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Immunohistochemical Reactivity of Monoclonal Antibodies Generated after Immunization of Mice with Cells from a Psoriatic Lesion

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Monoclonal antibodies were produced by immunizing mice with dispersed epidermal cells from a psoriatic plaque. We obtained 128 growing hybridoma clones. The immunohistochemical reactivity of the antibodies produced by these hybridomas was primarily screened on sections of skin biopsies from psoriatic plaques. Supernatants with positive staining were then tested on other dermatoses and human organs. Six supernatants stained infiltrating dermal cells and the whole epidermis in psoriatic lesions, but showed no reaction with sections from normal skin, ichthyosis, or acutely inflamed skin. With these antibodies, positive staining was also seen in some areas of the normal-appearing skin of patients with increasingly active psoriasis as well as after treatment in recently clinically healed lesions. A different and more heterogenous staining of epidermis with no or fewer infiltrating cells was seen in longstanding inflammatory disorders such as chronic eczema, neurodermatitis, lichen planus, discoid lupus erythematosus and mycosis fungoides. Scattered staining with these antibodies was in addition seen on infiltrating macrophage-like cells present also in other organs than skin and in some of the organs also other types of cells were stained, such as neck mucosal cells of the stomach, parts of duodenal mucosal cells, certain secretory cells in parotid gland, lung bronchiole and Hassall's bodies on the thymus. The cross-reactivity of antibodies to psoriatic lesions with other organs is of interest, since psoriasis might involve various tissues. Our findings support the idea that there exist unique cell surface antigens in psoriatic skin which might help elicit an immune reaction and/or be targets for such local immune reactions.

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Psoriatic lesions show a typical clinical picture which suggests that they may have a specific structural background. If there exist unique cell surface structures within the psoriatic lesions they may either be the target antigens for an immunological reaction and/or the result of such a reaction. It would therefore be of interest to know if such structures can also occur in normal-appearing skin of patients with psoriasis. In both cases such a knowledge could be of importance for our understanding of the pathophysiology of psoriasis and for the possibility of developing new diagnostic tools. By producing monoclonal antibodies to a suspension of epidermal cells from a psoriatic lesion we have searched for such unique cell surface molecules which are not found in epidermis from healthy subjects. We describe here the production of such antibodies and their immunohistochemical reactivity in skin biopsies from patients with psoriasis and other skin disorders as well as their reactivity with different other human organs.

MATERIAL AND METHODS

Donor patient

Skin was taken from a 25-year-old woman with psoriasis for 5 years, who was otherwise in good health. She had not received any drugs or exposure to UV-irradiation for 7 months. Her psoriasis was confined mainly to the scalp, but during the last 4 months, some typical plaque lesions had also grown on the trunk, elbows and knees. A 5–6 cm large psoriatic plaque on the thorax was washed with alcohol and anesthetized with lidocaine. Superficial biopsy samples were shaved off with a razor blade and immediately washed in sterile phosphate-buffered saline (PBS).

Cells used for immunization

After blotting on dry filter paper the specimens were laid with the epidermal side up in a Petri dish and soaked with 0.5% trypsin (type III Sigma Chemical Co, St Louis, Mo, USA) in PBS for 30 min at 37°C. The dermis was removed with fine forceps. The exposed epidermis was covered with 0.25% deoxyribonuclease I (Sigma) in PBS for 3–4 min at 37°C and was scrubbed with forceps. RPMI 1640 (Flow Labs, Irvine, Scotland), supplemented with 20% of patient's own serum, 20 mM HEPES, 2 mM L-glutamine, penicillin, streptomycin

Table I. *Reactivity of hybridoma to normal skin, psoriasis (pso) and other inflammatory skin disorders (see main text)*

