Virchows A Pathol Anat (1988) 413:113–122

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemistry (S 100, KL 1) and human papillomavirus DNA hybridization on Morbus Bowen and bowenoid papulosis*

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Summary. In this study 55 paraffin embedded samples defined as Bowen's disease or bowenoid papulosis were investigated with antibodies against S 100 protein and keratins (KL 1). S 100-positive cells were quantified and related to defined section area of the epidermal compartment by computer-assisted image analysis. The density of S 100-positive cells was compared with normal skin and was particularly related to growth patterns and keratinization of the different lesions under study. S 100-positive dendritic cells were found to be reduced overall in bowenoid lesions when compared with normal skin. Lesions with high counts of S 100-positive dendritic cells most frequently showed a solitary growth pattern with highly conserved architecture and differentiation and no tendency to stromal invasion. In contrast, cases with low counts of S 100-positive cells very often showed multifocal development, a high degree of architectural disturbance and dedifferentiation. In this group, stromal invasion (cases of invasive carcinoma associated with Bowen's disease) was seen more often. Interestingly, this latter group of cases also revealed a peculiar keratin pattern. Frequently, the basal cell layer was decorated with KL 1 antibody, which usually recognizes only suprabasaly located keratinocytes. No differences between Bowen's disease and bowenoid papulosis were found in terms of densities of S 100-positive dendritic cells and keratin pattern. In our experience, extragenital Bowen's disease and genital Bowen's disease can not be distinguished on purely morphological grounds or with the immunocytochemical approach presented here. Interestingly, when employing in situ hybridization with HPV 16 probes three of seven samples of genital Bowen's disease harboured HPV 16 DNA, whereas six cases of extragenital disease were negative.

Key words: Bowen's disease – Bowenoid papulosis – S 100 Protein – KL 1 Keratin – HPV-DNA

Introduction

Seventy-five years ago Bowen (Bowen 1912) described two cases of epidermal "atypical proliferations" for the first time. This carcinoma in situ of the skin – now widely known as Bowen's disease – has stimulated intensive epidemiological, clinical, and morphological investigations. The disease is frequent in light-skinned individuals and less prevalent in pigmented people (Mora et al. 1984). Bowen's disease may occur at sun-exposed or non-exposed skin sides, the trunk and the face being most commonly affected (Braun-Falco 1984). Some controversy exists whether or not Bowen's disease is predictive of visceral malignancy (Graham et al. 1959; Andersen et al. 1973; Callen and Headington 1980; Kao et al. 1986). Very early bowenoid lesions have been the subject of studies of chemical carcinogenesis, since exposure to arsenic salts has been observed to be followed not only by the development of arsenic keratosis but also of Bowen's disease and even invasive squamous cell carcinomas (Graham et al. 1961). In recent years, a fascinating approach to the aetiology of bowenoid lesions has opened with the molecular virological detection of HPV-DNA in certain types of bowenoid lesions at particular locations (Ikenberg et al. 1983; Gross et al. 1985). HPV-DNA type 11, 16 and 18 were found in anogenital bowenoid lesions which appear clinically at younger

* Supported by the Deutsche Forschungsgemeinschaft (Lo 285/2–4)

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ages as small brownish papules. Those studies supported the earlier clinical descriptions of so called bowenoid papulosis (Wade et al. 1979), which to the first time brought to mind that – although morphologically similar – bowenoid papulosis is biologically different from Bowen's disease. The morphological characteristics of Bowen's disease and bowenoid papulosis include profound disturbances of epithelial layering, a high degree of atypia of individual cells, and premature single cell keratinization (so called dyskeratosis) (Lever and Schaumburg-Lever 1983; Callen and Headington 1980). Bowenoid lesions are often associated with a dense inflammatory stromal infiltrate. The inflammatory response in squamous cell carcinoma of the mucocutanous surfaces has been discussed in recent publications, and the particular contribution of dendritic cells has been a matter of concern (Löning et al. 1983; Becker et al. 1985). The degree of dendritic cell density in certain types of mucocutaneous carcinoma and even other organ cancers has been regarded as being of prognostic signal (Furukawa et al. 1985; Tsujitani et al. 1987; Schröder et al. 1988). In this study of extragenital and genital bowenoid lesions, we wanted to get insights into the following questions:

1. How are dendritic cells related to growth patterns and differentiation?
2. What is the contribution of immunocytochemistry for keratins and in situ hybridization for HPV DNA in diagnostic pathology of bowenoid lesions?

For these purposes, we treated our samples with antibodies against S 100-protein which labels dendritic cells of the skin including Langerhans cells and melanocytes, and we looked for keratins, usually located at suprabasal locations (KL 1). Finally, a number of samples were examined for the presence of HPV 6, 11, 16 and 18 DNA employing in situ hybridization with biotinylated probes and with a modified immunogold-silver staining technique.

Materials and methods

Eighty-seven formalin-fixed and paraffin-embedded samples of Bowen's disease and bowenoid papulosis were retrieved from the files of the Institute of Pathology, University of Hamburg and the Clinic for Dermatology and Venerology, Medical University of Lübeck. All lesions showed the morphological criteria of cutanous carcinoma in situ; disordered layering, marked pleomorphism, and markedly disturbed keratinization (Lever and Schaumburg-Lever 1983). Among those cases, 10 lesions were located in the anogenital area (three vulvar, six peri- or intra-anal and one penile). In addition to lesion bearing skin, normal extragenital tissue (ten samples) was taken from surgical specimens.

Table 1. Age distribution in Bowen's disease

Age (years)	≦40	41–60	61–70	≧80
$n=$	8	10	55	14

Table 2. Location of Bowen's disease

Location	Head	Trunk	Arms	Hands	Legs	Genitals
$n=$	26	20	7	12	12	10

The clinical data obtained are summarized in Table 1 and 2. The paraffin embedded material was processed for conventional light microscopy (H & E), immunocytochemistry (S 100-protein and KL 1-keratin), and in situ hybridization with HPV 6, HPV 11, HPV 16, and HPV 18 probes.

