attempt to compensate for the variations in exposure to the ubiquitous allergens dealt with and for possible ethnic differences in the population.

Animal experiments have given overwhelming evidence of a linkage of genes controlling immune responses to the animal counterpart of the HLA locus in man. This linkage may not exist for all immunologic functions and not in all species. For instance, the genes controlling the level of IgE production in man are not linked to the HLA locus, in contrast to the conditions in mice (3). Our results are consistent with this latter study, as HLA antigens of at least the A and B series do not reveal any statistically significant associations with the various contact allergens studied in our population. There are still some indications, however, that HLA B7 may be associated with contact allergy. The HLA B7 frequency was somewhat higher in the patient group than in the controls and there was a significant difference in HLA B7 frequency between atopic and non-atopic patients. As an isolated phenomenon the tendency towards an overrepresentation of HLA B7 among our patients is not strong enough to indicate a real biological significance. However, HLA B7 has been reported to have an allergen-specific association with ragweed allergen E (8) and ragweed hayfever (6). Taken together, this information indicates that the possibility of a relationship between HLA factors and certain allergens should not be completely rejected, even if the majority of the associations found in our previous study could not be verified. This finding is in agreement with those of Roupe et al. (10) who did not find any association between HLA antigens of the A, B and C series and sensitivity to chromium, and of Silvennoinen-Kassinen et al. (12) who also failed to find any association between contact sensitivity to nickel and HLA antigens of the A, B, C and D series.

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REFERENCES

1. Beckman, L., Cedergren, B., Collinder, E. & Rasmusson, M.: Population studies in northern Sweden. III. Variations of ABO and Rh blood group gene frequencies in time and space. Hereditas *72:* 183, 1972.
2. Beckman, L., Beckman, G., Cedergren, B., Göransson, K. & Lidén, S.: Blood groups, serum groups and red cell enzyme types in allergic contact dermatitis. In manuscript.
3. Blumenthal, M. N., Amos, D. B., Noreen, H., Mendell, N. R. & Yunis, E. J.: Genetic mapping of Ir locus in man: Linkage to second locus of HL-A. Science *184:* 1301, 1974.
4. Bodmer, W. F.: Genetic factors in Hodgkin's disease: Association with a disease-susceptibility locus (DSA) in the HL-A region. Natl Cancer Inst Monogr *36:* 127, 1973.
5. Larsson, P. Å. & Lidén, S.: Prevalence of skin diseases among adolescents 12–16 years of age. Acta Dermatovener (Stockholm) *60:* 415, 1980).
6. Levine, B. B., Stember, R. H. & Fotino, M.: Ragweed hay fever: Genetic control and linkage to HL-A haplotypes. Science *178:* 1201, 1972.
7. Lidén, S., Beckman, L., Cedergren, B., Göransson, K. & Nyquist, H.: HLA antigens in allergic contact dermatitis. Acta Dermatovener (Stockholm), Suppl. *79:* 53, 1978.
8. Marsh, D. G., Bias, W. B. & Hsu, S. H.: Association of the HL-A7 cross-reacting group with a specific reaginic antibody response in allergic man. Science *179:* 691, 1973.
9. Pirilä, V.: Chamber test versus patch test for epicutaneous testing. Contact Dermatitis *1:* 48, 1975.
10. Roupe, G., Rydberg, L. & Swanbeck, G.: HLA-antigens and contact hypersensitivity. J Invest Dermatol *72:* 131, 1979.
11. Schultz, L. D. & Bailey, D. W.: Genetic control of contact sensitivity in mice: Effect of H-2 and non H-2 loci. Immunogenetics *1:* 570, 1975.
12. Silvennoinen-Kassinen, S., Ilonen, J., Tiilikainen, A. & Karvonen, J.: No significant association between HLA and nickel contact sensitivity. Tissue Antigens *14:* 459, 1979.

An Appraisal of Routine Direct Immunofluorescence in Vulvar Disorders

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Abstract. Sixty-four biopsies were obtained from patients with a variety of vulvar disorders for direct immuno-

Table I.

BM, Basement Membrane; BV, Blood Vessel; DEJ, Dermal–epidermal junction; UD, Upper dermal staining; ICS, Intercellular space

Disorder	No. of biopsies	Immunoglobulin noted on DIF				
		IgG	IgM	IgA	C3	Fibrin
Lichen sclerosus	24	–	BV(1)[a]	–	BV(1)	UD(12) DEJ(7)
Carcinoma in situ	6	–	BV(1)	–	BV(1)	BV(1)
Hyperplastic dystrophy	16	–	BV(1)	–	BV(1) BM(2)	BV(2)
Condyloma accuminatum	9	–	–	–	–	BV(1) BM(1)
Hailey–Hailey disease	1	–	–	–	–	–
Erythema multiforme	1	–	–	–	–	BM(1)
Fox–Fordyce disease	1	–	–	BV(1)	BV(1)	BV(1)
Pemphigus vulgaris	1	ICS(1)	–	–	ICS(1)	–
Pemphigoid	2	BM(1)	–	BM(1)	BM(2)	–
Normal vulva	3	–	–	–	–	–
Total	64					

