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Increased Cellular Immunity against Host Cell Antigens Induced by Vacciniavirus[1]

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With 2 Figures

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Summary

Guinea pigs show an increased cell-mediated immunity against host cell antigens after active immunization with vaccinia virus infected tissue culture cells. This immunologic adjuvant effect was observed in a heterologous system comprising primary rabbit kidney cells, primary fibroblasts from the mouse, permanent monkey kidney cells and *in vivo* infected mouse brain cells. The quantitative determination of the cellular immune response was carried out both in macrophage migration inhibition and in lymphocyte transformation tests. Cell-mediated immunity appears on the third day after immunization. A prerequisite for the adjuvant effect is the reproduction of the virus in the cell against which the increased cell-mediated immune response is directed.

1. Introduction

Ever since the publication of Ramon in 1925 (1) on the production of diphtheria and tetanus antitoxins, an increase in the immunogenicity of different substances has been aimed at the use of adjuvants. Now more than 70 different adjuvants are known, and in the meantime several immunological adjuvants have attained great importance in medicine, especially in the production of vaccines. Essentially three substances have won great importance: aluminium compounds, substances which are known as Freund's adjuvants and thirdly endotoxins of gramnegative bacteria. All these adjuvants are substances, which increase the immunogenicity of other substances in a completely unspecific way.

In preceding papers we have reported that viruses act as immunological adjuvants (2). Guinea pigs being actively immunized against heterologous virus-infected cells showed an increased production of humoral antibodies against the host cells used. These cytotoxic antibodies were directed only against the cell type

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in which the virus had been multiplied, thus indicating that the virus acts as a very specific adjuvant. In this connection we wanted to find out whether viruses, besides increasing the production of cytotoxic antibodies, can also improve the cell-mediated immune response. For the following experiments we chose the vaccinia virus, as in our previous studies on humoral antibodies it has proved to be a very good immunological adjuvant (3).

2. Materials and Methods

2.1. Virus and Cell Culture

Vaccinia virus strain WR was grown in cell cultures of BHK-cells ($10^{6.5}$ $TCID_{50}$/ml), Vero-cells (10^5 $TCID_{50}$), RK-cells (10^5 $TCID_{50}$/ml), embryonic guinea pig fibroblasts (10^5 $TCID_{50}$/ml) as well as *in vivo* in the brain of adult NMRI mice (10^8 $TCID_{50}$). Vaccinia virus strain Elstree was cultivated in human embryonic cells (10^6 $TCID_{50}$/ml).

Herpes simplex virus strain "Hof" was grown in BHK-cells ($10^{5.3}$ $TCID_{50}$).

All cell cultures were grown in Eagle's Minimum Essential Medium with Earle's BSS. Infectivity titrations were performed in AGMK cells.

2.2. Animals and Immunization

Pirbright guinea pigs, weighing 250 g, were used. Experiments were performed in groupes of 5 animals each. The virus infected and non infected cell suspensions were adjusted to 800 µg protein/ml. Inoculation of the suspensions took place as described before (2).

2.3. Assay for Blast Cell Transformation

Guinea pigs were bled out by cardiac puncture. The heparinized (25 U/ml Thrombowetren, Promonta, Hamburg) blood was sedimented with a 10 per cent added volume of 5 per cent dextran in saline (MG 250,000, Pharmacia, Uppsala, Sweden) for 45 minutes at 37° C to obtain a plasma rich in peripheral white blood cells. The spleens of the guinea pigs were removed aseptically, cut in fragments and homogenized by hand in a glass grinder with the addition of Eagle's Medium. The suspension was centrifuged at 1500 rev/min for 5 minutes, the sediment re-suspended in medium TC 199 with 30 per cent homologous heat-inactivated guinea pig serum, and left standing for 1 hour at +4° C. The cells in the supernatant fluid were then washed twice with Hanks' BSS, and finally resuspended in culture medium TC 199 with 15 per cent guinea pig serum. The suspension of the washed peripheral white blood cells as well as the spleen cells was adjusted to 10^6 viable lymphocytes per ml. Cell cultures were set up consisting of 1×10^6 mononuclear cells in a total volume of 1 ml in disposable culture tubes (Greiner, Nürtingen, Nr. 160S) at +37° C in a 5 per cent CO_2-air atmosphere. Twelve hours before processing the cultures, 1.5 µCi of tritiated thymidine (spec. activity 23,000 mCi/mMol thymidine, Radiochemical Centre Amersham), was added to each vial. Uptake of the isotope into DNA was assayed at the end of cultivation. The proliferative response was expressed as an isotope incorporation index, representing the ratio of the mean counts/minute of replicate culture vials containing antigen divided by mean counts/minute of replicate control culture vials in the same experiment.

