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EFFECT OF HUMAN ADENOVIRUS TYPE 6 ON THE PRIMARY IMMUNE RESPONSE IN MICE

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Summary. — CFLP and BALB/c mice inoculated intraperitoneally with large doses of adenovirus type 6 (Ad6) showed a decreased humoral immune response to sheep red blood cells (SRBC) and circulating interferon was detected in their serum. The timing of infection was critical. Infection of mice 3—11 days before SRBC administration led to depression of the 19 S haemolytic plaque forming cell (HPFC) response in the spleen. When mice were given Ad6 and SRBC simultaneously on Ad6 14 days before or 1 day after SRBC, there was no decrease in the number of HPFC. The suppressive effect was dependent on the dose of virus and antigen. Heat and UV treatment completely abolished the immunosuppressive effect of the virus, suggesting that a great amount of infectious adenovirus is needed to induce immunosuppression in mice.

Key words: human adenovirus type 6; mice; immunosuppression; interferon induction

Introduction

Chickens infected with various types of human adenoviruses 3—16 days before primary immunization with SRBC produced one tenth or less of the normal peak number of splenic direct HPFC (Béládi *et al.*, 1973). It was suggested that this immunosuppression is due to interferon induced by adenoviruses.

In the present study we investigated the effect of adenovirus type 6 on the primary immune response of mice to get more information about the mode of action of adenovirus on the immune system. We showed that AD6 produces immunosuppression and induces interferon in mice. The interferon, however, does not seem to be involved in the immunosuppression in this system.

Materials and Methods

Mice. Eight-twelve weeks old male CFLP and BALB/c mice were used.

Viruses. Human adenovirus type 6 (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 intraperitoneally (i.p.), usually with $10^{9.5}$ TCD_{50} of virus diluted

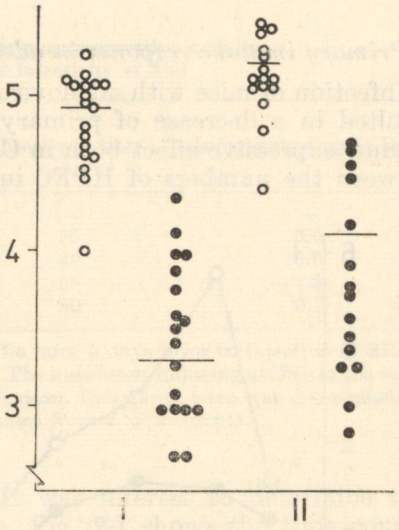

Fig. 1.

Primary direct HPFC responses to SRBC in CFLP (I) and BALB/c (II) mice measured 4 days after immunization
Control mice (○); mice infected with Ad6 by day 5 before immunization with SRBC (●). Means of HPFC counts (———). Each point represents 1 mouse. Data came from 3 independent experiments.
Ordinate: number of HPFC per spleen ($\log_{10}$ values)

in physiological saline. Vesicular stomatitis virus (VSV) was propagated in L cells and its infectivity was determined by the plaque method.

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

Assay for splenic plaque forming cells. The Jerne plaque technique was performed as modified by Dresser and Wortis (1967). Briefly, mice were killed by cervical dislocation and removed spleens kept in an ice-water bath. Spleen cell suspension was prepared in a glass homogenizer. Three dilutions of each spleen cell suspension were plated in two Petri dishes. The plates were incubated at 37 °C for 2 hr in an atmosphere of 5% CO_2-air. High efficiency haemolysin producing cells, referred to as 19 S HPFC, were detected following the addition of 1 ml of guinea pig complement diluted 1 : 10 and incubation for 45 min. The number of HPFC per spleen was calculated. The results indicate the mean HPFC counts from 5 mice.

Interferon induction and assay. Infectious or UV-irradiated virus was inoculated i.p. into mice. After appropriate intervals, groups of 5 mice were exsanguinated and their pooled serum interferon was assayed in mouse L cells. The cell cultures in either Petri dishes or Linbro plates were incubated with 2-fold dilutions of the sera for 4 or 24 hr and then challenged with VSV. Titres were recorded as the reciprocal of the dilution which caused a 50% reduction of PFU or prevented the cytopathic effect of VSV in 50% of cells.

UV irradiation of Ad6. Virus (1.3 ml) was irradiated in Petri dishes (4 cm diameter) by a Hanau germicidal lamp (18.8 erg/mm²/sec) under agitation.

Heat inactivation of Ad6. Virus was incubated at 56 °C in a water bath for 5 min. During this heat treatment the virus had completely lost its infectivity.

Results

Detection of interferon in adenovirus-infected mice

First, the production of interferon in adenovirus-infected mice was determined. Sera from these mice exerted an antiviral effect which was due to interferon. The latter was stable at pH 2, trypsin-sensitive, did not sediment at 100 000 ×g for 1 hr and actinomycin D inhibited its antiviral effect. The titre of interferon reached its maximum (32) 6 hr after inoculation, then quickly decreased; after 24 hr there was no detectable interferon in the serum.

