 1            | IgG + C in ICR                         |
| 1            | IgG + IgM in ICR                       |
| 1            | Not examined                           |
| 7            | Total                                  |

†† ICR = intercellular region

(Table 6). Examination of the serum in all patients revealed an antibody (IgG in six cases, IgA in one) of the pemphigus type.

We investigated specimens from eleven patients with lupus erythematosus (Table 7). Of these, nine were of the discoid type and two of the systemic type. In all cases immunoglobulins and/or complement were detected as a granular
(Table 7)

**Lupus Erythematosus – Skin Biopsies Results**

| No. of cases | Direct immunofluorescence of skin biopsy |
|--------------|-----------------------------------------|
| 2            | Granular IgG \( \div \) C at BM         |
| 1            | Granular IgG + IgA \( \div \) C at BM    |
| 1            | Granular C at BM                         |
| 2            | Granular IgG + IgM \( \div \) C at BM    |
| 1            | Granular IgG + IgM at BM                 |
| 3            | Granular IgM at BM                       |
| 1            | Granular IgM \( \div \) C at BM          |
| 2            | Negative (uninvolved skin)               |
| Total        |                                         |

deposit at the basement of the membrane region of the skin. Skin biopsies from unaffected skin in the two patients suspected to be suffering from systemic lupus erythematosus were negative.

Skin biopsies from ten patients suspected of suffering from a vasculitis were examined (Table 8). In eight of these immunoglobulins and/or complement were detected outlining the dermal blood vessels.

(Table 8)

**Vasculitis – Skin Biopsies Results**

| No. of cases | Direct immunofluorescence of skin biopsy |
|--------------|-----------------------------------------|
| 1            | IgA \( \div \) C outlining BV§         |
| 1            | IgA outlining BV                        |
| 3            | C outlining BV                          |
| 1            | IgM \( \div \) C outlining BV           |
| 1            | IgG, IgM \( \div \) C outlining BV      |
| 1            | IgG \( \div \) C outlining BV           |
| 2            | Negative                                |
| Total        |                                         |

§BV – blood vessels

Two patients suffered from herpes gestationis. Skin biopsies from both were examined by the direct immunofluorescent technique. In both, IgG and complement were detected as a granular, almost linear deposition along the basement membrane region. Examination of the sera failed to reveal any antibodies.

Immunofluorescent examination of the skin from two patients with lichen planus was performed. Characteristic fluorescent bodies were identified in the papillary dermis on treating the sections with fluorescein-labelled anti-IgG, anti-IgM and anti-complement.

Significant fluorescent findings were not found in any of the other cases.
CONCLUSIONS

Our experience over a two year period has confirmed the value of immunofluorescence in diagnostic dermatology. Thus, immunofluorescence may establish a diagnosis in cases where there is a doubt, and can on occasion be of value in assessing treatment regimes. There are, however, several points which must be emphasised. Firstly, the biopsy must be taken from the correct site (Fig 1), otherwise the possibility of false negative results arise. Secondly, an adequate clinical history with information of current treatment is essential, since an increasing number of drugs are being associated with a variety of auto-antibodies, e.g. pemphigus-type antibodies in patients taking rifampicin (Gange et al., 1976). Thirdly, in patients suspected to be suffering from dermatitis herpetiformis there is, unfortunately, a known (albeit low) tendency for a false negative result. The laboratory, therefore, must examine diligently a large number of sections in order to detect the IgA deposits. On occasion a repeat biopsy will be necessary.

To date we have not, to our knowledge, experienced either false positive or false negative results in either pemphigus or pemphigoid cases.

REFERENCES

DANTZIG, P. I., MAURO, J., RAYHANZADEH, S., and RODCFSKY, U. H. (1975). British Journal of Dermatology, 93, 531.

BEUTNER, E. H., JORDAN, R. E., and CHORZELSKI, T. P. (1968). Journal of Investigative Dermatology, 51, 63.

BEUTNER, E. H., CHORZELSKI, T. P. and JORDAN, R. E. (1970). Autosensitisation in Pemphigus and Bullous Pemphigoid. Thomas Springfield, 111.

BEUTNER, E. H. and JORDAN, R. E. (1964). Proceedings Society Experimental Biology and Medicine, 117, 505.

GANGE, R. W., RHODES, E. L., EDWARDS, C. O. and POWELL., M. E. A. (1976). British Journal of Dermatology, 95, 445.

HUDSON, M. and BLACK, M. M. (1975). Proceedings Royal Society Medicine, 68, 33.

THIOVLETT, J. and BEYVIN, A. J. (1968). Experimentia, 24, 945.

WINKKELMANN, R. K., CARAPETO, F. J. and JORDAN, R. E. (1977). British Journal of Dermatology, 96, 231.