Hybridoma	Normal-appearing skin: Healthy subjects and patients with stable psoriasis	Normal-appearing skin: Pat. with psoriasis in acute phase	Psoriatic plaque Donor + 6 patients	Different inflammatory skin disorders (see page 3)
C5	–	Areas as in pso. plaques	Epidermal + dermal cells	Heterogeneous in epidermis, no or a few dermal cells
D1	–	Ditto	Ditto	Ditto
D4	–	Ditto	Ditto	Ditto
F4	–	Ditto	Ditto	Ditto
F12	–	Ditto	Ditto	Ditto
B6	–	Ditto	St corneum + cells in upper dermis	St corneum
E4	Epidermal cell membranes	As normal skin	As normal skin	As normal skin
G5	Epidermal cell membranes	Ditto	Ditto	Ditto
A7	Elastic fibres and basement membrane	Ditto	Ditto	Ditto
D5	Ditto	Ditto	Ditto	Ditto
H9	Ditto	Ditto	Ditto	Ditto
C1	Epidermal and dermal cells	Ditto	Ditto	Ditto
F10	Donor basal cells	–	Donor basal cells	–

and amphotericin was then added. The epidermal cell suspension was repeatedly collected and spun down in the medium for 10 min at 500 *g*. Cornified flakes were left in the Petri dish. Following a triple wash in the medium with 10% autologous serum, the cells were filtered through sterile gauze to remove non-separated cells. The cells were counted and viability estimated by trypan blue exclusion was found to be 95%.

Immunization and production of hybridomas

Five hours after the biopsies were taken, 10×10^6 dispersed epidermal cells were washed in PBS before being mixed with an equal volume of Freund's complete adjuvant (Difco, Detroit, Mich., USA). Fifty µl of the emulsion (2.5×10^5 cells) was injected subcutaneously into each of the hind foot pads of 4 10-week-old male DBA/1 mice (1). The popliteal and inguinal lymph nodes were removed 9 days after immunization, and 90×10^6 dispersed lymph node cells were fused with 10×10^6 mouse myeloma cells (P3-X63-Ag8-653) using polyethylene glycol (PEG). After fusion, the cells were seeded on microtitre plates (Nunc, Roskilde, Denmark). Hypoxanthine-aminopterin-thymidine (HAT) was added the following day. Growing hybridomas were screened microscopically and the supernatants were removed and used for immunohistochemical screening.

Isotypes of the antibodies were determined using an ELISA with peroxidase-conjugated isotyped-specific goat antibodies (Nordic Laboratory, Tilburg, Holland).

Peroxidase–antiperoxidase (PAP) staining

Antigen expression was first evaluated on acetone-fixed 6 µm-thick frozen sections of diseased donor skin. Using the mouse PAP technique (2) positive supernatants were then tested for reactivity with sections of normal-appearing donor skin, normal skin from healthy volunteers, skin from various dermatoses and a number of other normal tissues as well as tumours (Tables I, II). Smears of injected epidermal cells used for immunization were also stained with this technique (Table III). Primary antibodies were either hybridoma supernatants (diluted 1:2) or commercially available monoclonal antibodies (Table III). Monoclonal anti-HLA-DR or anti-Leu 6 antibodies were used as positive controls. Omission of the primary antibodies served as negative control.

RESULTS

Screening of antibodies

The immunization with dispersed epidermal cells and subsequent fusion using regional lymph node cells resulted in 128 growing hydridoma clones. Six supernatants (Table I) reacted with epidermal and dermal structures in sections from the donor lesions, but did not react with her normal-appearing skin or normal skin from healthy volunteers. These supernatants also

Table II. *Reactivity of other tissues to hybridomas C5, D4 and F12*

MLC = Macrophage-like cells

Tissue	Positive cells
Esophagus[a]	Squamous epithelial cells
Stomach	Neck mucosal cells
Duodenum	Mucosal cells
Appendix	None
Colon	Scattered MLC in mucosa
Heart	A few MLC in stroma
Lung	Few scattered cells and terminal bronchi
Kidney	MLC mainly in glomeruli
Liver	A few scattered MLC
Cerebral cortex	Very few MLC
Cerebellum	Very few MLC
Parotid gland	Acini membranes + areas with MLC
Mammary glands	Very few MLC
Pancreas	Several MLC in stroma
Thyroid	Few MLC around follicles
Parathyroid	None
Thymus	Hassall's bodies
Ovary	None
Adrenal gland	Few MLC
Lymph node	None
Placenta	Cytotrophoblasts
Pheochromocytoma	Few MLC
Insulinoma	Few MLC

