Acta virol. **35**: 350-356, 1991

IMPAIRED INTERLEUKIN 2 PRODUCTION BY SPLEEN CELLS FROM MICE INFECTED WITH HUMAN ADENOVIRUS

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Received August 6, 1990

Summary. - Spleen cells from mice infected with human adenovirus type 6 (Ad6) showed defective interleukin 2 (IL2) production 3-5 days after the infection. The response of spleen cells to exogenous IL 2 was also deficient. The impaired capacity of concanavalin A-(Con A)-activated spleen cells from Ad6-infected mice to utilize IL 2 seemed to be related to the depressed capacity of the infected splenocytes to express IL 2 receptors. The immunologic dysfunction following infection with Ad6 may be a consequence of deficiencies in both the elaboration of and response to IL 2.

Key words: human adenovirus type 6; mice; immunosuppression; interleukin 2

Introduction

We earlier described a strong inhibition of humoral immunity by human Ad6 in mice. The mechanism of this immunosuppression is not clear, but the macrophages seem to be one of the direct targets for virus action (Berencsi *et al.*, 1982; Berencsi *et al.*, 1985). The macrophages play an important role in the regulation of lymphocyte responses, both by direct cellular interactions and by the production of soluble factors (Allison, 1978). As the Ad6-induced immunosuppression was only slightly reversed in mice treated with indomethacin (Berencsi *et al.*, 1987), it seemed very likely that other mechanisms than the enhanced prostaglandin synthesis are also involved in the immunosuppression observed. The present paper deals with experiments in which suppressed IL 2 production together with a defective expression of the IL 2 receptor have been found in adenovirus-infected mice.

Materials and Methods

Mice. Eight to twelve-week 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. Mice were inoculated intraperitoneally with 10^9 $TCID_{50}$ of virus diluted in physiological saline.

Generation of lymphokine supernatants. Spleen cell suspensions from control and virus-infected mice were prepared in RPMI 1640 medium supplemented with 10 % foetal calf serum. To generate

IL 2 supernatants, cultures of 10^7 spleen cells were prepared in 2 ml in 24-well cluster dishes (Costar). These cells were exposed to Con A (Calbiochem) at 3 μg/ml. Supernatants were harvested usually after a 24-hr incubation and were stored frozen at -20 ^{0}C until used.

Assay for IL 2. The thymus of 4 to 6-week old CBA mice was removed and thymocyte cultures were prepared in RPMI 1640 medium supplemented with 10 % foetal calf serum, phytohaemagglutinin M (Difco) at a dilution of 1:1000, and 2-mercaptoethanol at a concentration of 2×10^{-5} M. The cells were cultured in 96-well microtitre plates; the cell concentration was 10^6/well. The 50 μl aliquots of the Con A supernatants were added to the different wells in triplicate and were cultured for 2 days. After 48 hr, 37 kBq ^{3}H-thymidine (Amersham) was added to each well. Incubation proceeded for another 26 hr. Cells were harvested on an automatic cell harvester and counted in a Packard liquid scintillation counter.

Spleen cell response to exogenous IL 2 preparation. Spleens were collected 1 day after Ad6 infection, and spleen cells (2.5×10^6/ml) were cultured for 3 days in the presence of 3 μg/ml Con A. The cells were then washed twice and incubated (10^5/0.1 ml) for 24 hr with 0.1 ml of human IL 2 preparation (kindly provided by Dr. I. Rostóczy). Six hours before harvesting, the cells were pulsed with 37 kBq ^{3}H-thymidine according to the method of Colonna Romano and coworkers (1986).

Interleukin absorption by Ad6-infected cells. The method described by Gillis *et al.* (1982) was used. Briefly, spleen cells either from untreated mice or from mice infected with Ad6 were cultured for 3 days with Con A at a concentration of 3 μg/ml, then washed twice and incubated (10^8 cells/ml) with human IL 2 preparation for 1 hr at 4 ^{0}C and 1 hr at 37 ^{0}C. At the end of incubation, the supernatants were tested for IL 2 activity.