2.4. Assay for Macrophage Migration Inhibition

Macrophages were obtained from the peritoneal cavity of Pirbright strain guinea pigs 72 hours after injection of 20 ml of sodiumthioglycollate (Difco Laboratories, Detroit). The exudate cells were washed three times and incubated at 21° C for 60 minutes in TC 199 with 15 per cent homologous heat-inactivated guinea pig serum and 20 per cent antigen (virus cellsuspension or cell suspension alone, 1000γ protein/ml). Glass capillaries were filled with these cells adjusted to 10^7 cells/ml, then sealed with "Seal-Ease" (Adams, Parsippang, N.J.) at one end, and centrifuged at 1000 r.p.m. for

5 minutes. Each capillary was then cut at the border of the packed cells and placed in plastic culture chambers (Greiner, Nürtingen), where they were held in place by a small amount of sterile silicone. The chambers were filled with medium containing 10 per cent antigen (1000 γ/ml) or medium without antigen as control and incubated at 37° C. Migration areas were determined after 16 and 24 hours by tracing their outline on a microscope projection screen and measured by planimetry. The effect of antigens on macrophage migration was determined by comparison with the simultaneously prepared controls without antigen-containing medium and calculated as follows:

$$\text{Migration (\%)} = \frac{\text{average migration with antigen}}{\text{average migration without antigen}} \times 100$$

3. Results

3.1. Increased Cellular Immunity against Cell Antigens Induced by Vaccinia Virus

Pirbright guinea pigs were immunized against different cells infected *in vitro* with vaccinia virus. Controls were set up with non-infected cells. One week after the antigen-injection, cellular immunity against the respective host cell antigens was tested both by the macrophage migration inhibition test and the lymphocyte transformation test. The results thus obtained uniformly show that virus-infected heterologous cells induce a far better immune response than non-infected cells (Fig. 1). Both tests yielded closely correlated results (Fig. 2). The blast cell transformation was done with peripheral blood lymphocytes as well as spleen lymphocytes; yet the former ones provided much better results. This cellular immune response is demonstrable already three days after the antigen injection. In the autologous system, however, we did not detect any cellular immunity against primary embryonic guinea pig fibroblasts.

3.2. Intracellular Virus Replication as a Prerequisite for the Immunological Adjuvant Effect

In order to induce an increased cellular immune response against heterologous host cell antigens, the virus must replicate in the cell. Already 3 hours after infection—*i.e.* before any mature virus particles have been formed—increased immunogenicity can be demonstrated. Simply mixing the virus and non-infected cells immediately before injection does not lead to any adjuvant effect. Similarly, no adjuvant effect was observed when virus and non-infected cells were inoculated separately into the two hind legs.

In contrast, we found an increase of the cellular immunity directed against those cells—Vero cells in the present case—in which the virus had been grown. Live vaccinia virus does not appear necessary for the enhancing effect *in vivo*, since treatment of virus-infected cell homogenates with 0.075 per cent (v/v) of formaldehyde may inactivate virus completely, yet without reducing the activity of the cell antigen.

3.3. Specificity of the Increased Immune Response

Induction of lymphocyte mediated immunity by different heterologous cells proved to be highly specific. Guinea pig lymphocytes sensitized against one particular cell species did not cross react with other heterologous cells. The induced

effect proved to be strictly virus-dependent. Guinea pigs actively preimmunized against vaccinia virus showed no adjuvant effect. Likewise, if the virus-infected cell homogenate is incubated with anti-vaccinia virus antibody (Vacuman Berna) before being inoculated in guinea pigs, an increased cellular immune response can not be observed. This means, that virus-specific antigens are a prerequisite for

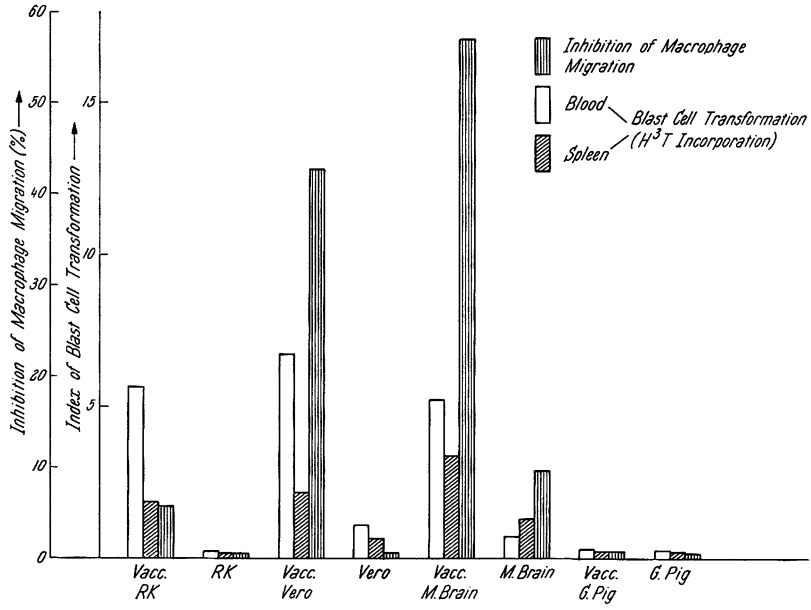

Fig. 1. Delayed hypersensitivity in guinea pigs which have been immunized with different cells infected by vaccinia virus