Fifty-five paraffin sections were stained with antibodies against S 100-protein (polyclonal rabbit antibodies purchased from Dako, Copenhagen, Denmark) and eighty-seven were stained with monoclonal mouse antibody raised against keratins (55–57 Kd, KL 1, distributed by Immunotech, Marseille, France).

Primary antibodies were diluted at 1:100 to 1:500 in PBS (phosphate buffered saline). Bound antibodies were detected with the avidin-biotin-peroxidase system (ABC Vectastain kit, Vector Laboratories, Inc., Burlingame, CA) purchased from Camon, Wiesbaden, FRG. The immunocytochemical protocol employed has been repeatedly described elsewhere (Hsu et al. 1981; Tsujitani et al. 1987). Following the 3,3′-diaminobenzidine reaction sections were counterstained with haemalaun. KL 1 stainings were evaluated in a semiquantitative manner. Differences of KL 1 staining at the different levels of the epidermis and the epidermal appendages were quoted and compared with staining of non-lesional marginal epidermis and with the staining of the ten normal control specimens. S 100-positive staining was quantified (by computer-aided image analysis) in addition to semi-objective evaluations of distributional variations. For a rough estimate of the number of active melanocytes in Bowen's disease, staining with HMB 45 antibodies (Ortho Pharmaceutical Co, Raritan, NJ USA) were also executed on fifteen sections.

Controls for S 100- and KL 1-staining included omission of the primary antibodies, staining with and without pretreatment with methanol and H_2O_2, and endpoint dilutions.

Quantitation of S 100-positive dendritic cells per mm^2 in lesional and normal skin was conducted by means of a semiautomatic image analysis system (Videoplan, Contron, Munich, FRG). Measurement of S 100-positive dendritic cells were made with a Leitz Laborlux 12 microscope equipped with a ×25 objective (Leitz, Wetzlar, FRG). The area of the epidermal compartment of each specimen was computed by the image analysis system, and S 100-positive dendritic cells were consecutively expressed in numbers per mm^2. Only those cells with clearly discernible nuclei were counted.

In situ hybridization was carried out as described elsewhere (Löning and Milde 1987; Henke et al. 1987). Briefly, sections were covered with 20 µl of the hybridization cocktail containing 2×SSC (1×SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2), 20% (v/v) deionized formamide, 10% (w/v) dextran sulphate, 0.1 mg/ml herring sperm DNA and 1 µg/ml biotinylated HPV probes (plasmids containing HPV 6, 11, 16 and 18

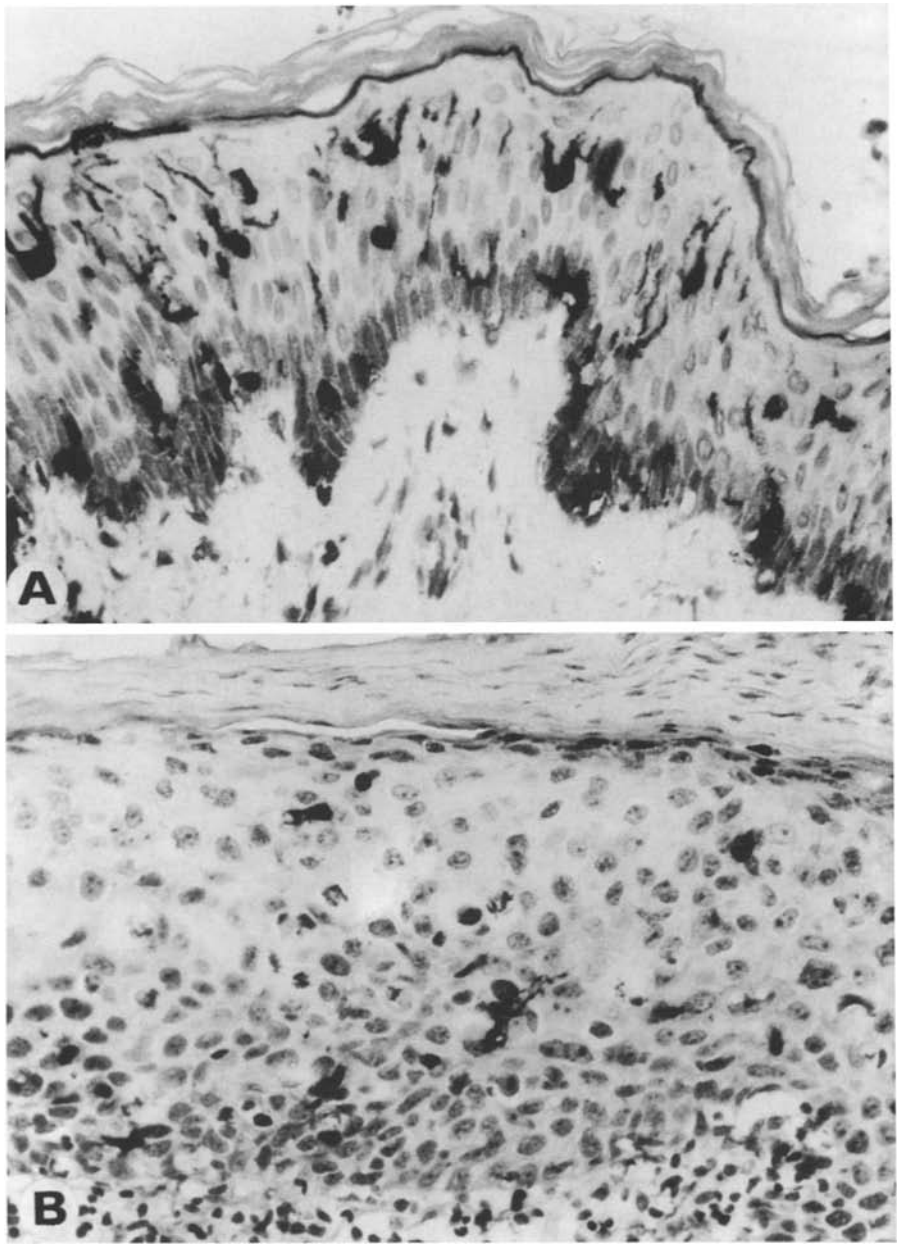

Fig. 1. A Normal skin from the trunk. Numerous S 100-protein positive dendritic/cells within suprabasal epidermal layers. × 500.
B Anogenital bowenoid lesion. Few S 100-positive dendritic/cells within the atypical epithelium. ABC technique. × 500