Results

Effect of Ad6 on in vitro IL 2 production

Spleen cells from control and Ad6-infected mice were stimulated *in vitro* with Con A for 24 hr and the supernatants were tested for IL 2 activity. Fig. 1

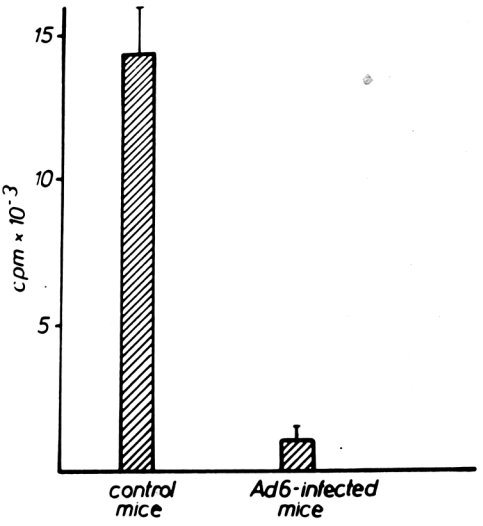

Fig. 1
Production of IL 2 by spleen cells of control and Ad6-infected mice 4 days after infection
The spleen cells were stimulated with Con A and the supernatants were tested for IL 2 activity. The data represent the ^{3}H-thymidine incorporation of mouse thymocytes in the presence of Con A supernatants. The mean value of data obtained from 3 mice±SD is indicated.

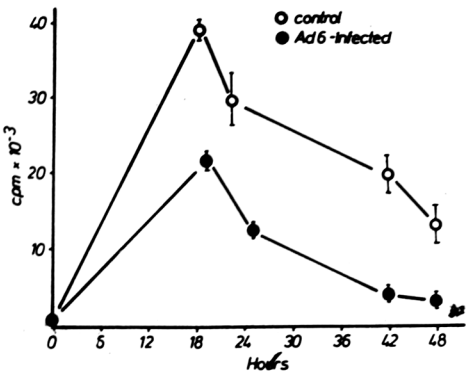

Fig. 2
Kinetics of IL 2 production by spleen cells of control and Ad6-infected mice 3 days after infection
The spleen cells were stimulated with Con A and the supernatants were tested for IL 2 activity at different intervals. The data represent the ^{3}H-thymidine incorporation of mouse thymocytes in the presence of Con A supernatants. The mean value from 4 cultures±SD is indicated.

shows that the spleen cells of untreated mice produced a higher amount of IL 2 than the spleen cells of mice 4 days after virus injection. A depressed IL 2 production was found 1–7 days after Ad6 inoculation (data not shown). The kinetics of the *in vitro* IL 2 production was also studied. Fig. 2 shows that in cell cultures from the spleens of the control mice the maximum level of IL 2 was found 19 hr after the initiation of cultures, after which the IL 2 level decreased. The kinetics of IL 2 production of the spleen cells from the Ad6-infected mice was similar, but the level of IL 2 activity was constantly lower during the whole course of the 48-hr incubation, indicating that Ad6 infection depresses the ability of spleen cells to elaborated IL 2.

Effect of Ad6 infection on the proliferative response of spleen cells to IL 2

The effect of Ad6 on IL 2 responsiveness was analysed by measuring the DNA synthesis of spleen cells from Ad6-infected mice in the presence of human IL 2 preparation. Fig. 3 shows that Con A-activated spleen cells from the Ad6-infected mice 4 days after infection proliferate when cultured with exogenous IL 2, but this response is significantly lower than the proliferative response of the control cells. The difference between the DNA syntheses of

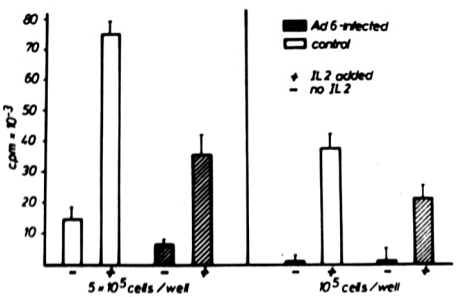

Fig. 3
Response of Con A-stimulated spleen cells taken from control and Ad6-infected mice to exogenous IL 2
The spleen were taken 4 days after the infection. The data represent the ^{3}H-thymidine incorporation of spleen cells in the presence of human IL 2 preparation. The mean value of data obtained from 3 mice±SD is indicated.

