LETTERS to the EDITOR

Immunohistochemical detection of papillomavirus antigens in Kaposi's sarcoma

SIR,—In this week's issue (p 515) some of us, with other colleagues, in New York, report molecular biological data indicating a possible relation between human papillomavirus (HPV) and Kaposi's sarcoma (KS). Because epithelial cells seem to be the dominant source for replication of HPV and productive infection in the skin,[1] we decided to examine biopsy specimens from patients with cutaneous KS immunohistochemically, to see if keratinocytes express HPV-related antigen (PRA).

From reviewing paraffin-embedded archival KS tissue 11 skin biopsies from nine AIDS patients were immunostained, as were 6 skin samples from four elderly patients with classic KS and 3 from a chronic renal failure patient who had received a kidney transplant. The antiserum (Dako Corp, Santa Barbara, California)[2] was used at 1 in 500 dilution in combination with avidin-biotin peroxidase staining, following blocking of endogeneous peroxidase activity, with 3-amino-9-ethylcarbazole as the chromogen.[3] In no specimen was any epidermal cell (keratinocyte or Langerhans') PRA positive and no keratinocytes in KS displayed koilocytotic changes, multinucleation, or viral inclusions. (In both vulvar condyloma and cutaneous verruca, the same antibody only reacted with the nuclei of koilocytotic cells in the upper epithelial layers, and not with any dermal cells.) However, beneath the basement membrane zone, dermal dendrocytes in both perilesional tissue and within the KS lesion itself (fig 1) were stained positively in 14 (70%) of the 20 KS lesions (8/11 AIDS KS, 4/6 classic KS, 2/3 transplant-associated KS). At least two different lots of antisera against HPV were tested (with equivalent results); no staining was seen when normal rabbit serum was used as control or if the primary antibody was omitted; and 3 biopsies of skin from AIDS patients with dermatitis were also negative (both epidermis and dermis). While 1 AIDS-related psoriasis specimen was negative a study of 10 additional non-AIDS-related psoriatic plaques revealed that 6 had PRA immunoreactive dermal dendritic cells but no epidermal positivity. This suggests that there may be some situations in which dermal dendritic cells express a cross-reactive protein not related to HPV (unless HPV has a role in psoriasis).

We did two other experiments. We used a different monoclonal antibody diluted 1 in 5000 (MAB 837; Chemicon International, Temecula, California) to detect HPV proteins (HPV 1, 6, 11, 16, 18, and 31) on the same set of tissues. Except for the condyloma and verruca, which had positive nuclear staining of epidermal koilocytotic keratinocytes, all the KS and psoriatic specimens were uniformly non-reactive in both epidermal and dermal compartments. We also used a polyclonal rabbit anti-HPV-16-specific antibody to the early region protein, E7 (provided by Dr Douglas Lowy and Dr John Shiller, National Institutes of Health). This antibody was used at a 1 in 1000 dilution with 3,3-diaminobenzidine as chromagen and non-immune rabbit serum as negative control. A KS lesion found to contain HPV-16 by polymerase chain reaction (PCR) revealed positive intranuclear staining of only the dermal spindle-shaped cells, whereas KS lesions that were negative by PCR had no intranuclear staining in dermis or epidermis (fig 2). The HPV-16-specific E7 antibody may be a more specific marker than the other commercially available antibodies, which detect capsid or late-appearing HPV gene products.

The striking PRA immunoreactivity of dermal dendrocytes in these KS specimens was unexpected. The lack of epidermal reactivity is also very surprising in the light of current understanding of the pathophysiology of cutaneous HPV infection.[1] Confirmatory studies with molecular probes are in progress. Although the positive immunostaining results, especially those in non-AIDS-related psoriasis, demand DNA/RNA studies, the uniformly negative epidermal reactivity in AIDS-related specimens suggests that keratinocytes are not the site of productive infection in these patients. It should be noted that transgenic mice containing bovine papillomavirus genome develop dermal tumours with prominent collections of spindle-shaped cells resembling dermatofibromas.[4] We demonstrated previously that human dermatofibromas and KS lesions share histological features, including increased collections of dermal dendrocytes.[5]