inserts were kindly provided by Dr. Harald zur Hausen and Dr. Lutz Gissmann), hybridization was allowed to take place at 37° C overnight. Consecutively, sections were carefully washed in two changes of SSC buffer (1 × SSC, 45% formamide) at 37° C (corresponding to Tm −17° C), each for 10 min, and in further three changes of 2 × SSC at room temperature each for 5 min. Hybridized nucleic acids were detected with the immunogold-silver staining technique. Sections were treated consecutively with rabbit-anti-biotin-antibodies (Enzo, New York) biotinylated goat anti-rabbit-antiserum (Dianova, Hamburg), and streptavidin-colloidal gold (5 nm)-conjugates (Janssen, Beerse, Belgium). All reagents were diluted at 1:250 in Tris-HCl buffer (pH 7.5 for steps 1 and 2, pH 8.2 for step 3), and incubated for 1 h at 37° C. For the final silver development, a commercially distributed kit was used (IntenSE, Janssen, Beerse). Sections were counterstained with hematoxylin and examined by standard and interference reflection microscopy (Verschueren 1985; Ambros et al. 1986).

Controls included omission of the specific viral probe, application of heterologous HPV probes (HPV 6, 11, 16, 18), and positive controls (condylomas, papillomas, cervical epithelial dysplasias).

Results

The clinical profiles of the patients (age, distribution of lesions) are illustrated in Table 1 and 2. More than 80% of the patients were older than 60 years. Fifty-two were females, 34 were males. Clinical follow-up data was obtained from 31 pa-

tients. Three had recurrences of their lesions, six patients were reported to suffer from other internal malignancies. Nine individuals had other skin cancer, mostly basal cell carcinoma. One patient, treated with arsenic salts for psoriasis, showed both internal malignancy and other skin cancers. Ten bowenoid lesions were obtained from anogenital (vulvar, peri- or intraanal, penile) skin (6 females, two of them younger than 40 years, one male). Two of these ten lesions were already referred to us with the clinical diagnosis of bowenoid papulosis. The residual eight lesions were diagnosed as Bowen's disease.

All lesions examined showed the criteria of a carcinoma in situ of the skin by light microscopy, including disturbed epithelial layering, high grade of atypia of individual cells and premature single cell keratinization. Analogous morphological changes were seen in extragenital and in anogenital bowenoid lesions. We notably found no differences of true Bowen's disease and bowenoid papulosis with respect to the involvement of skin appendages (acrotrichia/acrosyringia).

Multicentric development of bowenoid lesions was seen in 69% of cases, whereas solitary lesions were observed in 31%. Thorough reevaluation of all cases revealed ten lesions containing invasive foci, thus representing invasive Bowen carcinoma. With regarding to the growth pattern, 58% of cases were flat lesions, 27% showed an exophytic, and 15% an endophytic growth pattern.

Morphometric evaluation of S 100-positive cells was carried out in 55 cases. The residual 32 cases were excluded from this study for varying reasons (lack of material, poor tissue preservation). S 100-positive dendritic cells were found to be reduced overall in bowenoid lesions when compared with normal skin (Fig. 1 A, B). In fifteen bowenoid lesions stained with antibodies against active melanocytes, we found only few cells to be positive for this antigen (HMB 45). The mean value for all bowenoid lesions was 53.3 cells per mm^2 with a wide range of 5 to 205 cells per mm^2. In seven genital lesions mean value was 22.5 cells per mm^2 with a range of 6 to 43 cells per mm^2. 57.3 cells per mm^2 were counted in the non-genital lesions. In normal controls, the quantitative analysis yielded a mean value of 147 cells per mm^2 (Fig. 2).

In the series of cases investigated a group of extragenital lesions (7 cases) was distinguished, which showed the highest counts of S 100-positive dendritic cells (111 to 205 cells per mm^2). These cases were usually solitary lesions with highly conserved architecture and differentiation.

In contrast, another group of cases (13 lesions)

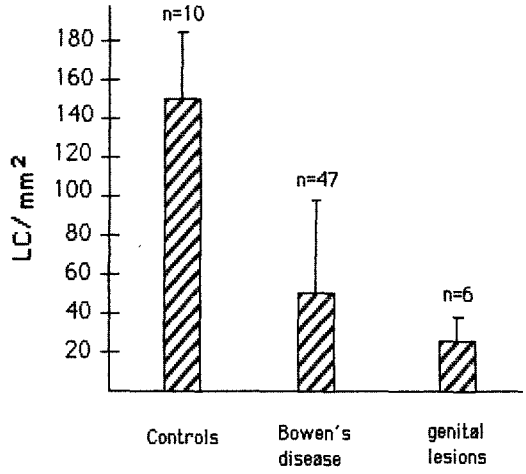

Fig. 2. Comparison of quantitation of S 100-positive dendritic/Langerhans cells in bowenoid lesions and normal controls

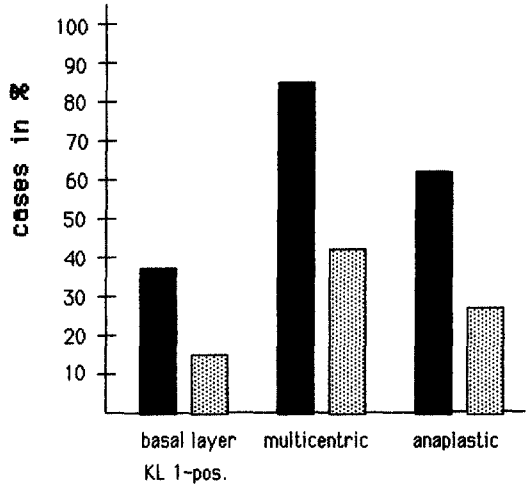

Fig. 3. Comparison of cases with very low (*dark columns*) and very high counts (*bright columns*) of S 100-positive dendritic/Langerhans cells. The degree of basal layer staining with KL 1 ($n=23$) of multicentric development of lesions ($n=59$) and individual cellular atypia ($n=32$) is compared