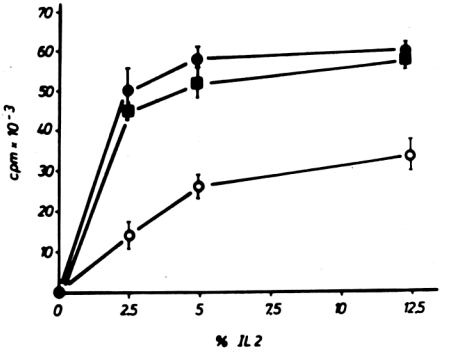

Fig. 4
IL 2 absorption by spleen cells of control and Ad6-infected mice 24 hr after infection
^{3}H-thymidine incorporation of thymocytes in the presence of serial dilutions of human IL 2 preparation previously absorbed on Con A-stimulated spleen cells of Ad6-infected mice: ■, and of control mice: o, unabsorbed IL 2 preparation: ●.

cells taken from the control and infected animals was similar when different numbers of IL 2-stimulated cells were tested.

Effect of Ad6 on the binding of IL 2

The failure of exogenous IL 2 to induce a similar proliferative response in the spleen cells from the Ad6-infected mice to that of the control mice may be a consequence of a perturbation in the capacity of the lymphocytes to express IL 2 receptors. This possibility was examined in the next experiment. Con A-activated spleen cells were incubated with human IL 2 preparation. The supernatants were then tested for residual IL 2 activity. Fig. 4 shows that the absorption of IL 2 by Con A-stimulated spleen cells from the control mice resulted in a pronounced loss of IL 2 activity. In contrast, the absorption of IL 2 by the same number of spleen cells from the Ad6-infected mice resulted in only a few per cent loss of IL 2 activity. The effect of unabsorbed IL 2 on the ^{3}H-thymidine incorporation of thymus cells from CBA mice is also shown in Fig. 4. The data obtained indicate that the Con A-stimulated spleen cells of the Ad6-infected mice express a lower amount of IL 2 receptors.

Discussion

Many viruses bring about a state of immunosuppression in the host and this may be caused by direct or indirect effects upon one or more cell types of the immune system. The immunosuppressive effect of human adenoviruses has been demonstrated in various experimental systems. We earlier reported that the adenovirus infection of chickens resulted in a transient suppression of the humoral immune response to sheep red blood cells (SRBC) (Béládi *et al.*, 1973). The natural cytotoxicity reaction is also suppressed 2 days after adenovirus inoculation in chickens (Mándi *et al.*, 1987). A transient suppression of the antibody response to unrelated antigens was found in hamsters (Hamburg *et al.*, 1970). We have previously shown that Ad6 also has the ability to induce