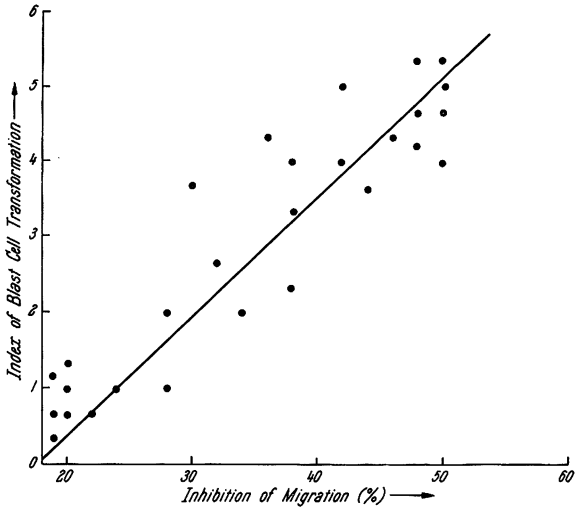

Fig. 2. Correlation of blast cell transformation and inhibition of macrophage migration

the adjuvant effect. On the other hand, the adjuvant effect is not only inducible by vaccinia virus. Indeed, we were able to show the same, though less effective enhanced immunogenicity of cells by using vesicular stomatitis virus as well as herpes simplex virus.

4. Discussion

Vaccinia virus acts as an immunological adjuvant, that is, it increases cellular immune reaction against heterologous cell antigens in guinea pigs. The same effect could also be demonstrated by using herpes simplex virus and vesicular stomatitis virus. Parameters for the delayed type of hypersensitivity were incorporation of tritiated thymidine into transformed guinea pig lymphocytes as well as the inhibition of macrophage migration. Both tests, which have been used successfully in the guinea pig system by several authors (4, 5, 6), correlated closely. Our experiments show that virus-enhanced immunogenicity can be demonstrated with different heterologous cells, infected *in vitro* and *in vivo* by vaccinia virus. The enhancement of the heterologous cell antigen activity produced by vaccinia-virus is highly specific for the cell species that has been infected by the virus: immunization of guinea pigs with homogenates of different virus-infected heterologous cells did not confer any cross immunity. Against syngeneic normal host cells we found no such virus-enhanced immunogenicity.

The mode of action of the adjuvant effect induced by vaccinia virus differs in several respects from that of commonly used adjuvants such as aluminium hydroxyde or Freund's adjuvants. One prerequisite for the immunological adjuvant effect is the replication of the virus in the cell, against which the cellular immunity will be directed. Non-infected cells mixed with virus before inoculation failed to immunize against the cells. Likewise ineffective is the separate injection of vaccinia virus and non-infected cell homogenates. This provides further evidence that the cell antigen enhancing effect of the virus is not simply due to a nonspecific stimulation of the immune system.

Live vaccinia virus does not seem necessary for the observed adjuvant effect. Virus-infected cell homogenates that had been completely inactivated with formaldehyde retained the increased immunogenicity. These experiments confirm the findings of LINDENMANN (7), HÄKKINEN (8) and BOONE (9) who showed that treatment of virus-infected tumor cells with formaldehyde did not reduce their virus-induced immunogenicity. Immunization of mice with homogenates of influenza virus-infected tumor cells conferred immunity to challenge with tumor cells, whereas immunization with the same dose of homogenate of non-infected tumor cells did not.

Already 2—3 hours after vaccinia virus infection the immunogenicity of the host cell is increased, that is before any mature particles of vaccinia viruses are produced. This coincides with a change of the host cell surface which also appears 2—3 hours after virus infection (10). Therefore the adjuvant effect produced by vaccinia virus seems to be due to a close association between host cell antigens and antigens produced by the virus. These complex antigens apparently occur in the plasma membrane of the host cell, as we were able to observe the same enhancing effect by using plasma membranes isolated from infected BHK-cells (10). BOONE (11) recently demonstrated that membrane fractions prepared from tumor

cells infected with influenza virus showed an enhanced tumor-transplantation-antigen-activity. This and our own observations lead us to believe that the potential immunogen is a host-cell-virus antigen complex formed in the plasma membrane of the host cell. The virus-specific part of this supposed complex antigen can be abolished by treating the virus-cell homogenates with virus specific antibody and thus, the adjuvant effect does not work any more.

One may suppose that the protective immune reaction against tumor specific antigens is primarily due to cellular immunity. Our experiments presented here may give evidence that viruses can take over the role of immunological adjuvants in a tumor system and possibly increase a specific cellular immune response directed against tumor cell antigens. Further experiments about virus enhanced tumor cell antigens are in progress.

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