[a] Monkey; all other tissues are human.

reacted with the epidermis and certain dermal cells from plaque lesions of other patients with psoriasis (Fig. 1). Supernatant D4 showed a rather different pattern of antibody distribution than the other five supernatants (Fig. 2). Clinically, apparently completely healed psoriatic lesions treated with potent topical corticosteroids under occlusion or with anthralin for 3 weeks displayed a considerable reactivity in both epidermis and dermis. Also in normal-appearing skin from patients with psoriasis in an active phase, small areas of reactivity could be seen in both dermis and epidermis with these six hybridomas (Fig. 3). One supernatant (C1) reacted with epidermis and dermis-infiltrating cells of both normal and diseased skin (C1) and another (F10) reacted with the donor's basal cells both in lesion and in normal-appearing skin, but not in other biopsies. Two supernatants (E4, G5) stained cell envelopes or intercellular areas of epidermal cells in all biopsies (Fig. 4). Three supernatants (A7, D5, H9) stained the basement membrane and elastic tissue, especially in the upper dermis (Fig. 5).

Biopsies from patients with dermatitis herpetiformis, scleroderma, ichthyosis, urticaria and strongly positive allergic patch tests 48 h after application, 20 h UV-B erythema, and tape-stripped normal skin showed no staining of epidermis with the six supernatants positive to psoriatic lesions, though occasionally a few infiltrating cells were seen in the dermis. However, in lesional skin from patients with longstanding, infiltrated eczema, neurodermatitis, lichen planus, mycosis fungoides parapsoriasis, erythema multiforme, discoid lupus erythematosus (LE), pemphigoid, and graft versus host reaction, a variable staining of epidermis and certain dermal cells was obtained with these six antibodies (Table I). However, these lesions looked different from the psoriatic lesions, as no (or only a few) positive dermal cells were stained with the six antibodies. The reactivity of the epidermis was homogenous in active psoriatic plaque lesions, whereas in eczema it was most marked in the upper stratum spinosum (Fig. 6) and in discoid LE in the basal layer, especially in hair follicles (Fig. 7).

The reactivity of various organs other than skin was tested with the supernatants from hybridomas C5, D4 and F12 (Table II). In some organs certain specific cell epithelial structures were stained (stomach, duodenum, lung, pancreas, placenta, salivary glands) in addition to scattered macrophage-like cells, which were also seen in tumours and other organs (Figs. 8–10). In the thymus the Hassall's bodies reacted with C5, D4, F4 and F12. Lymph nodes showed no reaction except with the supernatants D5 and H9 which reacted with collagen and/or elastic fibres. Guinea pig ears showed no reactivity with any of the supernatants.

Some of the cells intended for immunization were frozen at $-70°C$, thawed at a later date and stained immunohistochemically after being attached to glass slides. Almost all cells displayed immunostaining with the six supernatants that showed reactivity with the frozen sections from psoriatic epidermis (Table III). Monoclonal antibodies identifying different infiltrating cells were in addition used to further characterize the phenotypes of the cells used for immunization. The results shown in Table III indicate that a majority of the cells used for immunization were indeed keratinocytes but that also a number of other cells, mainly of macrophage lineage, were present in this suspended cell population from psoriatic epidermis.