immunosuppression in mice. The number of anti-SRBC antibody-forming cells in the spleen of infected mice was less than in controls 3–11 days after virus inoculation. Our observation that silica, an agent known to be toxic for macrophages, almost completely reversed the immunosuppressive effect of Ad6, indicates that in our experimental system macrophages mediate the suppressor activity of Ad6 (Berencsi *et al.*, 1985). Macrophages are able to produce prostaglandins, which are known to be important mediators of the suppressive activity of macrophages (Metzger *et al.*, 1980; Nathan *et al.*, 1980). Treatment of mice with the prostaglandin synthetase inhibitor indomethacin did not result in a pronounced reversal of immunosuppresion, suggesting that other factors might also be involved (Berencsi *et al.*, 1987). The intention of our present study was to extend our knowledge about the role of the regulatory lymphokines in the immunosuppression induced by Ad6. IL 2 was chosen for study due to its pivotal role in the immune response. Our results provide evidence of a deficiency in IL 2 production. Another defect found in Ad6-infected mice was the failure to respond to IL 2. As IL 2 exerts its multiple biological activities upon binding to its specific cell surface receptors, the unresponsiveness to IL 2 could be a result of the lack of IL 2 receptors. Our data show that the spleen cells from the Ad6-infected mice after *in vitro* stimulation with Con A have an impaired capacity to develop receptors for IL 2, as their capacity to bind exogenous IL 2 was markedly decreased. It is not known, however, whether this is a direct effect of Ad6 on the elaboration of IL 2 receptors or it is secondary to the depressed IL 2 production, as IL 2 stimulates its own receptors (Smith and Cantrell, 1985). The impaired IL 2 production and IL 2 receptor expression are common phenomena observed in protozoal (Sileghem *et al.*, 1987; Ho *et al.*, 1988; Tarleton, 1988; Cillari *et al.*, 1988) and bacterial (Turcotte, 1987; Makonkawkeyoon and Kasinkerk, 1989; Deschenes et al., 1986) infections. Immunosuppressive viruses have also been found to influence IL 2 production. Immunologic dysfunction secondary to infection with malignant rabbit fibroma virus reflects deficiencies in both the elaboration of and response to IL 2 (Strayer *et al.*, 1986). Spleen cells from Newcastle disease virus-infected mice failed to produce detectable amounts of IL 2 (Colonna Romano *et al.*, 1986). Blackett and Mims (1988) demonstrated a notable reduction in Con A-induced IL 2 production by spleen cells from mice infected with murine cytomegalovirus. It is not clear how the various pathogens inhibit IL 2 production and response. There are several possible explanations for the failure of IL 2 production. The production of IL 2 is enhanced by the interaction between IL 1 and sensitized T lymphocytes. We can not exclude this possibility in Ad6-infected mice, as macrophages, the main source of IL 1, are involved in the Ad6-induced immunosuppression in mice (Berencsi *et al.*, 1985). The role of prostaglandin E_2, a known inhibitor of IL 2 production (Rappaport and Dodge, 1982), is very probably exluded by the failure of indomethacin to reverse the immunosuppression due to Ad6 in mice (Berencsi *et al.*, 1987). On the basis of our previous observation that macrophages are

important targets of Ad6-induced immunosuppression, we assume that adenovirus disturbs various macrophage functions, which may result in incomplete IL 2 production and responsiveness.

Acknowledgement. The skilful technical assistance of Anikó Salaki is gratefully acknowledged.

References

Allison, A. C. (1978): Mechanisms by which activated macrophages inhibit lymphocyte responses. *Immunol. Rev.* **40**, 3–8.

Berencsi, K., Bakay, M., and Béládi, I. (1985): The role of macrophages in adenovirus-induced immunosuppression in mice. *Acta virol.* **29**, 61–65.

Berencsi, K., Bakay, M., and Béládi, I. (1987): Involvement of prostaglandins in immunosppression caused by adenovirus in mice. *Acta virol.* **31**, 74–77.

Berencsi, K., Bakay, M., and Kovács, P. (1982): Effect of human adenovirus type 6 on the primary immune response in mice. *Acta virol.* **26**, 340–345.

Béládi, I., Pusztai, R., Mucsi, I., Bakay, M., and Bajszár, Gy. (1973): Effect of human adenoviruses on the response of chickens to sheep erythrocytes. *Infect. Immun.* **7**, 22–28.

Blackett, S., and Mims, C. A. (1988): Studies of depressed interleukin-2 production by spleen cells from mice following infection with cytomegalovirus. *Arch. Virol.* **99**, 1–8.