showed very low counts of S 100-positive dendritic cells (below 30 per mm^2). These cases were associated with multifocal growth, a high degree of architectural disturbance, striking cellular atypia, and with a higher tendency of stromal invasion (cases of invasive carcinoma associated with Bowen's disease) (Fig. 3). In addition, cases with low counts of S 100-positive dendritic cells showed a peculiar keratin staining. Whereas KL 1 antibodies usually stain suprabasal cells only, these antibodies decorated basal layer cells heavily in this group of cases (Fig. 4 A, B). In the suprabasal layers keratins showed a patchy distribution. Areas with absent keratin staining were seen as well as focal intensive keratin expression (Fig. 5 A). Keratin staining was not seen to be different in extragenital

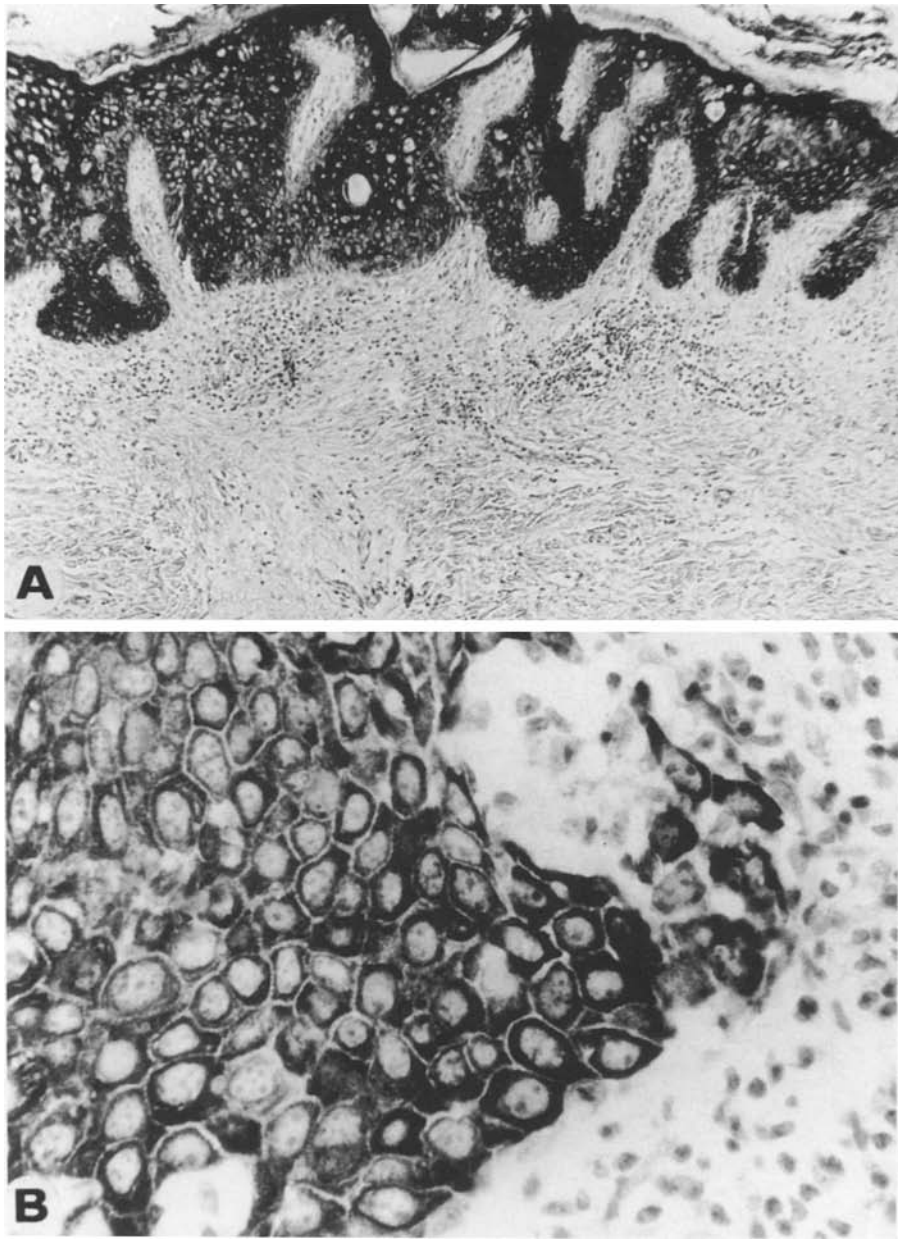

Fig. 4. A Extragenital Bowen's disease. The basal layer is positive for 55–57 Kd keratins. Monoclonal antibody KL 1. ABC technique. × 80.
B Higher magnification. Invasive focus of keratin positive cells. × 500

or anogenital bowenoid lesions. S 100-positive dendritic cells were reduced in extragenital as well as in genital lesions (Fig. 5B). Thus, we found the quantity and distribution of the applied marker molecules (S 100, KL 1) to be similar at the different locations. Moreover, even bowenoid papulosis (defined by clinical data and hybridization) could not be distinguished from extragenital Bowen's disease by means of S 100-protein and KL 1 keratin staining.

In situ hybridization was performed on seven genital bowenoid lesions and six cases of Bowen's disease, which showed koilocytosis and were thus already suspicious of HPV infection. The six cases of extragenital Bowen's disease as well as seven genital bowenoid lesions were hybridized with HPV 6, 11, 16, and 18 probes. All extragenital lesions were negative for HPV-DNA. In contrast, three genital lesions hybridized with HPV 16 probes. Hybridized cells were all located in the upper layers of the epidermis and within the stratum corneum (Fig. 6A, B). No hybridization with HPV 6, 11, 16 and 18 probes was observed in these cases.