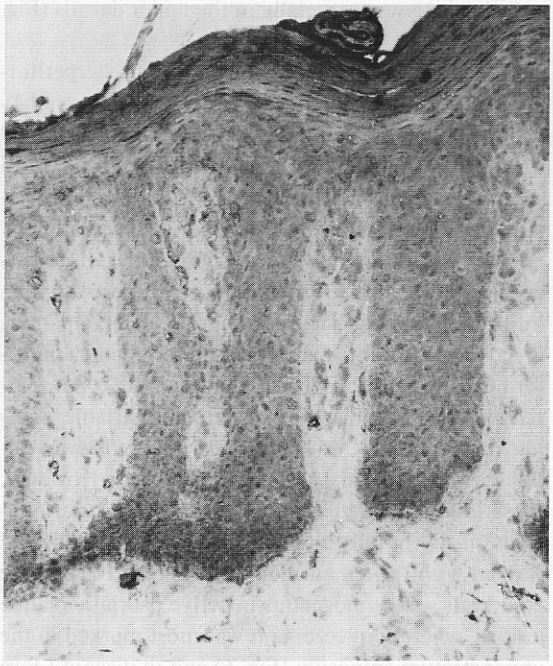

Fig. 1. Psoriatic plaque reacting with C5 in epidermis and dermal macrophage-like cells. ×40.

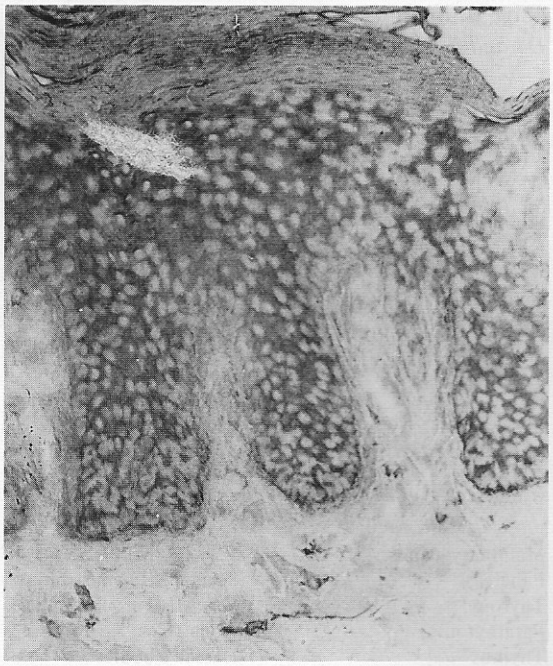

Fig. 2. Same psoriatic plaque as in Fig. 1, reacting with D4 in epidermis and dermal macrophage-like cells. ×140.

Table III. *Percentages of cells used for immunization showing reactivity to hybridoma supernatant and other commercially available mouse monoclonal antibodies*

Positive cells were identified by immunocytochemistry. A minimum of 200 cells were counted

Hybridoma	Ig chain composition	Percent reactive cells	Reactivity
C5, D4	$IgGl^k$	96	See Tables I and II
F, F12	$IgGl^k$	94	See Tables I and II
H9	$IgGl^k$	15	See Table I
MoAbs from other sources			
PAL-E[a]	IgG 2a	<1	Endothelial cells (3)
RF D[b]	–	8	Certain macrophages, Kupffer cells (4)
Anti Leu-M1 (CD15)[c]	IgM^k	16	Monocytes, granulocytes
Anti Leu-M2[c]	IgM	0	Monocyte subset
Anti Leu-M3[c]	IgG $2b^k$	10	Mature monocytes
OKM5 (CD36)[d]	IgG $2b^k$	23	Monocytes, macrophages, thrombocytes
Anti Leu 4 (CD3)[c]	IgG^k	20	T-cells
Anti Leu 6 (CD1)[c]	IgG $2b^k$	5	Langerhans' cells, thymocytes
Anti HLA-DR[c]	IgG $2a^k$	25	B-cells, macrophages, Langerhans' cells activated T-cells, monocytes and keratinocytes
Anti-transferrin[c] receptor	IgG $2a^k$	14	Activated cells of various types

[a] Bio-Zac, Järfälla, Stockholm, Sweden.
[b] A gift from Dr Poulter, Royal Free Hospital, School of Medicine, London, England.
[c] Becton Dickinson Corp, Sunnyvale, Calif., USA.
[d] Ortho Diagnostic Systems, Raritan, NJ, USA.