The aetiology and pathogenesis of KS remain a puzzle. The cell of origin used to be thought of as the endothelial cell[6] but a relation between cultured KS cells and dermal dendrocytes (a subset of bone-marrow-derived monocyte/macrophages) has also been considered.[7] Dermal dendritic cells were prominent constituents of the multicellular population observed in early KS[5,7] and unpublished studies of KS cell lines containing HPV DNA sequences revealed that they expressed monocyte/macrophage but not endothelial cell markers. Mahoney et al[8] have demonstrated HIV transcripts co-localising to dermal dendrocytes in three of eight HIV-infected patients. However, there is epidemiological evidence[9,10] that some sexually transmitted infectious agent other than HIV may have a critical role in KS. Much more work is needed to find out if the immunoreactivity reported here is recognising a known strain of HPV or whether a novel non-epidermotropic strain is involved in KS (or, indeed, an entirely different virus with a cross-reactive capsid protein); and the possibility of endogenously derived non-virus-associated cross-reactive proteins also deserves

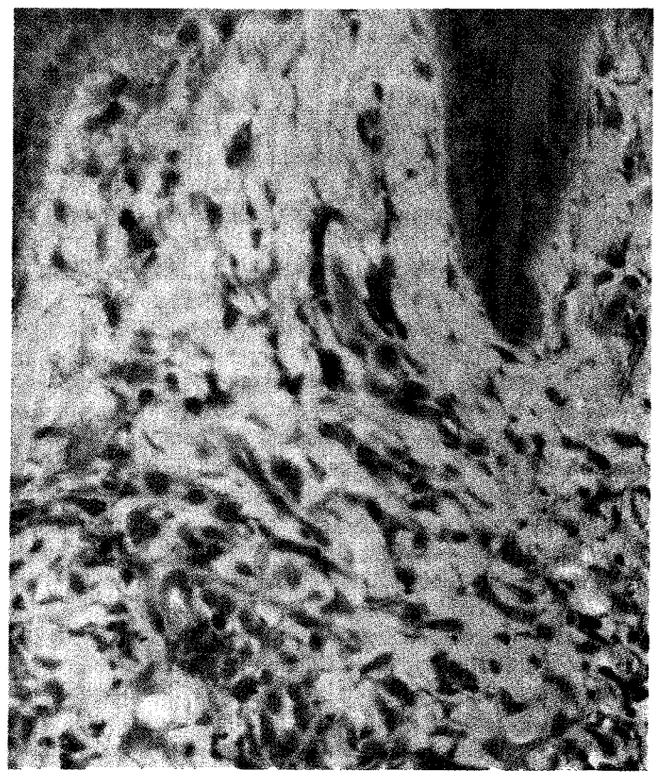

Fig 1—Cutaneous KS lesion with prominent PRA immunoreactive dermal dendritic cells in both angiocentric and interstitial pattern.

Immunoperoxidase staining with red chromogen reaction product, counterstained with haematoxylin, × 60.