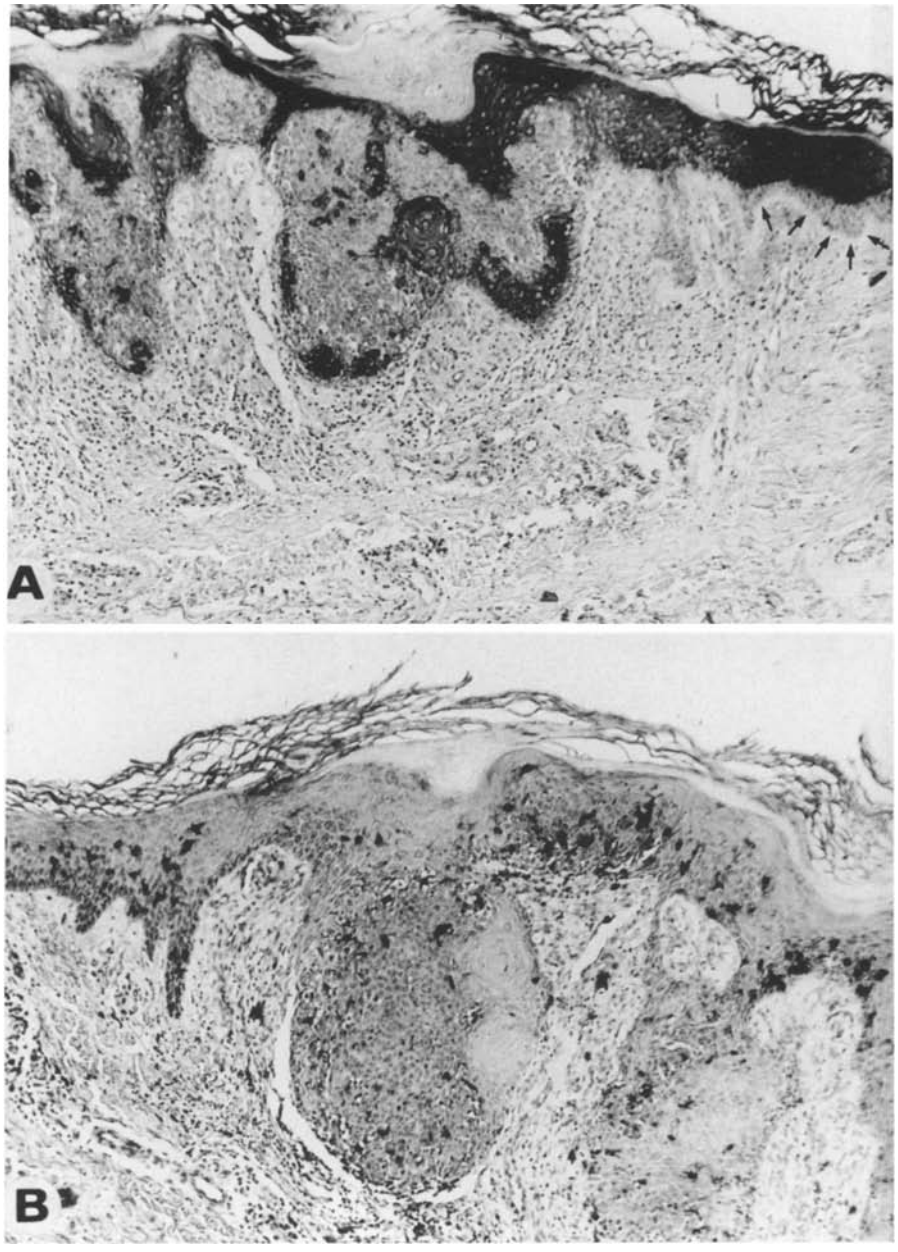

Fig. 5. A Extragenital Bowen's disease. Demonstration of patchy keratin staining pattern within the lesional skin. The basal layer of the adjacent normal skin is not labelled (*small arrows*). Monoclonal antibody KL 1. ABC technique × 80.
B Extragenital Bowen's disease. Low density of S 100-positive dendritic/cells within the bowenoid lesion and high density in the adjacent skin. ABC technique. × 80

Discussion

Antibodies against S 100-protein decorate dendritic cells in all squamous epithelia and lymphoid organs (Ralfkiaer et al. 1984; Vanstapel et al. 1986). In the skin, S 100-protein is present in Langerhans cells and their precursors and in melanocytes (Cocchia et al. 1981; Halliday et al. 1986). Although antibodies for S 100-protein do not discriminate between members of the accessory cell system (Langerhans cells, indeterminate cells) and melanocytes, evaluation of S 100-positive intraepidermal cells remains the only valuable approach for retrospective analysis of paraffin-embedded specimens and still provides interesting information on the general state and shifts of intraepidermal dendritic cells in normal skin, inflammatory conditions, and in skin derived tumours (Egan et al. 1986; McArdle et al. 1986a). The presence and concentration of S 100-positive cells have been reported to be indicative of prognosis in nasopharyngeal carcinomas, papillary thyroid cancer as well as in lung and in stomach cancer (Nomori et al. 1986; Schröder et al. 1988; Furukawa et al. 1985; Tsujitani et al. 1987). Thus, S 100-positive cells may turn out to be a marker of the biological dif-