Primary immune response in adenovirus-infected mice

Infection of mice with adenovirus 5 days before immunization with SRBC resulted in a decrease of primary immune response (Fig. 1). Ad6 had an immunosupressive effect both in CFLP and in BALB/c mice. The difference between the numbers of HPFC in the spleens of control and Ad6-infected

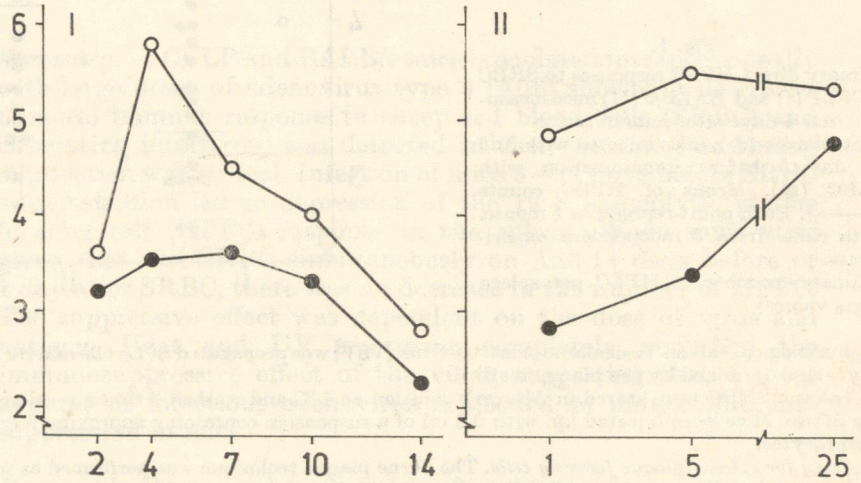

Fig. 2.
Effect of Ad6 on kinetics of the primary immune response to SRBC
I. The virus was given 5 days before the inoculation of SRBC and the number of HPFC in the spleen was determined at different intervals.
Abscissa: days after inoculation of SRBC
II. BALB/c mice were infected with Ad6 5 days prior to injection of various doses of SRBC; the number of HPFC in the spleen was determined 4 days later.
Abscissa: number of SRBC given to a single mice ($\times 10^8$).
Ordinates: number of HPFC per spleen ($\log_{10}$ values).
Each point indicates the mean value out of 5 mice.
Control mice (○); Ad6 infected mice (●).

mice exceeded one log unit. The inhibition of immune response, however, proved to be transient. CFLP mice were given an i.p. injection of Ad6 on days −14, −11, −6, −3, 0, +1 in relation to the injection of SRBC. When mice were inoculated simultaneously or 1 day after SRBC administration, the virus infection resulted rather in a slight enhancement of the number of HPFC in the spleen. Adenovirus injected at intervals from −11 to −3 days inhibited the immune response. A remarkable suppression of 93% was observed on day −6. The suppression was 82 and 63% on days −11 and −3, respectively. Virus infection on day −14 did not influence the number of HPFC.

The results of the preceding experiments showed that Ad6 treatment was associated with an inhibition of antibody formation when spleen HPFC were

Table 1. Effect of UV irradiation on immunosuppressive activity, interferon inducing ability and infectivity of Ad6

UV irradiation time of Ad6 (min)	HPFC/spleen	Interferon titre	Infectivity (log TCD_{50})
—	1.84×10^5	—	—
0	1.07×10^4	96	9.6
5	5.73×10^4	80	9.0
15	1.13×10^5	96	7.3
30	1.29×10^5	80	6.1

The virus was UV irradiated and injected into BALB/c mice 5 days prior to injection of SRBC and the number of HPFC was determined 4 days later. The interferon-inducing ability of the same virus samples was tested in CFLP mice 6 hr after infection. Interferon titre was determined in L cells in Linbro plates. The data indicate the mean values from 2 experiments.

determined 4 days after immunization. It was interest to determine the kinetics of the antibody response in mice. Fig. 2-I shows the time course of the antibody response to SRBC of control and Ad6-infected mice. Ad6 was given 5 days before SRBC and the numbers of HPFC in the spleens were determined at different days after immunization. An inhibition of HPFC was observed in Ad6-infected mice regardless of the day of assay. The effect of Ad6 on the antibody response to various doses of SRBC is shown in Fig. 2-II. Mice were infected 5 days before SRBC administration. At SRBC doses of 10^8 or 5×10^8 the virus had a pronounced inhibitory effect. A slight suppression was still obtained when the number of SRBC injected was as high as 2.5×10^9.

