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IMMUNOENHANCEMENT AND SUPPRESSION INDUCED BY ADENOVIRUS IN CHICKEN

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Summary. - Chickens injected intravenously (i.v.) with human adenovirus type 6 (Ad6) reveal a 2-17-fold increase in the number of plaque-forming cells producing antibody (Ab) against sheep red blood cells (SRBC) 2-6 days after virus infection. Further, polyclonal B-cell activation has been demonstrated by the quantitation of immunoglobulin-producing cells (IgPC) and cells producing immunoglobulin (Ig) of IgM isotype (IgPCμ) in the spleen of chicken inoculated with Ad6. Ad6 infection in chicken results in immunosuppression against SRBC when this unrelated antigen is given after virus infection. It seems that coincidence occurs between the B-cell mitogenic activation and the immunosuppression caused by Ad6, as the most pronounced change in both activities appears on the fourth day following virus infection. These findings suggest that the B-cell mitogenicity of the virus contributes to the impairment of the humoral immune response to SRBC.

Key words: adenovirus; chicken; polyclonal activation

Introduction

Immunosuppression is a frequent consequence of infections due to viruses. However, details of the interactions between the viruses and the host immune system are incompletely understood. It has been demonstrated in this laboratory that the human adenoviruses induce interferon production in chickens (Pusztai *et al.,* 1969) and a transient immunosuppression against SRBC when the SRBC are inoculated into chickens 3 to 14 days after virus infection (Béládi *et al.,* 1973).

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We now present data on polyclonal B-cell activation by Ad6 in chickens and suggest a close relationship between the B-cell-activating and immunosuppressive effect of this virus.

Materials and Methods

Birds. Six-twelve-week-old chickens originated from a local supplier; in some experiments, birds of the White Leghorn strain (Hyline Poultry Farm, Johnston, Iowa) were used.

Virus. Ad6 (kindly provided by Dr. R. Wigand, Homburg - Saar) was propagated in HEp-2 cells and purified by CsCl gradient centrifugation.

Sera, antisera, antibodies. Normal chicken and guinea pig sera were used as sources of complement (C).

Antiserum to chicken Ig and antiserum to mouse Ig were applied as developing antisera. Both antisera were raised in rabbits hyperimmunized with the respective Ig. Antiserum to chicken Ig was used at a dilution of 1:100, and antiserum to mouse Ig at a dilution of 1 : 200. (It was previously shown that these dilutions lead to detection of the maximum number of IgPC.)

Affinity-purified antibody (Ab) to chicken Ig was isolated from hyperimmune rabbit antiserum to chicken Ig according to the method described by Gourvich *et al.* (1968).

Two monoclonal Ab (mAb), designated $HCHM_1$ and $HCHMA_2$ directed to different epitopes of the chicken IgM heavy chain, were produced in mice. Chicken IgM was prepared according to the method described by Drén and Németh (1985) and used for the immunization of Balb/c mice. The Sp-2/0 non-secreting parental myeloma cell-line was used for fusion as described by Shulman *et al.* (1978). To detect IgPCμ , the two mAb were mixed in a ratio of 1 : 1 and used at a final dilution of 1 : 100.

Infection/immunization of chickens. Chickens were injected i.v. with 10^{11} $TCID_{50}$ of Ad6. After Ad6 injection, some birds were immunized with SRBC (1 ml of a 10% suspension i.v.). Spleens were removed on different days after infection/immunization, and splenocytes were washed with RPMI 1640 medium and used for Ab-producing cell (AbPC) and IgPC determination.

AbPC and IgPC assay. AbPC to SRBC were detected by the direct haemolytic plaque assay (Jerne and Nordin, 1963), using 0.6% agarose (Sigma Chemical Co., St. Louis, MO) for the bottom and top layers and normal chicken serum as a source of C.

The number of IgPC was determined by the reverse haemolytic plaque assay (Molinaro *et al.*, 1975), using SRBC sensitized with affinity-purified Ab to chicken Ig. Rabbit antiserum to chicken Ig was used as a developing serum and normal guinea pig serum (1 : 10) as a source of C.