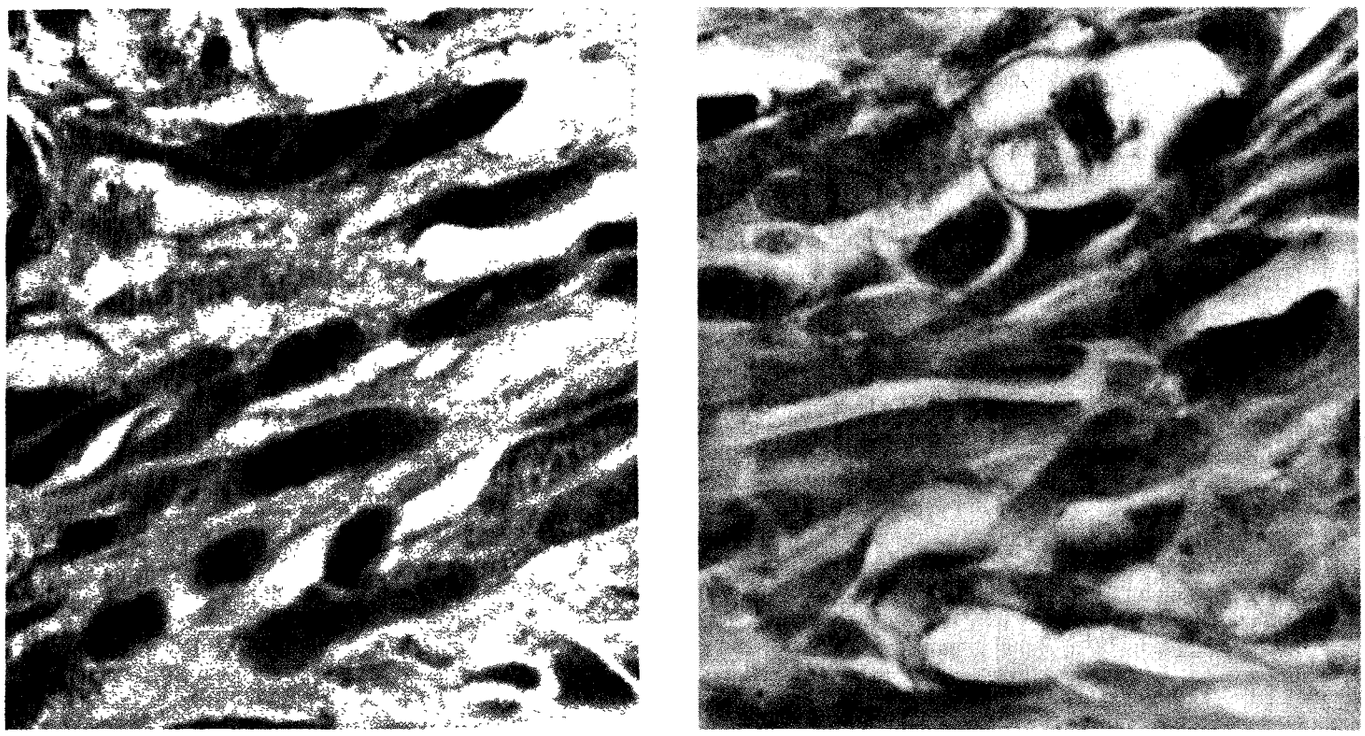

Fig 2—Cutaneous KS lesions examined by PCR and concomitant immunoreactivity for HPV-16-specific E7 protein.

Left strong nuclear immunoreactivity in dermal spindle-shaped cells from PCR-positive case (brown chronogen reaction product), × 200. Right. absence of immunoreactivity in PCR-negative case, × 200

attention. Nonetheless, this letter and the article in this issue will, we hope, prompt colleagues to consider papillomaviruses as possible KS agents.

Dr J. T. Headington kindly reviewed slides and the text.

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Thalidomide for systemic lupus erythematosus

SIR,—Treatment for systemic lupus erythematosus (SLE) is largely symptomatic, with antimalarials, glucocorticoids, and immunosuppressive drugs.[1] Thalidomide has proved successful in the treatment of chronic discoid lupus erythematosus (CDLE),[2] antimalarial-resistant CDLE,[3] subacute cutaneous lupus erythematosus,[4] and lupus erythematosus profundus.[5] However, thalidomide treatment of SLE has not yet been investigated in detail.[6] We report 3 patients with SLE, confirmed according to the American Rheumatism Association 1982 criteria.

Patient 1—A 30-year-old woman had progressive acute SLE for 6 years (onset 1985) with multivisceral features—cutaneous, articular, muscular, and neurological (severe polyneuropathy of the lower limbs)—and impure renal nephrotic syndrome with segmental and focal glomerulonephritis, pancytopenia, and Raynaud's syndrome. Immunological screening showed positive antinuclear antibodies (ANA), anti-DNA-antibodies (titre 1/280), anti-smooth muscle (SM) antibodies, and hypocomplementaemia. Various treatments were instituted successively: corticosteroid (per os or bolus injection) with corticodependence (25 mg per day), cyclophosphamide, plasmapheresis, and cyclosporin. She had severe infectious episodes with renal deterioration with these treatments. $3\frac{1}{2}$ years after onset of SLE (July, 1988) thalidomide (100 mg daily) combined with glucocorticoids (40 mg daily) were instituted. Tolerance of this treatment was adequate with no side-effects. Clinical improvement, with appreciably reduced skin and articular features and without neuropathological deterioration, led to a progressive decrease in glucocorticoid dose. Thalidomide therapy was maintained for 2 years at daily doses of 25–100 mg. No changes in immunological indices (ANA, anti-DNA antibodies, and complement) were seen. The patient has since been kept on maintenance corticosteroid therapy.

Patient 2—A 27-year-old woman had SLE for 5 years (onset 1986) with skin lesions and articular features (but no other visceral symptoms) and immunological disorders: ANA (1/640), anti-DNA antibodies, and anti-SM antibodies. Initial treatment with synthetic antimalarials failed, and thalidomide (200 mg daily) was begun in March, 1988. Cutaneous features almost completely disappeared after 3 months of treatment and articular symptoms decreased slightly. After 6 months on thalidomide she had serious drowsiness, despite a reduction in dose. Treatment was stopped in November, 1988, which led to recrudescence of skin and articular features. General corticosteroid therapy was then instituted with adequate therapeutic response.

Patient 3—A 26-year-old woman had SLE for 5 years (onset 1986) with skin lesions and articular features and immunological disorders: ANA (1/1024), anti-SM antibodies, and hypocomplementaemia. General corticosteroid therapy after initial