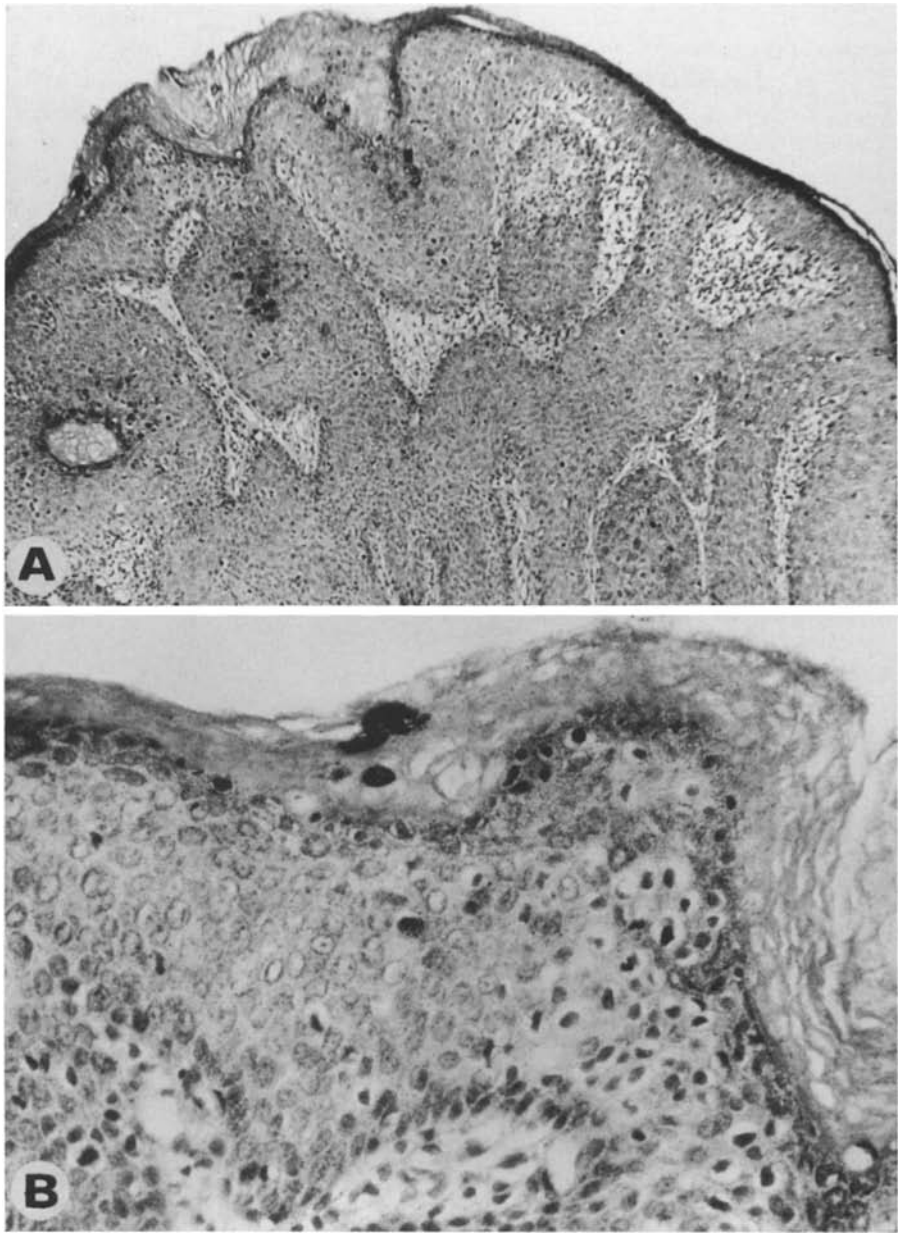

Fig. 6. A Bowenoid papulosis. In situ hybridization. Hybridized cells within the upper epidermal layers and the stratum corneum. Biotinylated HPV-16 probe. Immunogold-silver staining. × 80. **B** Higher magnification. Positive cells show nuclear and cytoplasmatic hybridization with HPV 16 probes. × 500

ferences known to exist within particular tumour groups despite of their close morphological relatedness.

Our studies on S 100-positive dendritic cells had two major concern: firstly, we wanted to evaluate the general differences of the amount of S 100-positive dendritic cells of normal skin and bowenoid lesions. We have clearly shown, that S 100-positive dendritic cells were reduced within the atypical epidermis when compared with normal. We consider these findings to be an expression of reduced numbers of Langerhans cells and their precursors, since melanocytes reside within the epidermis as a relatively stable cell population (Quevedo et al. 1987). Antibodies against inactive melanocytes are not available, thus at present, we could only compare our results with antibodies against active melanocytes. As expected, we found only very few melanocytes to be positive for this antigen in a selected number of fifteen bowenoid cases. We know from our own studies and those of other research groups carried out on frozen sections of tumours of squamous cell origin, that Langerhans cells recognized by their CD1 antigens are indeed reduced in basal cell epitheliomas and squamous cell carcinomas. The antigen processing and pre-

senting functions of Langerhans cells have been well documented in numerous experimental studies (Stingl et al. 1980; Braathen et al. 1980). Langerhans cells have been ascribed to play a key role in T cell help (Ralfkiaer et al. 1984) and in nearly all cell mediated skin reactions against extrinsic (e.g. virus infections) and intrinsic factors (Nakajima et al. 1982; Morris et al. 1983; Gatter et al. 1984; Chardonnet et al. 1986). Tumours apparently elicit a Langerhans cell response to a varying extent dependent on the tumour origin (skin, thyroid, stomach, lung) (Löning et al. 1983; Schröder et al. 1988; Tsujitani et al. 1987; Furukawa et al. 1985) the tumour type (basal cell epithelioma, squamous cell carcinoma) (Gatter et al. 1984, Meissner et al. 1986; Egan et al. 1986), and its differentiation (highly versus low differentiated squamous cell carcinomas) (Smolle et al. 1986).

Looking closer at our quantitative data on bowenoid lesions a second question developed, the answer to which may supplement the former discussion: were there quantitative differences of S 100-positive dendritic cells within the tumour group itself? We were surprised to find very low counts of dendritic cells to be present in "high grade" bowenoid lesions (multiple tumour foci in the same specimen, greater prevalence of stromal invasion, profound architectural disturbance and cellular atypia). In contrast, very high counts of S 100-positive dendritic cells were found in "low grade" lesions which were most often solitary, with highly conserved epidermal architecture and differentiation and with a minor tendency to stromal invasion. When looking at differences between extragenital and genital bowenoid lesions, we saw lower counts of dendritic cells in the genital group, although these data have to be substantiated in a larger number of samples. Within the group of genital bowenoid lesions, we were not able to discriminate clinically diagnosed bowenoid papulosis and Bowen's disease by means of quantitation of S 100-positive cells. Again, these data have to be interpreted with caution since the number of cases is small. The number of Langerhans cells has been reported to be reduced in HPV infected tissues, and has been discussed as a possible influence on malignant outgrowth (McArdle et al. 1986b). Keratin staining was generally extremely irregular, sometimes patchy or even absent in the majority of bowenoid lesions. Absence of staining of basal layer cells was usually observed in normal epidermis and in most bowenoid lesions as well. However, in striking contrast to these usual observations, basal layer cells were frequently decorated in "high grade" Bowen's disease. This aberrant keratinization may be due to the profound loss of polarization in this group of lesions (Löning et al. 1980; Chardonnet et al. 1986; Moll et al. 1986). No differences of staining patterns were seen between extragenital and genital lesions and within the group of genital bowenoid lesions itself.