Cillari, E., Liew, F. Y., Lo Campo, P., Milano, S., Mansueto, S., and Salerno, A. (1988): Suppression of IL-2 production by cryopreserved peripheral blood mononuclear cells from patients with active visceral leishmaniasis in Sicily. *J. Immunol.* **140**, 2721–2726.

Colonna Romano, G., Dieli, F., Abrignani, S., Salerno, A., and Collozzi, V. (1986): Inhibition of lymphocyte mitogenesis in mice infected with Newcastle disease virus: viral interference with the interleukin system. *Immunol.* **57**, 373–378.

Deschenes, M., Guenounou, M., Ronco, E., Vacheron, F., and Nauciel, C. (1986): Impairment of lymphocyte proliferative responses and interleukin-2 production in susceptible (C57BL/6) mice infected with *Salmonella typhimurium. Immunol.* **58**, 225–230.

Gillis, S., Mochizuki, D. Y., Conlon, P. J., Hefeneider, S. H., Ramthum, C. A., Gillis, E. A., Frank, M.B., Henney, C. S., and Watson, J. D. (1982): Molecular characterization of interleukin 2. *Immunol. Rev.* **63**, 167–171.

Hamburg, V., Scherbakova, O., and Svet-Moldavsky, G. (1970): Suppression of antibody formation against Sendai virus in SV40 and adenovirus 16 infected hamsters. *Experientia* **26**, 532–534.

Ho, M., Webster, H. K., Green, B., Looareesuwan, S., Kongchareon, S., and White, N. J., (1988): Defective production of and response to IL-2 in acute human falciparum malaria. *J. Immunol.* **141**, 2755–2759.

Makonkawkeyoon, S., and Kasinrerk, W. (1989): *In vitro* suppression of interleukin 2 production by *Mycobacterium leprae* antigen. *Clin. exp. Immuol.* **76**, 398–403.

Mándi, Y., Pusztai, R., Baranji, K., Seprényi, Gy., Taródi, B., Bakay, M., and Béládi, I. (1987): The role of interferon in the adenovirus-induced augmentation of granulocyte-mediated cytotoxicity in chicken. *Immunobiol.* **174**, 210–220.

Metzger, Z., Hoffeld, J. T., and Oppenheim, J. J. (1980): Macrophage-mediated suppresion. I. Evidence for participation of both hydrogen peroxide and prostaglandins in suppression of murine lymphocyte proliferation. *J. Immunol.* **124**, 983–986.

Nathan, C. F., Murray, H. W., and Cohn, Z. A. (1980): The macrophage as an effector cell. *N. Engl. J. Med.* **303**, 622–626.

Rappaport, R. S., and Dodge, G. R. (1982): Prostaglandin E inhibits the production of human interleukin 2. *J. Exp. Med.* **155**, 943–948.

Sileghem, M., Hamers, R., and De Baetselier, P. (1987): Experimental *Trypanosoma brucei* infections selectively suppress both interleukin 2 production and interleukin 2 receptor expression. *Eur. J. Immunol.* **17**, 1417–1421.

Smith, K. A., and Cantrell, D. A. (1985): Interleukin 2 regulates its own receptors. *Proc. Natl. Acad. Sci. U.S.A.* **82**, *864–868.*

Strayer, D. S., Horowitz, M., and Leibowitz, J. L. (1986): Immunosuppression in viral oncogenesis. III. Effects of virus infection on interleukin 1 and interleukin 2 generation and responsiveness. *J. Immunol.* **137**, 3632–3638.

Tarleton, R. L. (1988): *Trypanosoma cruzi*-induced suppression of IL-2 production. II. Evidence for a role for suppressor cells. *J. Immunol.* **140**, 2769–2773.

Turcotte, R. (1987): A suppressor B lymphocyte inhibiting IL-2 consumption in spleen cell cultures from *Mycobacterium bovis* BCG-infected mice. *Immunol.* **62**, 439–444.