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INVOLVEMENT OF PROSTAGLANDINS IN IMMUNOSUPPRESSION CAUSED BY ADENOVIRUS IN MICE

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Summary. — The effect of intraperitoneally (i.p.) inoculated human adenovirus type 6 (Ad6) was tested for humoral immune response against sheep red blood cells (SRBC) in normal and indomethacin-treated mice, with the aim to elucidate the mode of the virus action in immunosuppression. The results indicate that inhibition of prostaglandin synthesis slightly influences the immunosuppressive effect of the virus. It is verly likely that also other mechanisms are involved in the immunosuppression observed.

Key words: human adenovirus type 6; mice; prostaglandins; immunosuppression; macrophages

Introduction

Infection with Ad6 severely inhibits the humoral immune response in mice. One of the direct targets for virus action is the macrophage, since immunosuppression is prevented by pretreatment of mice with silica, which depresses macrophage function (Berencsi *et al.*, 1982, 1985). It is not known how macrophages influence the immune response. One possibility is that macrophages mediate immunosuppression by releasing suppressor factors. From all products released by macrophages, prostaglandins seem a likely candidate since they inhibit B and T lymphocyte functions (Goodwin, 1980). We, therefore, examined the effect of indomethacin, a well-known inhibitor of prostaglandin synthetase, on immunosuppression caused by Ad6.

Materials and Methods

Mice. Eight-twelve weeks old male CBA mice were used. *Virus.* Ad6 (kindly provided by Dr. R. Wigand, Homburg/Saar) was grown in HEp-2 cells, purified by CsCl gradient centrifugation and stored at −70 °C in 20 % glycerol. Mice were inoculated i.p., usually with 10^9 TCD_{50} of virus diluted in physiological saline.

Antigen. SRBC were stored in Alsever's solution at 4 °C and washed 3 times in physiological saline on the day of use. Mice were injected i.p. with 0.2 ml of a suspension containing approximately 5×10^8 erythrocytes.

Drug treatment. Indomethacin (Sigma) was used to inhibit prostaglandin production. The drug was dissolved in 7.5 % sodium bicarbonate. Further dilutions of the drug were prepared in phosphate buffered saline. Indomethacin (5 mg/kg) was administered i.p. 2 hr before and 24 hr after virus treatment. This amount of indomethacin was comparable to the doses used by others (Hart, 1985).

Assay for splenic haemolytic plaque forming cells (HPFC). The Jerne plaque technique was performed as modified by Dresser and Wortis (1967). High-efficiency haemolysin producing cells, referred to as 19S HPFC, were detected. The number of HPFC per spleen was calculated. The reported results are the mean HPFC counts from 6 mice.

Results

First, the toxicity of indomethacin was tested in mice. CBA mice were inoculated i.p. with 5 and 10 mg/kg indomethacin on two consecutive days and observed for survival during an 11-day period. The 5 mg/kg indome-

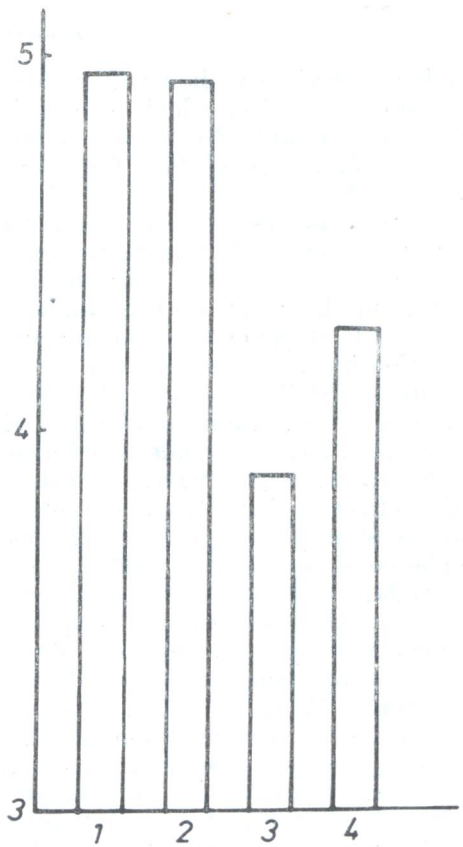

Fig. 1.
Primary direct HPFC responses to SRBC in CBA mice
Mice were inoculated with 5 mg/kg indomethacin 2 hr before and 24 hr after Ad6 infection. SRBC were given 5 days after virus inoculation and the number of HPFC was determined 4 days after immunization. Each bar indicates the mean HPFC of 6 mice.