In the next experiment, various amounts of virus were injected into BALB/c mice 5 days before SRBC. A rather high amount of infectious virus was needed to get suppression. Doses of $10^{10.24}$ and $10^{9.24}$ TCD_{50} of virus decreased the formation of HPFC by 93% and 71%, respectively. Less virus had no influence on the immune response.

Primary immune response of mice inoculated with UV-irradiated or heat-inactivated Ad6

Heat-inactivation completely destroyed the ability of Ad6 to diminish the SRBC response of mice. BALB/c mice were inoculated by infectious or heat-inactivated Ad6 by 5 days before SRBC administration. The average number of HPFC in the spleens of control mice was 2.1×10^5. Ad6 infection decreased the number of HPFC to 9×10^3 per spleen. Heat-inactivated Ad6 had no inhibitory effect, the number of HPFC per spleen was 3.2×10^5. To elucidate whether infectious virus, large amount of virus antigens or the interferon produced were responsible for immunosupression, the virus was UV-irradiated for different periods and its immunosuppressive activity, interferon inducing ability and infectivity were studied. Table 1 demonstrates that on UV-irradiation the virus rapidly lost its immunosuppressive effect and its infectivity while its interferon inducing capacity was not influenced by irradiation lasting for up to 30 min.

Discussion

We found that infection of mice with human adenovirus type 6 can modify anti-SRBC PFC response. Ad6 appears to be able to decrease the humoral immune response to SRBC antigen depending on the experimental conditions; furthermore, Ad6 has been shown to induce circulating interferon. The dose of Ad6, the time of its administration relative to antigen challenge and the dose of antigen were critical factors in determining the nature of the effect. The immunosuppressive effect was observed when Ad6 was inoculated 3—11 days before SRBC administration. When Ad6 was inoculated at the same time as antigen or 1 day later, the virus had no suppressive effect but rather a slight immunoenhancing effect could be observed. In mice inoculated with Ad6 14 days before immunization, no effect could be demonstrated. These data indicate that Ad6 has no inhibitory effect on an ongoing immune response, that the immunosuppressive effect is exerted on some early event involved in the induction of antibody production and, furthermore, that the immunosuppression is transient. Our results are compatible with the previous finding on the suppressive effect of human adenoviruses on the formation of HPFC in the spleen of chickens (Béládi *et al.*, 1973).

Many viruses have been shown decrease the immune response (Notkins *et al.*, 1970; Specter *et al.*, 1978). The mechanism by which viruses can depress the immune response of the host is not known. The virus may act by altering or destroying antibody-producing cells, their precursors or macrophages Notkins *et al.*, 1970; Hibbs *et al.*, 1980). In the case of Ad6, a purely toxic effect seems unlikely since UV-irradiated and heat- inactivated virus had no immunosuppressive effect. Although there it no evidence that Ad6 can infect mouse lymphocytes and macrophages, it cannot be excluded that early steps in virus replication occur. Human adenovirus type 5 undegoes an abortive infection in mice. After intravenous injection of 10^9 PFU of adenovirus type 5, mice showed signs of clinical disease and death occurred between the fourth and seventh days in 50% of mice (Duncan *et al.*, 1978). Similarly to adenovirus type 5, Ad6 might alter the function of immunocompetent cells and macrophages by infecting them abortively, although i.p. injection of $10^{9.24}$—$10^{10.24}$ TCD_{50} of Ad6 did not result in death or in any sign of illness. The only abnormality was the enlargement of spleens by 30—40% (data not shown).

Another possibility is that virus-induced interferon is responsible for the immunosuppression. It is widely accepted that interferon affects normal cell function and exerts important effects on the immune system (Gresser and Tovey 1978; Epstein, 1979; Johnson and Baron, 1977). Viruses capable of stimulating the production of interferon can theoretically shut down the immune response. The circulating interferon was suggested to be responsible for immunosuppression in various virus — host systems (Béládi *et al.*, 1973; Berencsi *et al.*, 1974; Berencsi and Béládi, 1977; Virelizier *et al.*, 1976). Interferon was not found to be involved in immunosuppresion caused by simian foamy virus (Hooks and Detrick-Hooks, 1979). In the Ad6 — mouse system, an immunosuppresive effect of virus-induced interferon is also

unlikely. We were able to demonstrate circulating interferon in mice inoculated with UV irradiated virus which was shown not to be immunosuppressive. These data indicate that Ad6-induced interferon had no supressive effect on the immune response in mice.

Viruses can activate suppressor lymphocytes or macrophages which then act directly or indirectly on those cells which would otherwise be responsible to antigenic stimuli (Hibbs *et al.*, 1980; Israel *et al.*, 1980). It is possible that a similar mechanism for suppression was operating in our mouse system. Demonstration of suppressor cells or a suppressor factor might contribute to the understanding of the mechanism of immunosuppression caused by Ad6.

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