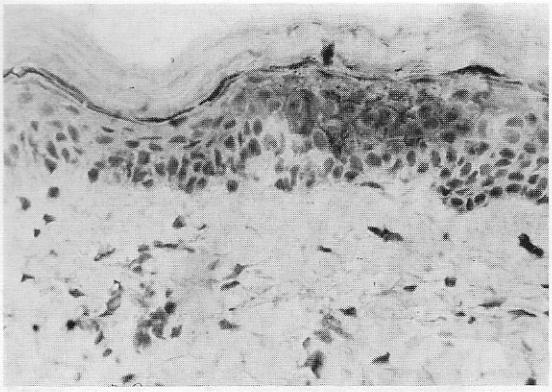

Fig. 3. Reactivity to C5 in epidermal and dermal areas of normal-appearing skin of a patient with active psoriasis. ×180.

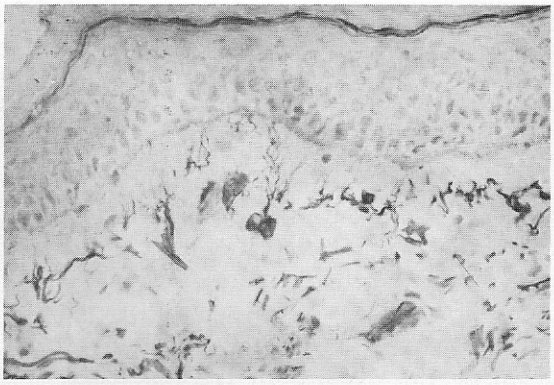

Fig. 5. Basement membrane and elastic tissue of upper dermis in normal skin reacting with D5. ×100.

Some of these 'reference' antibodies were also used for further characterization of the psoriatic skin lesions. Thus in active psoriasis, anti-transferrin receptor antibodies showed some reactivity on the basal cells, but the patterns obtained with anti-transferrin receptor antibodies did not resemble those patterns that were seen with the presently produced antibodies, thereby excluding the possibility that these antibodies might simply react with the transferrin receptor.

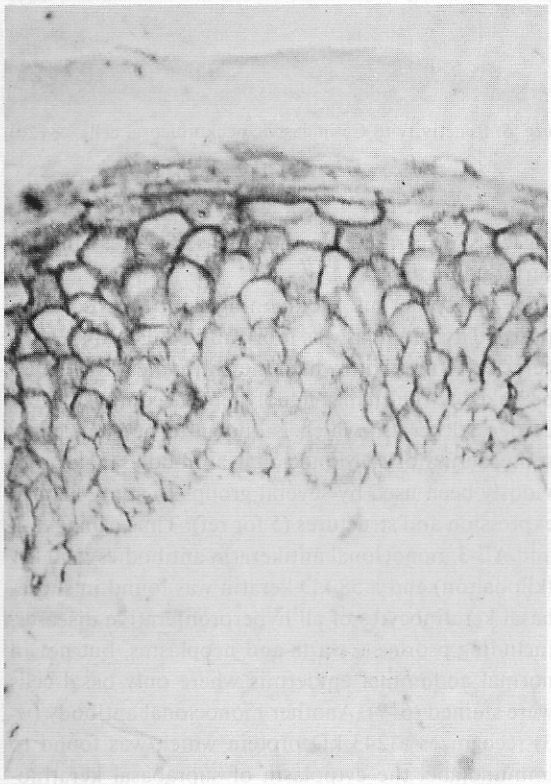

Fig. 4. Cell membranes or intracellular areas of normal skin reacting to G5. ×430.