Abscissa: 1 — SRBC; 2 — indomethacin and SRBC; 3 — Ad6 and SRBC; 4 — indomethacin, Ad6 and SRBC
Ordinate: number of HPFC per spleen/ ($\log_{10}$ values)

thacin proved not to be toxic, but 80 % of the mice treated with 10 mg/kg indomethacin did not survive. Therefore, in the experiment comparing the immunosuppressive activity of Ad6 in normal mice with that in indomethacin-treated mice, a nontoxic dose of the drug, 5 mg/kg, was used. Mice were inoculated i.p. with the drug, and 2 hr later they were infected with Ad6, and another injection of indomethacin was given 24 hr later. Five days after virus infection, SRBC were administered and the number of HPFC in the spleen was determined on the 4th day after antigen inoculation (Fig. 1). The results indicated that Ad6 treatment was associated with inhibition of antibody formation. Indomethacin treatment itself did not result in enhancement of the number of antibody forming cells. In mice treated with Ad6 and indomethacin, the number of antibody forming cells was higher than in mice infected with Ad6 only. Five mg/kg indomethacin given in two doses resulted in slight reversal of immunosuppression. The number of HPFC in the spleen of drug-treated mice was 2.5× higher than that for mice infected with Ad6 only.

Discussion

We have previously shown that Ad6 has the ability to induce immunosuppression in mice, which mechanism, however, is not well understood. Ad6 is able to induce circulating interferon, which has an important immunoregulatory function (Johnson, 1982, De Maeyer *et al.*, 1982), but the role of interferon in immunosuppression was ruled out by experiments testing the immunosuppressive effect and interferon inducing capacity of UV-inactivated virus. It was found that in response to UV irradiation Ad6 had rapidly lost its immunosuppressive activity, but the interferon inducing ability did not change. The suppressive effect of Ad6 depended on the inoculation sites of the virus and SRBC. Inoculation by the same i.p. route resulted in immunosuppression, but when the virus and SRBC were administered at different sites, no inhibitory effect could be detected. The results indicate that the effect of the virus was manifested locally. Next we made experiments with silica, an agent known to be toxic for macrophages, which almost completely reversed the immunosuppressive effect of Ad6. The above observations indicate that in our experimental system peritoneal macrophages mediate the suppressor activity of Ad6 (Berencsi *et al.*, 1982, 1985). It is widely recognized that macrophages not only behave as scavenger cells, but also play a central role in the induction and control of immune response. Among their immunoregulatory functions, macrophages possess a strong suppressor activity. Macrophage-mediated suppressor activity can be modified by several agents (Wing and Remington, 1977, Knop, 1980). It is very likely that function of macrophages is influenced by Ad6 infection and this altered function accounts for the immunosuppression observed. Macrophages are able to produce prostaglandins, which are known to be an important mediator of the suppressive activity of resident and activated peritoneal macrophages (Metzger *et al.*, 1980, Nathan *et al.*, 1980). Little is known about the effects of infections

resulting in host immunosuppression on prostaglandin production by macrophages. Fierer *et al.* (1984) reported changes in synthesis of prostaglandins during the course of trypanosoma infection, these changes contributing to the immune dysfunction. Infection by a number of viruses is associated with immunosuppression, but it was shown in dengue-virus infected mice only that the inhibition of the immune response is partly mediated through prostaglandin pathway (Shukla and Chaturvedi 1981). In our system, treatment of mice with the prostaglandin synthetase inhibitor indomethacin did not result in pronounced reversal of immunosuppression: only a slight enhancement of the immune response could be observed. This suggests that different mechanisms, possibly involving other suppressive molecules or other impaired functions of macrophages, might be more relevant.

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