[a] Number of positive cases.

fluorescence study. Twelve of 24 vulvar biopsies from patients with lichen sclerosus demonstrated deposition of fibrin in the upper dermis, while 7 vulvar biopsies showed fibrin deposition at the dermal-epidermal junction. Blood vessel immunofluorescence was observed in several patients but was not a disease-specific finding. Patients with pemphigus and pemphigoid yielded characteristic reactions and served as positive controls. Direct immunofluorescence may be of value in assessing patiens with vulvar disorders, and in particular lichen sclerosus.

Direct immunofluorescence (DIF) has found application as a diagnostic technique for a variety of dermatologic disorders. Its usefulness in determining and differentiating bullous pemphigoid from pemphigus is now firmly established. It is of central importance in the diagnosis of cicatricial pemphigoid, herpes gestationis and dermatitis herpetiformis. Direct immunofluorescence is of prognostic and diagnostic value in systemic lupus erythematosus (6).

Diseases of the human vulva are essentially dermatologic problems but for most patients who suffer from these conditions, the primary physician is often a gynecologist. Gynecologists are learning to recognize and differentiate vulvar disorders with increasing accuracy (2). Nevertheless, many conditions are clinically similar or clinically subtle and the diagnosis is often confused.

The present study was undertaken in order to evaluate the use of DIF in the diagnosis of various vulvar disorders. While the histologic hallmarks of lichen sclerosus are well defined (5) and familiar to pathologists, the etiology of this disease remains obscure. The demonstration of immunoglobulin and/or complement deposition in affected tissue may shed light on the obscure origins of this disease. Hyperplastic vulvar dystrophy is frequently assumed to be the result of external (contact) irritants. Others have noted immunoglobulins on DIF study of contact dermatitis (1). Therefore, patients with hyperplastic dystrophy were included in order to assess this correlation. Finally, the histologic findings characteristic of Fox-Fordyce disease are frequently elusive and the diagnosis rests largely on history and clinical presentation. Any technique that would allow histologic confirmation of this disease without the time-consuming process of preparing and examining serial sections would be of distinct advantage.

MATERIALS AND METHODS

Patients reporting to the Vulvar Clinic of the Milwaukee County Medical Complex were examined and evaluated according to a routine protocol (3) usually involving skin biopsy. Vulvar biopsies were taken from affected skin sites using a 4 mm Keyes punch after the area had been sufficiently infiltrated with 1% lidocaine solution. Double biopsies, adjacent to one another, were taken on all study patients.

One specimen was fixed in 10% formalin and submitted for routine histologic examination. The adjacent specimen was snap frozen in liquid nitrogen and subsequently stored at −70°C until sectioned on a cryostat at −30°C and mounted unfixed on glass slides. These study sections were then stained as described by Jordon (6) with fluorescein-conjugated antisera to human IgG, IgM, IgA, IgD,

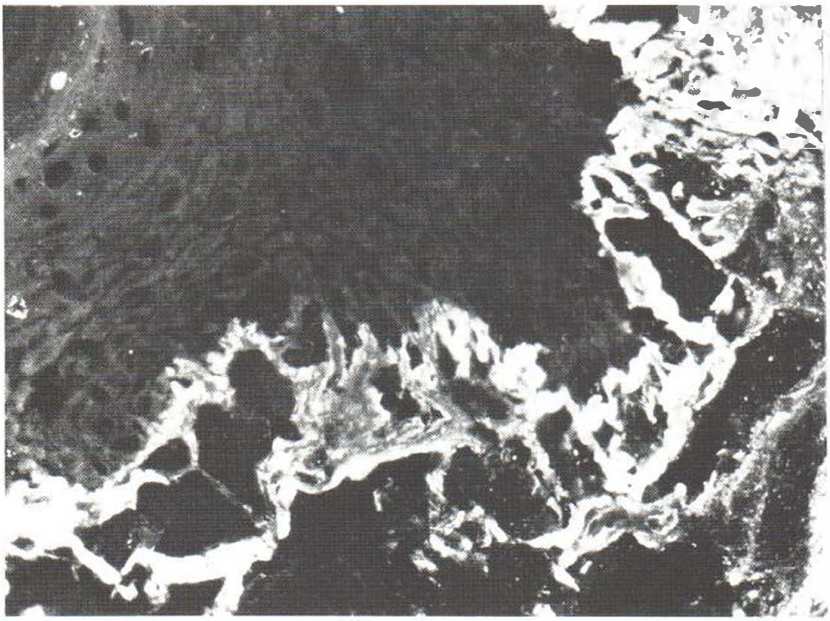

Fig. 1. Upper dermal fibrin deposition in vulvar lichen sclerosus. (×175).