Reversible vulvar atypia, so-called multicentric pigmented Bowen's disease, and bowenoid papulosis are terms for anogenital skin lesions which show the same histopathological features as true Bowen's disease. In contrast to Bowen's disease, these recurrent lesions occur in younger patients and show often a multicentric distribution (Scherer and Haensch 1984; Patterson et al. 1986).

A relation of anogenital bowenoid lesions, cervical intraepithelial neoplasia and genital condylomas has been reported by several authors (Grussendorf and Bär 1977; Gross et al. 1985; Jablonska et al. 1986) who found human papilloma virus in these groups of lesions.

Human papillomaviruses are supposed to induce genital condylomas as well as bowenoid skin lesions or cervical neoplasia (Zur Hausen 1977; Reid et al. 1982 and 1984). Whereas in genital condylomata with only mild cellular atypia and no tendency to stromal invasion HPV 6/11 DNA is a common finding (Gupta et al. 1987; Wells et al. 1987), bowenoid papulosis of the genitalia is very often associated with HPV 16 DNA (Ikenberg et al. 1983; Gross et al. 1985).

There is some evidence that malignant transformation of genital bowenoid lesions depends on the type of human papillomavirus. A higher degree of malignancy is seen in vulvar or cervical lesions which contain HPV 16 DNA (Jablonska et al. 1986; Macnab et al. 1986; Gupta et al. 1987; Wells et al. 1987).

In our material 3 cases of anogenital bowenoid lesions (all specimens initially diagnosed as Bowen's disease) hybridized with HPV 16 DNA, but not with HPV 6/11 DNA. All lesions showed increased cellular atypia and koilocytosis. Quantitation of Langerhans cells in these cases revealed markedly decreased counts for S 100-positive dendritic cells when compared with normal controls. Interestingly, all our HPV 16 positive specimens were taken from a younger woman who underwent intensive surgical treatment for recurrent vulvar bowenoid lesions. Nevertheless, lesions were recurrent over a period of five years. Only a final therapeutic approach with 5-fluorouracil led to a remission of all lesions. All other specimens of genital and extragenital sites ($n=10$) were negative for HPV DNA and recurrence was only reported in one female patient with bowenoid papulosis.

References

Ambros PF, Matzke MA, Matzke AJM (1986) Detection of a 17 kb unique sequence (T-DNA) in plant chromosomes by in situ hybridization. Chromosoma 94:11–18

Andersen S, Nielsen A, Reymann F (1973) Relationship between Bowen disease and internal malignant tumors. Arch Dermatol 108:367–370

Becker J, Behem J, Löning T, Reichart P, Geerlings H (1985) Quantitative analysis of immunocompetent cells in human normal oral and uterine cervical mucosa, oral papillomas and leukoplakias. Arch Oral Biol 30:257–264

Braathen LR, Thorsby E (1980) Studies on human epidermal Langerhans cells. I. Allo-activating and antigen-presenting capacity. Scand J Immunol 11:401–408

Braun-Falco O, Plewig G, Wolff HH (1984) Dermatologie und Venerologie. 3., neubearbeitete Auflage, Springer-Verlag Berlin Heidelberg New York Tokyo

Callen JP, Headington J (1980) Bowen's and Non-Bowen's squamous intraepidermal neoplasia of the skin. Arch Dermatol 116:422–426

Chardonnet Y, Viac J, Leval J, Begoud G, Morgan A, Thivolet J (1986) Laryngeal papillomas: local cellular immune response, keratinization and viral antigen. Virchows Arch B 51:421–428

Cocchia D, Michetti F, Donato R (1981) Immunochemical and immunocytochemical localization of S-100 antigen in normal human skin. Nature 294:85–87

Egan MJ, Crocker J, Newman J, Collard M (1986) Immunohistochemical localization of S 100 protein in skin tumors. Arch Pathol Lab Med 110:765–767

Furukawa T, Watanabe S, Kodama T, Sato Y, Shimosato Y, Suemasu K (1985) T-zone histiocytes in adenocarcinoma of the lung in relation to postoperative prognosis. Cancer 56:2651–2656

Gatter KC, Morris HB, Roach B, Mortimer P, Fleming KA, Mason DY (1984) Langerhans' cells and T cells in human skin tumors: an immunhistological study. Histopathology 8:229–244

Graham JH, Helwig EB (1959) Bowen's disease and its relationship to systemic cancer. Arch Dermatol 80:133–159

Graham JH, Mazzanti GR, Helwig EB (1961) Chemistry of Bowen's disease: Relationship to arsenic. J Invest Dermatol 37:317–330

Gross G, Wagner D, Hauser-Brauner B, Ikenberg H, Gissmann L (1985) Bowenoide Papulose und Carcinoma in situ der Cervix uteri bei Sexualpartnern. Ein Beispiel für die Übertragbarkeit der HPV-16-Infektion. Hautarzt 36:465–469

Grussendorf EI, Bär Th (1977) Condylomata acuminata associated with Morbus Bowen (Carcinoma in situ). A light and electron microscopic study. Dermatologica 155:50–58

Gupta J, Pilotti S, Rilke F, Shah K (1987) Association of human papillomavirus type 16 with neoplastic lesions of the vulva and other genital sites by in situ hybridization. Am J Pathol 127:206–215

Halliday GM, McArdle JP, Knight BA, Muller HK (1986) New methodology for assessment of the Langerhans cell network. J Pathol 148:127–134

Henke RP, Milde-Langosch K, Löning Th, Stromme Koppang H (1987a) Human papillomavirus type 13 and focal epithelial hyperplasia of the oral mucosa: DNA hybridization on paraffin-embedded specimens. Virchows Arch A 411:193–198

