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SKIN VIRULENCE OF ATTENUATED PSEUDORABIES VIRUS (PRV) STRAINS

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Summary. - After intradermal inoculation of the attenuated pseudorabies virus (PRV) strains, Bartha, MK 35 (gI^+) and MK 35-T-2 (gI^-) to rabbits, they caused local inflammatory nodules with oedema reddiness and necrosis. The specifity of these reactions was confirmed by their inhibition with anti-PRV serum. The minimal dose that caused visible skin reaction was about 10^2 $TCID_{50}$/ml for Bartha and MK 35-T-2 (gI^-) strains and over 10^4 $TCID_{50}$/ml for the MK 35 (gI^+) strain. It is assumed that the established residual skin virulence can serve as an additional distinction marker for attenuated PRV strains.

Key words: pseudorabies virus; virulence; attenuated vaccines

Although many herpesvirus infections cause local skin reactions, the PRV virulence is settled using its ability to cause lethal encephalitis in susceptible animals. Many attenuated strains that do not cause letal encephatilits after subcutaneous inoculation have been obtained during the last decades (Pensaert and Kluge, 1989). These are successfully used as vaccines and differ mainly by deletions in their genomes, determinning the absence of certain viral proteins (Lomniczi *et al.*, 1984; Mettenleiter *et al.*, 1989). The differences between their residual virulence are not completely specified and are currently under investigation (Mengeling *et al.*, 1990; Rziha *et al.*, 1990). In order to establish the differences in residual virulence of some attenuated PRV strains, we examined their ability to cause skin alternations in susceptible animals.

Attenuated strains MK 35 (gI^+) and MK 35-T-2 (gI^-) (Tatarov, 1990) and the Bartha strain obtained from „Filaxia" - Budapest were used. The strains were propagated in chick embryo cells (CEC) and lyophylized with infectious titres of 10^7, 10^6 and $10^{5.5}$ $TCID_{50}$/ml respectively (Fig. 3). Rabbits with 3 kg body weight, guinea pigs, one year old sheep and two months old pigs, negative for PRV antibodies were used, three animals were included into each experimental group. Ten-fold dilutions of each PRV strain were inoculated intradermally into the cut skin or rubbed into the scarificated skin. As controls, each animal was inoculated in the same way with uninfectious cell culture medium. The results were read by appearance of skin changes and examined histologically after fixation with 10 % formalin, and haemotoxylin-eosin staining. Tissue samples were treated and inoculated in cell cultures for virus reisolation. To prove the specifity of the skin changes, 10^4 $TCID_{50}$ of the strain MK 35-T-2 (gI^-) were mixed with two-fold dilutions of a anti

PRV serum with a neutralization titre 1:32 or with control foetal swine serum. After one hour incubation at 37 °C these mixtures were inoculated intradermally to rabbits.

We inoculated undiluted virus strains intradermally or by scarification to pigs, sheep, guinea pigs and rabbits (3 animals each). After scarification the skin changes did not appear in each animal. Neither appeared visible changes after intradermal inoculation of pigs and sheep. Slight reddening was observed after intradermal inoculation of guinea pigs.

By 24 hours after intradermal inoculation of the PRV strains to rabbits distinctive local indurations appeared, namely red local swellings with a diameter of 1.5 - 2.0 cm around the inoculation spot (Fig. 1). By time the local inflammatory nodules extented slightly and necrotic lesions appeared in their centre.

Microscopically we found a marked inflammatory reaction (Fig. 2a, b), which involed the epidermis, dermis and the subcutaneous connective tissue. Necrotic areas were seen in the epidermis. In the dermis hyperaemia, oedema, haemorrhages and marked round cell infiltration were seen. In other areas granulocytes and increased number of eosinophilic cells were found. Large cells with dark nuclei were also observed as well as vasculitis and folliculitis. PRV strains were reisolated from some inflammatory nodules.

Intradermal inoculation of mixtures of MK 35-T-2 strain and dilutions of anti-PRV serum caused local inflammatory nodules only when serum dilutions were over 1:16. The control mixture with foetal serum caused marked nodules confirming the specifity of skin reaction.