To detect IgPC producing Ab of IgM isotype, a modification of the reverse plaque assay was developed. SRBC sensitized with affinity-purified Ab to chicken Ig were used as indicator cells. The mixture of the two mAb to chicken μ-chains was used as a first developing antiserum. After the incubation of splenocytes and indicator SRBC with mAb at 37 °C for 45 min, the mAb were substituted by the second developing antiserum directed to mouse Ig (1 : 100) and, after an additional incubation at 37 °C for 45 min, guinea pig C was added. In separate experiments, it was shown that IgPCμ can be detected only in the samples treated with mAb and antiserum to mouse Ig and C; the omission of any of these reagents resulted in the absence of plaques. (It should be noted that the attempt to use a mixture of two mAb to γ chain of chicken Ig to detect IgPC producing Ab of IgG isotype was unsuccessful.)

Results were expressed as the mean number of IgPC ± standard error.

Sensitization of SRBC. SRBC were sensitized with affinity-purified Ab to chicken Ig by the chromium chloride method (Ling *et al.*, 1977). Briefly: SRBC mixed with Ab (300–400 μg of Ab per 0.1 ml of packed SRBC in 0.2–0.3 ml of 0.9% NaCl solution without phosphates) were treated with freshly-prepared $CrCl_3$ solution (drop by drop, with mixing). To prepare a "working" solution of $CrCl_3$, 10 μl of an "aged" 1% stock solution of $CrCl_3 \cdot 6H_2O$ and 6 μl of 0.05 N NaOH solution were mixed with 1 ml of 0.9% NaCl solution without phosphates. 0.4–0.6 ml of this "working" Cr-solution was added to 0.1 ml of packed SRBC.

Statistical analysis. Paired T-tests of summary data were used to demonstrate the statistical significance (p) of the differences between the means of the number of plaques in the spleens of control and Ad6-infected chickens.

Results

Kinetics of polyclonal B-cell activation to erythrocyte antigen

To determine the kinetics of polyclonal B-cell activation, Ad6-infected chickens were sacrificed at different intervals after inoculation, and the number of AbPC to SRBC in the spleens was determined. As shown in Fig. 1, chickens infected with Ad6 exhibited an AbPC response to SRBC as early as 2 days after virus inoculation. This *in vivo* polyclonal response peaked on day 4, when the number of AbPC to SRBC in the infected chickens was more than 17 times higher than in the controls.

Effect of Ad6 infection on appearance of IgPC

As a rise in the number of AbPC was found in Ad6-infected chickens, it was interesting to learn whether, additionally to SRBC-specific AbPC, the quantity of other IgPC also increased. Determination of the number of IgPC after Ad6 inoculation showed that the virus induced a marked increase in the number of IgPC. On the fourth day after Ad6 inoculation, the number of IgPC was about 4 times higher than that in the controls. The majority of the IgPC appearing in response to Ad6 were of IgM isotype (Table 1).

Correlation between polyclonal and immunosuppressive activities of Ad6

To test the relationship between the mitogenic and immunosuppressive activities of Ad6, one group of chickens was injected i.v. with Ad6, and the numbers of AbPC to SRBC in the splenocyte suspension were determined on

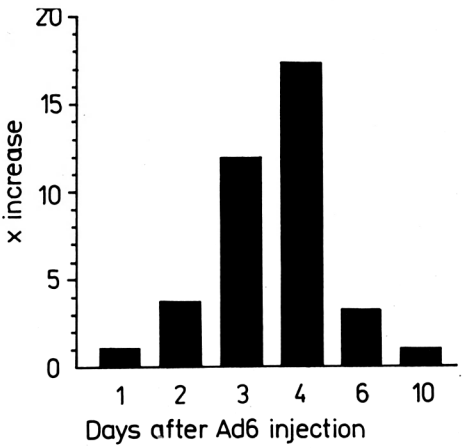

Fig. 1
Increase relative to controls of number of AbPC to SRBC in Ad6-infected chickens on different days after virus infection
Results are expressed as the stimulation index relative to the AbPC count of the control, i.e. $76 \pm 59/10^8$ spleen cells. Values are means of the results on 3 chickens, except for day 4, when 8 chickens were used.