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxydase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Ikenberg H, Gissmann L, Gross G, Grussendorf-Conen EI, Hausen H zur (1983) Human papilloma virus type 16 related DNA in genital Bowen's disease and in bowenoid papulosis. Int J Cancer 32:563–565

Jablonska St, Obalek S, Orth G (1986) Bowenoide Papulose und ihr Zusammenhang mit Bowen'scher Krankheit und atypischen Kondylomen. Z Hautkr 61:563–565

Kao GF (1986) Carcinoma arising in Bowen's disease. Arch Dermatol 122:1124–1126

Lever WF, Schaumburg-Lever G (1983) Histopathology of the skin. Ed. 6. J.B. Lippincott, Philadelphia, pp 377–378, 496–498

Löning Th, Staquet MJ, Thivolet J, Seifert G (1980) Keratin polypeptides distribution in normal and diseased human epidermis and oral mucosa. Virchows Archiv A 388:273–288

Löning Th, Broemel H, Becker WM, Otto HF (1983) Identification of inflammatory cell phenotypes in human oral carcinomas by means of monoclonal antibodies. Arch Oral Biol 28:1093–1100

Löning Th, Milde K (1987) Viral tumor markers. Curr Top Pathol 77:339–365

Macnab JCM, Walkinshaw SA, Cordiner JW, Clements JB (1986) Human papillomavirus in clinically and histologically normal tissue of patients with genital cancer. N Engl J Med 315:1052–1058

McArdle JP, Knight BA, Halliday GM, Muller HK, Rowden G (1986a) Quantitative assessment of Langerhans cells in actinic keratosis, Bowen's disease, keratoacanthoma, squamous cell carcinoma and basal cell carcinoma. Pathology 18:212–217

McArdle JP, Muller HK (1986b) Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection. Am J Obstet Gynecol 154:509–515

Meissner K, Haftek M, Arlot M, Mauduit G, Thivolet J (1986) Quantitative analysis of T 6-positive Langerhans cells in human skin cancers. Virchows Arch A 410:57–63

Moll R, Moll I, Franke WW (1986) Intermediärfilamente als Kriterium bei der Diagnostik von Hauttumoren. Pathologe 7:164–174

Mora RG, Perniciaro C, Lee B (1984) Cancer of the skin in blacks. III A review of nineteen patients with Bowen's disease. J Am Acad Dermatol 11:557–562

Morris HB, Gatter KC, Sykes G, Casemore V, Mason DY (1983) Langerhans' cells in human cervical epithelium: effects of wart virus infection and intraepithelial neoplasia. Br J Obstet Gynecol 90:412–420

Nakajima T, Watanabe S, Sato S, Shimosato Y, Motoi M, Lennert K (1982) S-100 protein in Langerhans' cells, interdigitating cells and histiocytosis X cells (1982) Gann 73:429–432

Nomori H, Watanabe S, Nakajima T, Shimosato Y, Kameya T (1986) Histiocytes in nasopharyngeal carcinoma in relation to prognosis. Cancer 57:100–105

Patterson JW, Kao GF, Graham JH, Helwig EB (1986) Bowenoid Papulosis. A clinicopathologic study with ultrastructural observations. Cancer 57:823–836

Quevedo WC Jr., Fitzpatrick TB, Szabo G, Jimbow K (1987) Biology of melanocytes. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (eds) Dermatology in general medicine, 3rd Edition. McGraw-Hill Company, New York, pp 230–233

Ralfkiaer E, Stein H, Plesner T, Hou-Jensen K, Mason D (1984) In situ immunological characterization of Langerhans cells with monoclonal antibodies: comparison with other dendritic cells in skin and lymph nodes. Virchows Arch A 403:401–412

Reid R, Stanhope CR, Herschman BR, Booth E, Phibbs GD, Smith JP (1982) Genital warts and cervical cancer. I. Evidence of an association between subclinical papillomavirus infection and cervical malignancy. Cancer 50:377–387

Reid R, Crum CP, Herschman BR, Fu YS, Braun L, Shah KV, Agronow SJ, Stanhope CR (1984) Genital warts and cervical cancer. III. Subclinical papillomaviral infection and cervical neoplasia are linked by a spectrum of continuous morphologic and biologic change. Cancer 53:943–953

Scherer C, Haensch R (1984) Bowenoide Papulose der Genitoanalregion. Fortschr Med 5:31–32

Schröder S, Schwarz W, Rehpenning W, Löning Th, Böcker W (1987) Dendritic/Langerhans cells and prognosis in patients with papillary thyroid carcinomas. Am J Clin Pathol: 89:295–300

Smolle J, Soyer HP, Ehall R, Bartenstein S, Kerl H (1986) Langerhans cell density in epithelial skin tumors correlates with epithelial differentiation but not with the peritumoral infiltrate. J Invest Dermatol 87:477–479

Stingl G, Katz SI, Green I, Shevach EM (1980) The functional role of Langerhans cells. J Invest Dermatol 74:315–318

Tsujitani S, Furukawa T, Tamada R, Okamura T, Yasumoto K, Sugimachi K (1987) Langerhans cells and prognosis in patients with gastric carcinoma. Cancer 59:501–505

Vanstapel MJ, Gatter KC, de Wolf-Peeters C, Mason DY, Desmet VD (1986) New sites of human S-100 immunoreactivity detected with monoclonal antibodies. Am J Clin Pathol 85:160–168

Verschueren H (1985) Interference reflection microscopy in cell biology: methodology and applications. J Cell Sci 75:279–301

Wade TR, Kopf AW, Ackermann AB (1979) Bowenoid papulosis of the genitalia. Arch Dermatol 115:306–308

Wells M, Griffiths S, Lewis F, Bird CC (1987) Demonstration of papillomavirus types in paraffin processed tissue from human anogenital lesions by in-situ DNA hybridization. J Pathol 152:77–82

Zur Hausen H (1977) Human papillomaviruses and their possible role in squamous cell carcinomas. Curr Top Microbiol Immunol 78:1–30

Accepted August 29, 1987