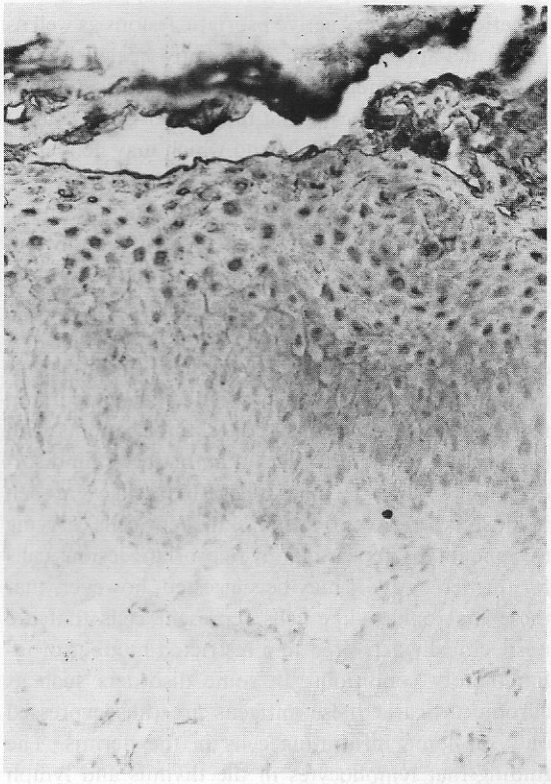

Fig. 6. Contact dermatitis reacting to C5 in upper spinal layer. ×180.

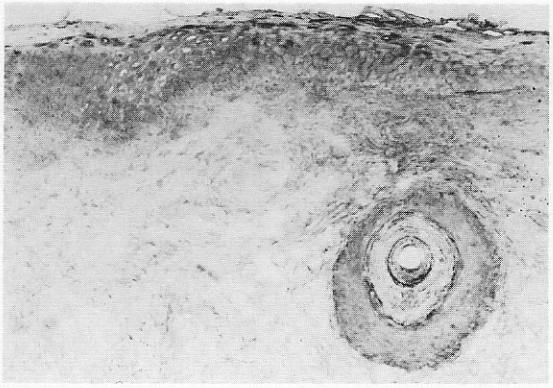

Fig. 7. Discoid LE reacting to C5 mainly in the basal layer in a hair follicle. ×100.

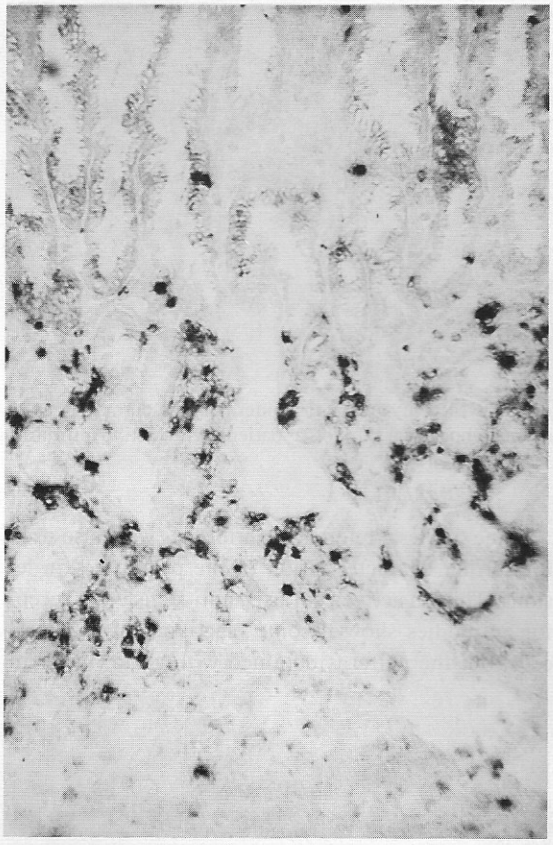

Fig. 8. Reactivity to C5 in gastric neck mucosal cells. ×120.