Table 1. Effects of Ad6 infection on the appearance of IgPC in chicken

Groups	IgPC/10^6 spleen cells[a]	
	total[b]	IgM isotype
Control	8015±1985	3749±1738
Ad6-infected[c]	28210±11838	22110±11896

[a] - Mean ± standard deviation of results from four independent experiments.
[b] - $p = 0.01$
[c] - Four days after Ad6 infection.

different days after infection. The second group of birds was inoculated with the same dose of virus and received SRBC on the same days as when the numbers of AbPC in the first group were determined. The number of AbPC to SRBC was determined on the fourth day after SRBC injection. The results of this experiment are presented in Fig. 2. It can be seen that the injection of SRBC at the time of maximum polyclonal response to Ad6 induces the minimum immune response to SRBC. Thus, in Ad6-inoculated and SRBC-immunized birds the decrease in responsiveness to exogenous antigen coincides with the peak of polyclonal B-cell activation in chickens infected only with Ad6.

Discussion

Viruses may cause immunosuppression by a variety of mechanisms affecting one or more cell types of the immune system by direct or indirect ways. The virus-induced effect may be temporary or prolonged.

Viruses have been shown to be immunosuppressive in chickens, as reviewed by Hudson (1985). Marek's disease virus produces a general state of immuno-

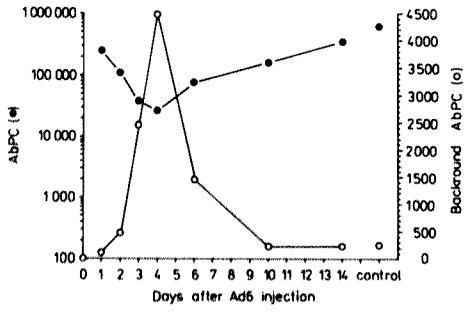

Fig. 2
Polyclonal B mitogenic and immunosuppressive effects of Ad6 to SRBC in chickens
Chickens were injected with Ad6 and the number of AbPC to SRBC was determined on the days indicated (○). Chickens were injected with Ad6, which was followed by SRBC immunization on the days indicated. The number of AbPC to SRBC was estimated four days later (●). Each value represents the number of AbPC/10^8 spleen cells.

suppression, it impairs the responsiveness to antigens, the delayed hypersensitivity reaction, and the response of the spleen cells to mitogens. Another chicken virus, infectious bursal disease virus, affects primarily the B lymphocytes, resulting in a defective gammaglobulin synthesis. The immunosuppression of avian retroviruses is mediated by an immunosuppressive factor released by adherent spleen cells.

In our experimental model, the effects of Ad6, which is non-pathogenic for chicken, were studied on the immune function of chickens. We found that after Ad6 infection a transient suppression of the humoral immune response to SRBC occurred, lasting from day 2 to days 10–14 after virus inoculation. However, the AbPC response to SRBC was slightly increased when Ad6 and SRBC were inoculated on the same day (Béládi *et al.,* 1973). Adenovirus also influences other functions of the immune system in chickens. The natural cytotoxicity reaction is suppressed following a marked increase on the first day after virus infection (Mándi *et al.,* 1987). Further, 12–24 hr after virus infection the antibody-dependent cellular cytotoxicity (ADCC) was enhanced (Mándi *et al.,* 1982). The influence of human adenovirus on the immune system has also been studied in other experimental models, and several mechanism of the immunomodulating effect have been suggested. A transient suppression of the Ab response to unrelated antigens was found in hamsters and mice (Hamburg *et al.,* 1970; Berencsi *et al.,* 1982). These alterations have been attributed to antigenic competition or, in the case of adenovirus-infected mice, to a change in the macrophage function. Horváth *et al.* (1983) reported on the impairing effect of adenovirus on the function of human peripheral lymphocytes.

Human adenoviruses induce interferon in chickens and mice (Pusztai *et al.,* 1969; Berencsi *et al.,* 1982).