IgE, C3 and fibrin. The sections were read with a Leitz microscope using exciter filters BG12 and UG-1; barrier filter was UV 430. Clinical examination and H & E stained biopsy sections were used to establish the diagnosis.

RESULTS

A total of 64 biopsies were examined by DIF, as shown in Table I. No positive fluorescence was noted in any specimen with IgD or IgE antisera. Positive results were noted in blood vessels, the dermal–epidermal junction (DEJ), basement membrane zone (BMZ) and upper dermis in a variety of vulvar disorders using antisera to IgG, IgM, IgA, C3 and fibrin.

Of 24 patients with clinical and histologic evidence of lichen sclerosus, 12 (50%) showed immunofluorescent fibrin deposition in the upper dermis (Figs. 1 and 2). Seven specimens (29%) also

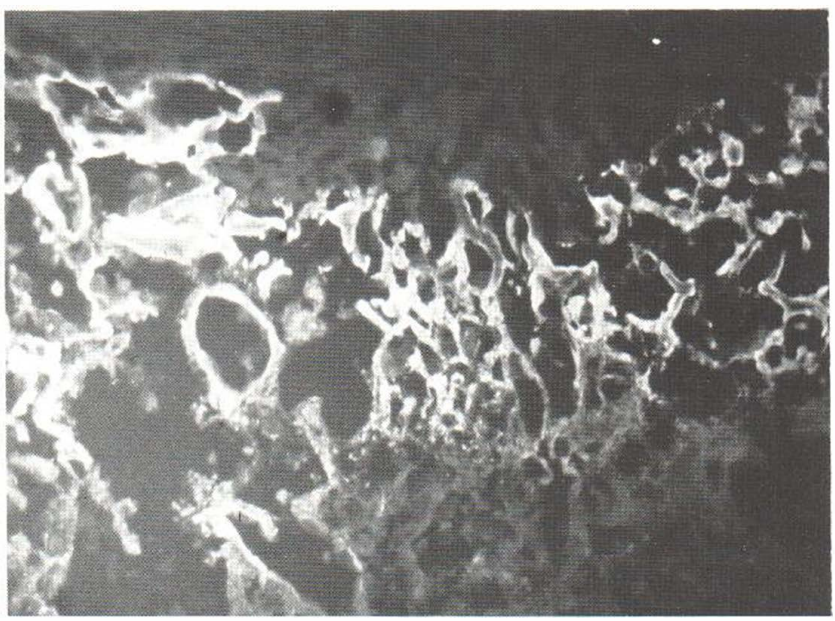

Fig. 2. Upper dermal fibrin deposition in vulvar lichen sclerosus. (×175).

Fig. 3. IgA basement membrane fluorescence in vulvar cicatricial pemphigoid. (×350).

showed fibrin deposition at the DEJ beneath the BMZ. The BMZ is a distinct area between the plasma membranes of the basal cells and the dermis. The DEJ is the area below the BMZ (basal lamina) made up of anchoring fibrils and upper dermal elements. In addition, one individual showed a positive reaction to both IgM and C3 in the dermal vessels.

Of 6 cases submitted, one case of carcinoma in situ showed positive dermal blood vessel IF. The single patient with Fox-Fordyce disease showed positive DIF in the dermal vessels with IgA, C3 and fibrin.

Similarly, 4 cases (25%) of hyperplastic dystrophy from a sample of 16 cases showed evidence of positive DIF. Positive reactions were noted in the dermal vessels with anti-IgM, C3 and fibrin, and the BMZ was positive with fibrin as well in two biopsies.

Patients with pemphigus and pemphigoid gave characteristic reactions and in that way acted as positive controls for the technique. Fig. 3 depicts a vulvar biopsy of a patient with cicatricial pemphigoid. Negative controls were provided by 3 patients with normal vulvar skin whose specimens failed to react to DIF.

DISCUSSION

The dermatologic diseases which have been found to exhibit characteristic patterns of DIF include pemphigus, bullous pemphigoid, lupus erythematosus, herpes gestationis, dermatitis herpetiformis and cutaneous vasculitis. In addition, pemphigus and pemphigoid regularly have oral and ocular DIF which is positive (6).