DISCUSSION

Six monoclonal antibodies raised by us reacted with infiltrating dermal cells and the whole epidermis in psoriatic lesions, but showed no reaction in normal skin, ichthyosis or acutely inflamed skin. Positive reactions were also seen in the normal-appearing skin of patients with very active psoriatic lesions as well as in normal-appearing skin of recently healed lesions following treatment with potent corticosteroids. These findings indicate that unique cell surface antigens may exist in psoriasis and which may help elicit an immune reaction with a certain specificity for psoriatic lesions and/or be the targets for such immune reactions. The diffuse staining of the epidermis in psoriatic lesions could also be seen in other inflammatory conditions, although the staining in these cases was most often restricted to cells within certain epidermal layers. The relevance of these findings is at present obscure, but they may reflect some common features of cell activation in the skin of patients with psoriasis and in some other inflammatory skin disorders. The infiltrating macrophage-like cells were seen in most tissues except normal skin. Why cells carrying this phenotype appear mainly in psoriatic lesions calls for further study. It may be suggested, however, that these macrophage-like cells represent cells that are unspecifically activated in a restricted number of inflammatory conditions. In some disorders such as chronic urticaria these antigens are thus expressed only on some infiltrating cells in the dermis. The finding that lymphocytes in the thymus and lymph nodes showed no staining with any of the six antibodies in question also indicates that the dermal infiltrating cells in certain chronic diseases express unique surface antigens.

In the epidermis the staining with the psoriasis-positive hybridomas was more diffuse. Possibly, antigenic structures with which the antibodies react could be linked to the change in polypeptide composition of epidermal keratin which is known to be changed in some dermatosis. Monoclonal antibodies have previously been used by several groups to study keratin expression and structures (5 for ref). Thus using AE-1 and AE-3 monoclonal antikeratin antibodies a 50 kD (kilodalton) and a 58 kD keratin was found in suprabasal keratinocytes of all hyperproliferative diseases, including psoriasis, warts and neoplasms, but not in normal abdominal epidermis where only basal cells were stained (6–7). Another monoclonal antibody (ψ-3) recognizes a 243 kD protein which was found to immunolabel the cytoplasm of suprabasal keratinocytes in psoriatic lesions, but not in normal skin (8). These antibodies were later also found in tape-

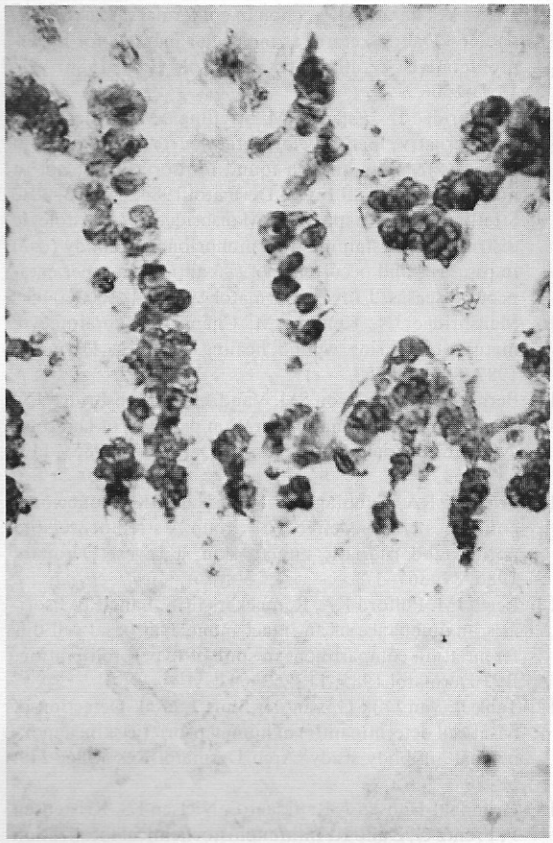

Fig. 9. Reactivity to C5 in duodenum. ×120.