As interferon is a known immunomodulator, it is plausible to consider its role in the alterations observed in adenovirus-infected animals, as its productions always preceded the immunological changes. The role of interferon in augmenting the natural cytotoxicity and ADCC of chickens has been described (Mándi *et al.,* 1987). The effect of interferon in influencing the humoral immune response has been suggested in adenovirus-infected chickens (Béládi *et al.,* 1973). However, in the mouse model the UV-inactivated adenovirus retained the interferon-inducing capacity, while its suppressive activity was lost, excluding the role of interferon (Berencsi *et al.,* 1982). Thus, other mechanisms due to direct or indirect effects of the virus may exist in adenovirus-induced immunosuppression.

Here, we present data concerning the increase of SRBC-specific AbPC in spleens of Ad6-infected chickens. The number of AbPC to SRBC is markedly higher 3–4 days after Ad6 infection than in the controls. We have demonstrated not only that SRBC-specific AbPC appeared, but also that the number of total IgPC rose. The development of a modified method for reverse haemolytic plaque detection permitted the demonstration that the majority of the IgPC arising upon Ad6 inoculation are of the IgM isotype of chicken Ig.

Polyclonal B-cell activation by different pathogens is well documented. *Trypanosoma cruzi* - infected mice are polyclonally stimulated (d'Imperio Lima *et al.*, 1986; Ortiz-Ortiz *et al.*, 1980). Viruses such as lactic dehydrogenase virus in mice, Epstein-Barr virus and human immunodeficiency virus in man stimulate B-cells polyclonally (Cautelier and van Snick, 1985; Garzelli *et al.*, 1989; Lifson and Engelman, 1989). Immunosuppression with the demonstrated involvement of B-cell proliferation has been observed in hosts infected with parasites (Grosskinsky and Askonas, 1981; Sacks *et al.*, 1984), bacteria (Vendrell *et al.*, 1985), or virus (Cautelier and van Snick, 1985). LPS, one of the most effective B-cell mitogens also induces immunosuppression (Persson, 1977). It can be suggested, that polyclonal B-cell stimulation *in vivo* may lead to immunosuppression, as the immune system is a homeostatic one and any alteration in the steady state may result in immunosuppression. Our observation that the time point of the marked polyclonal B-cell activation coincides with the significantly impaired capacity to respond to an exogenous antigen suggests that the Ad6-induced polyclonal B-lymphocyte stimulation contributes to the immune dysfunction associated with the infection. A similar conclusion was drawn by Ferreira *et al.* (1988) from experiments with an immunosuppressive protein secreted by *Streptococcus mutants*. However, they observed the maximum decrease in AbPC 2 days later than the maximum polyclonal stimulation, while in Ad6-infected chickens the immunosuppressive and polyclonal B mitogenic activities are mirror images of each other.

The human adenoviruses do not multiply in chick cells. No sign of pathogenicity can be observed in chickens. The only alterations found are relative granulocytosis and lymphopenia on the first day of infection, followed by relative granulopenia and lymphocytosis on the fourth day after infection (unpublished data). Our results suggest that, in contrast with viral infection of human and animals, in which the effects of the virus on the host immune response seem to be correlated with its pathogenicity (Cautelier and van Snick 1985; Garzelli *et al.*, 1989), the human adenovirus (though not pathogenic for chicken) results in similar disorders of the immune system, i.e. polyclonal B-cell activation and immunosuppression.

Gibson and coworkers (1982) showed the fiber protein of type 12 human adenovirus to be mitogenic for lymphocytes of BALB/c mice *in vitro*. They also found an increased IgM synthesis to SRBC in mice immunized with SRBC along with fiber protein. Recently it has been observed that murine adenovirus infection resulted in an increased production of IgG2a in mice (Cautelier *et al.*, 1990). It is obvious that the results obtained with chickens and mice cannot be converted to adenovirus infections in humans, but the polyclonal B-cell stimulating effect may contribute to the pathogenicity of the virus in man. On the basis of the available data, the lymphopenia that accompanies adenovirus infection is attributed to the production of cytotoxic antibodies of the IgM class directed against autologous lymphocytes (Huang *et al.*, 1973; Huang and Hong, 1975). The appearance of cytotoxic antibodies can be a consequence of polyc-

lonal B-cell stimulation. Accordingly, the potential role of adenovirus in autoimmune diseases may be considered.

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