Activation of latent papillomavirus genomes by chronic mechanical irritation

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The skin of animals of a laboratory strain of Mastomys natalensis carrying endogenous, latent papillomavirus genomes was irritated by scratching with glasspaper. Hyperproliferation of the epidermis and amplification of viral DNA followed this treatment, and in approximately 27% of the animals virus-producing papillomas were induced.

Recurrent virus infections are a severe medical problem and a curative treatment strategy has not been developed. The mechanism by which virus infections become latent and the events which reactivate such viruses are not known. Certain papillomavirus types show a high frequency of latency; in man, human papillomavirus (HPV) types 6, 11, 16 and 18 (Boshart et al., 1984; Dürst et al., 1983) persist in clinically normal tissues of the larynx and the urogenital tract (Steinberg et al., 1983; de Villiers et al., 1987). Latent HPV genomes can be detected in more than 60% of normal tissue samples taken from areas adjacent to surgically removed papillomas from cases of recurrent genital papillomatosis (Ferenczy et al., 1985), and papillomas reoccur in close proximity to laser surgery wounds or directly in the healed wound a few weeks after surgery.

To determine whether skin regeneration, induced by mechanical irritation, might activate latent papillomavirus genomes, we studied a laboratory strain of the rodent Mastomys natalensis, which carries an endogenous, latent papillomavirus (MnPV) (Amtmann et al., 1984; Amtmann & Wayss, 1987).

Mechanical irritation was performed by scratching the skin with fine glasspaper. As this treatment affects only the upper layers of the epidermis we expected epidermal cell proliferation to be induced. To test this assumption, the skin of animals was irritated with glasspaper and, at various times, the number of cell nuclei per mm of interfollicular epidermis was determined (Fig. 1); the epidermis became hyperplastic within 12 h, the most intensive hyperplasia being observed between 48 and 72 h after treatment, followed by reversion to the normal epidermal appearance within a few days. When the skin of animals was irritated twice a week with glasspaper, a stationary hyperplasia of the epidermis was induced (data not shown).

The effect of chronic mechanical irritation on the number of MnPV genomes in the epidermis was studied by Southern blot analysis after 8 weeks of treatment. The DNA of treated and untreated areas of skin from the back of 15 animals was isolated, and the amount of viral DNA per cell was determined by Southern blotting. As an example, the results obtained from four animals are shown in Fig. 2; in all cases more viral DNA was detected in the irritated samples. The number of viral DNA molecules per skin cell was estimated by densitometry, which was calibrated using defined amounts of MnPV DNA. On average, the amount of viral DNA in irritated skin was 58.7-fold that in untreated skin ($P < 0.05$). In control experiments, we found that the amount of viral DNA in two different skin samples from the same animal did not differ by more than 10% on average (data not shown).

To study the effects on tumour formation the skin of animals was irritated with glasspaper for 67 weeks (Fig. 3). After this period, eight of 30 animals had tumours on the back, the first tumour appearing after 12 weeks; in contrast, of the animals in the control group, only one developed a tumour on the back, which appeared after 49 weeks. The difference between these groups was statistically significant ($P < 0.05$). All back skin tumours were histologically identified as so-called keratoacanthomas (Fig. 4a), the typical lesion induced by MnPV, and they contained several thousand copies of MnPV DNA per cell (data not shown).

The tumours which were induced by scratching were screened for MnPV production by indirect immunofluorescence using an MnPV-specific $M. natalensis$ serum and fluorescein isothiocyanate-conjugated sheep anti-mouse IgG antiserum (Amtmann & Wayss, 1987). In all cases fluorescence was observed in the nuclei of the keratinized layers of the tumour. The basal layers of the
Fig. 1. Induction of epidermal hyperplasia after scratching of the skin once with glasspaper. The hair was removed from 10-week-old *M. natalensis* with electric clippers and 1 week later the animals were scratched with glasspaper. After various times animals were killed, treated and untreated skin was removed, and microtome sections were prepared after fixation in formalin and embedding in paraffin. After staining with haematoxylin and eosin, the number of epidermal nuclei per mm interfollicular skin (IFE) was evaluated microscopically. ■, Treated males; ○, treated females; ●, untreated males; □, untreated females. Each point represents the average of three animals (±s.d.).

Fig. 2. Amplification of MnPV-specific DNA sequences in the epidermis after chronic wounding. Ten-week-old animals were scratched with fine glasspaper twice a week on the back skin; an area of approximately 200 mm² was treated. After 8 weeks animals were killed, and DNA was isolated from treated and untreated skin. Each DNA preparation (20 µg) was run uncleaved on 1% agarose gels and MnPV-specific DNA sequences were detected by Southern blotting (Amtmann & Sauer, 1982; Krieg *et al.*, 1983). Lanes 1 and 2, animal 1; lanes 3 and 4, animal 2; lanes 5 and 6, animal 3; lanes 7 and 8, animal 4; lanes 1, 3, 5 and 7, treated skin samples; lanes 2, 4, 6 and 8, untreated skin samples.

Fig. 3. Induction of keratoacanthomas in the back skin of animals by scratching with glasspaper. The skin of a group of 30 animals (10 weeks old) was scratched twice weekly with glasspaper. Another 30 animals were not treated and served as controls. The animals were examined for tumour formation every week. ○, Control animals; ●, treated animals.

tumour and the adjacent normal tissue displayed no specific fluorescence. The results from one tumour are shown in Fig. 4(b and c).

By scratching with glasspaper a stationary hyperplasia was induced in the epidermis of *M. natalensis*. As a consequence of this treatment, endogenous, latent papillomavirus genomes were amplified and MnPV-producing skin tumours appeared at the site of treatment. In a previous paper we have shown that the same effect can be achieved by topical application of the tumour promoter tetradecanoyl-phorbol-13-acetate (TPA) (Amtmann *et al.*, 1984), which is also a strong inducer of epidermal hyperplasia. The virus-inducing activity of TPA probably resides in its hyperplasiogenic activity rather than in its tumour-promoting property because retinoyl-phorbol-13-acetate, which has only hyperplasiogenic activity (Fürstenberger *et al.*, 1981), activates MnPV genomes to the same extent as TPA (unpublished results). TPA induces trans-acting factors, such as the serum response factor, that bind to the regulatory regions of a number of viruses and induce the
expression of viral genes. The same trans-acting factors are induced by growth factors which are liberated after wounding in order to initiate tissue regeneration.

In those cases in which latent viral genomes persist in the neighbourhood of a papilloma after surgical removal of the lesion and tissue regeneration, papillomavirus genomes are activated and new warts appear. Therefore a cure for recurrent laryngeal papillomatosis or genital condylomas must avoid the activation of latent viral genomes.

References


(Received 28 February 1991; Accepted 8 July 1991)