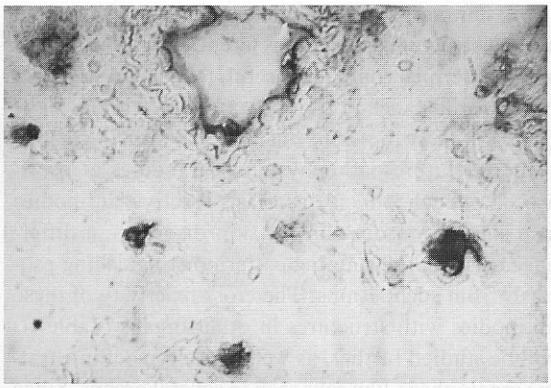

Fig. 10. Reactivity to C5 in lung. ×310.

stripped skin of healthy subjects (9–10). They were located in all epidermal layers except the basal layer (9–12). Thus the staining pattern of these antibodies differs in several respects from the staining patterns of our own antibodies, thus leaving us with uncertainty as to whether our antibodies react with previously undefined structures of keratin or with epidermal structures unrelated to keratin.

Streptococcal monoclonal antibodies have been shown to cross-react with epidermal components. When such antibodies were used, however, no difference was found between the staining of normal skin and that of lesional or non-lesional psoriatic skin (12).

Leigh et al. (13) raised a monoclonal antibody (LP34) against psoriatic plaque scale stratum corneum, but the antibody reacted with both normal skin and with cultured keratinocytes. Tank et al. (14) tested monoclonal antibodies against mesothelioma cells on normal and psoriatic skin. With immunoperoxidase technique they found a staining of the basal cell layer in normal skin but also of the suprabasal in psoriatic lesions. Grøndahl-Hansen et al. (15) reported abnormal focal epidermal localization of monoclonal antibodies against plasminogen activator in psoriatic lesions but not in non-involved or normal skin. Their data for tissue type plasminogen activator was confirmed with an autohistographic technique (16). Our antibodies do not seem to be directed against any of the above-mentioned antigens and they therefore merit further biochemical characterization.

Our antibodies against epithelial tissues also reacted with the Hassall's bodies in the thymus. Such a thymus–epidermis relationship has recently been studied by Schmitt et al. (17), who used a panel of anti-thymic cell monoclonal antibodies on human keratinocytes and Langerhans' cells. The antibodies which reacted with all the thymic epithelial cells including Hassall's corpuscles decorated all the epidermal cell layers. Our hybridomas, which were positive in psoriatic lesions, reacted only with Hassall's bodies. Hence they are probably not identical with those described by others.

The reactivity of three hybridomas (D5, H9 and A7) with elastic fibres and basement membrane is similar to that described by Schmitt et al. (18) with a monoclonal antibody, HD8, which recognized the microfibrillar component of the dermal fibres and the oxytolan fibres of the dermal–epidermal junction. Another monoclonal antibody, NKH-1, recently described, labelled the same structures (19). Antivitronectin anti-SAP and antifibrillin also stain the papillary oxytolan fibres, but not the basement membrane zone (20–23). The reactivity of these antibodies, however, also seems to differ somewhat from ours, since they have a maximum staining of the thicker fibres.

In summary, monoclonal antibodies generated against structures present preferentially in the dermis and epidermis could be useful for diagnostic purposes, for instance in psoriatic arthritis without signs of psoriasis in the skin, but may have their greatest value for future pathophysiological studies of psoriasis. Although our six psoriasis-reactive antibodies also stained various chronic skin disorders, a difference exists in the staining pattern distinguishing psoriasis from such lesions. The cross-reactivity of these antibodies with structures in other organs (Table II) will be studied further, as well as the type of infiltrating macrophage-like cells seen in several organs. Since psoriasis might involve other organs than the skin, the distribution of antigens could perhaps increase our knowledge about this aspect. The method of immunization and production of monoclonal antibodies seems to be a possible way to further study psoriatic inflammatory skin lesions as well as the mechanisms behind the involvement also of disorders other than the skin in psoriasis.

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