Fibrin-related DIF was present in a wide variety of vulvar disorders, but usually in single cases with the noted exception of lichen sclerosus et atrophicus, where it was identified in 18 of the subjects (75%). Twelve of these biopsies were heavily stained in the upper dermis with fibrin. Although this occurs on an occasional DIF biopsy, the regularity of its appearance in lichen sclerosus was unique. This fluorescence may be demonstrating an immunological product seen in routine biopsies as a diffuse eosinophilic deposition below the epidermis. Such a possibility would be consistent with the findings of Harrington (4) who noted a higher incidence of tissue antibodies and auto-immune diseases in patients with lichen sclerosus than in a control population. Six of the seven DEJ positive biopsies did not reveal diffuse upper dermal fluorescence and may have been a limited form of the diffuse upper dermal pattern.

The presence of IgA, C3 and fibrin DIF in the dermal vessels of a biopsy representative of Fox-Fordyce disease is interesting. If this finding can be confirmed in other cases, it may represent a significant adjunct in the histological diagnosis of this perplexing entity.

This study demonstrated also that in bullous dis-

eases of the vulva, immunofluorescence can be of assistance in diagnosis. The examples of pemphigus vulgaris and bullous pemphigoid all gave their characteristic DIF findings (6).

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REFERENCES

1. Cormane, R. H., Husz, S. & Hammerlinck, F.: Immunoglobulin and complement bearing lymphocytes in allergic contact dermatitis and atopic dermatitis (eczema). Br J Dermatol *90:* 597, 1974.
2. Friedrich, E. G.: Vulvar Disease. W. B. Saunders Co., Philadephia, Pa 1976.
3. Friedrich, E. G., Burch, K. & Bahr, J. P.: The vulvar clinic: An eight-year appraisal. Am J Obstet Gynecol *135:* 1036, 1979.
4. Harrington, C. I.: An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus et atrophicus. Br J Dermatol *101* (Suppl. 17): 12, 1979.
5. International Society for the Study of Vulvar Disease: New Nomenclature for Vulvar Disease. J Cutan Pathol *3:* 159, 1976.
6. Jordon, R. E.: Immunohistopathology of the Skin. *In* Manual of Clinical Immunology. American Society for Microbiology, Washington, D.C., 1976. 932 pp.

Focal Contact Sensitivity to Nitrogen Mustard in Lesions of Cutaneous T-Cell Lymphoma (*Mycosis Fungoides*)

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Abstract. A report is presented on a patient with mycosis fungoides who developed a remarkable focal contact sensitivity to topically applied nitrogen mustard in aqueous solution (1/5000). Although the nitrogen mustard was applied over the entire skin surface, repeated challenge demonstrated that the eczematous contact dermatitis reaction was limited precisely to the areas of clinically identifiable mycosis fungoides. The surrounding normal skin showed no response. The basis for the surprising focal induction and localization of contact dermatitis is not known but might refleet the presence of a clone of malignant T-lymphocytes which became specifically sensitized to the nitrogen mustard.

Key words: Mechlorethamine; Nitrogen mustard; Contact dermatitis; T-cells; Lymphoma; Mycosis fungoides

Application of a powerful contact allergen to the entire skin surface certainly offers a unique opportunity to explore further the nature of contact dermatitis in man. Such an opportunity is provided the physician who uses topical chemotherapy in the treatment of mycosis fungoides. The entire skin is painted daily with a known sensitizing antigen, nitrogen mustard (mechlorethamine HCl). Once sensitization has occurred the response to any further contact with nitrogen mustard is an eczematous contact dermatitis similar to that seen in poison ivy sensitized or DNCB sensitized patients. In keeping with the basic immunologic tenets of allergic contact dermatitis, the entire skin is sensitized.

The present report describes a patient in whom such an allergic contact dermatitis was induced, but in whom the sensitivity was sharply limited to the lesions of his mycosis fungoides. On painting his entire skin with nitrogen mustard, only his plaques of mycosis fungoides developed eczematous contact dermatitis. The uninvolved skin showed no response whatsoever. This finding merits study since it could enlarge our understanding of the immunology of contact dermatitis.

CASE REPORT

This 59-year-old male first noted pruritic inflammatory plaques appearing over his trunk, arms and legs in 1973. Two biopsies in November 1974 failed to reveal changes other than those of dermatitis. The condition persisted and in May 1976 two additional biopsies showed the histologic changes of mycosis fungoides. Steroid creams were applied, affording some relief.

He was hospitalized in June 1976 for staging of his disease. A skin biopsy confirmed the diagnosis of mycosis fungoides. A blood count, urinalysis, automated 12 unit blood chemistry profile, and bone marrow were all normal. Upper and lower gastrointestinal films, as well as a urogram, lymphangiogram and liver–spleen scan were normal. Patch tests to 30 common contact allergens as well as intradermal tests to Candida, Staphylococcus and trichophytin antigens were negative.

A course of ten treatments with topical nitrogen mustard (mechlorethamine HCl 1/5 000 aqueous) was applied to the entire skin surface. This was effective and tolerated