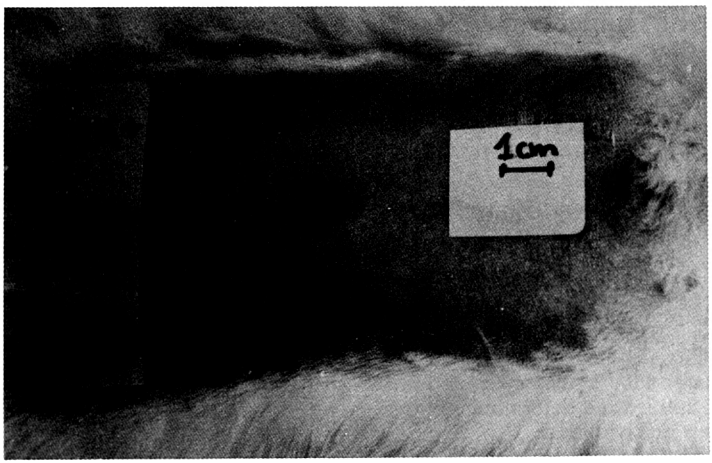

Fig. 1
Inflammatory skin reactions 48 hr after intradermal inoculation of dilutions of attenuated PRV strains

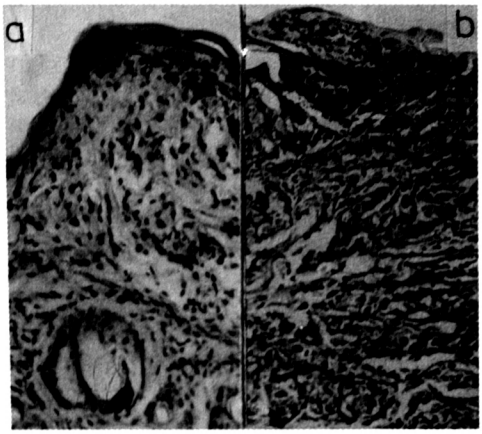

Fig. 2
Histologic picture of the local skin lesion. Inflammatory reaction with cell infiltration, haemorrhages and partial epidermal necrosis (b)

In order to compare the infectious titres in CEC with skin virulence, we inoculated intradermally 10-fold dilutions of each strain. The titre of the established skin virulence as compared with the infectious titres in CEC is presented in Fig. 3. It shows that the skin virulence was by 2 $\log_{10}$ lower as the titres in CEC for strains Bartha and MK 35-T-2 (gI^-), but for the strain MK 35 (gI^+) this difference was over 4 $\log_{10}$. Thus the minimal skin reactive dose for the strains Bartha and MK 35-T-2 (gI^-) was about 10^2 $TCID_{50}$ while for the strain MK 35 (gI^+) it was over 10^4 $TCID_{50}$.

Our investigations show that some attenuated PRV strains, not causing death, reproduce locally in the skin in intradermally inoculated rabbits and cause local inflammatory nodules. The histological changes in the lesions are represented by inflammatory round cell infiltration, haemorrhages and partial necrosis of the epidermis. The inhibition of the inoculated strains by

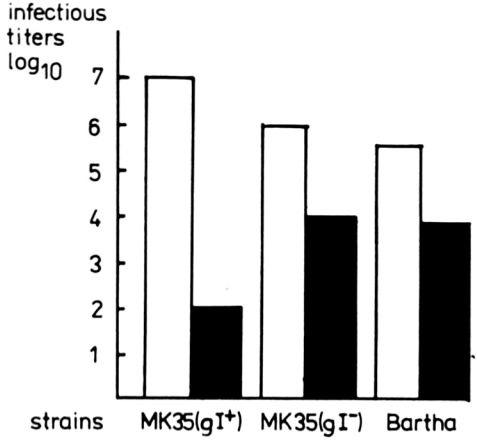

Fig. 3
Comparison between the infectious titres of the attenuated PRV strains measured in Ch. F. cell culture and determined by their skin virulence
It is obvious that the minimal dose that causes visible skin reaction is about 10^2 $TCID_{50}$/ml for Bartha and MK 35-T-2 (gI^-) strains and over 10^4 $TCID_{50}$/ml for the MK 35 (gI^+) strain.

anti-PRV serum confirmed the specifity of the local changes and point at the possibility to detect anti-PRV antibodies by inhibition of the skin reaction.

Our results show that the attenuated PRV strains although loosing their ability to cause lethal encephalitis, still possess residual skin virulence at intradermal inoculation in rabbits. It is interesting that this residual virulence is weaker in strain MK 35 (gI^+) than in strains Bartha and MK 35-T-2 (gI^-). Presumably this residual skin virulence is determined by some viral proteins and may serve as a differentiating marker of the attenuated PRV strains.

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