INDUCTION OF SKIN RESISTANCE TO VACCINIA VIRUS IN RABBITS BY VACCINIA-SOLUBLE EARLY ANTIGENS

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A specific antigen (S antigen) induced by poxvirus was found on the surface of vaccinia-infected cells by use of immunofluorescence (1) or immune hemadsorption (2). S-specific antibody, which stained by immunofluorescence the S antigen but not cytoplasmic inclusions of vaccinia-infected cells, was prepared by immunizing rabbits with rabbit kidney cells infected with a conditional lethal mutant of vaccinia virus (3). This mutant (4) grows well in the chorioallantoic membrane of embryonated hens' eggs as well as in chick embryo cell cultures, but fails to induce viral DNA and late protein syntheses in rabbit kidney and HeLa cells, although early antigens detectable by immunofluorescence or complement fixation are produced. The soluble early antigen preparation made from mutant-infected HeLa cells also elicited in rabbits the production of antibody of the same nature. These immune sera contained complement-fixing and precipitating antibodies against vaccinia-soluble antigens, but no detectable neutralizing antibody (3). Although the relationship between the antigen(s) responsible for surface immunofluorescence of infected cells (S antigen) and those for complement fixation or immunodiffusion remains to be elucidated, the above data indicate that the soluble early antigen preparation made from mutant-infected HeLa cells contained the S antigen, but not the antigen(s) concerning the induction of vaccinia-neutralizing antibody. This led the authors to examine the immunological role of this antigen preparation in animals against vaccinial infection, and the present paper deals with the induction of skin resistance to vaccinia virus, probably of cell-mediated type, in rabbits by this antigen preparation without eliciting the production of circulating neutralizing antibody. The antigen preparation used here may contain more than one antigen, but not all of the early antigens produced in vaccinia-infected cells as will be discussed later. The antigen preparation will be referred to as the Es antigen (vaccinia-soluble early antigen preparation including the S antigen) in the text.

Materials and Methods

Viruses.—DI strain of vaccinia virus and a conditional lethal mutant derived from it, DIs strain (4), were propagated in the chorioallantoic membrane (CAM)[1] of embryonated hens' eggs. Vaccinia virus used for intradermal challenge of rabbits was a commercial lot of calf lymph vaccine prepared with the Ikeda strain of vaccinia virus. Extracellular vaccinia virus

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[1] *Abbreviations used in this paper:* CAM, chorioallantoic membrane; CE, chick embryo; CF, complement fixation; MIF, migration inhibitory factor; m.o.i., multiplicity of infection; PFU, plaque-forming units; p.i., postinfection; VSV, vesicular stomatitis virus.

for neutralization test was obtained from DI virus-infected HeLa cell cultures as described by Appleyard et al. (5). Vesicular stomatitis virus (VSV) was propagated in HeLa cell cultures.

Cell Cultures.—HeLa and chick embryo (CE) cell cultures were prepared as described previously (1, 6). RK-13, a cell line derived from rabbit kidney, was grown in synthetic 199 medium supplemented with 5% bovine serum.

Assay of Infectivity and Neutralizing Antibody.—Plaque technique on CE monolayers was used for vaccinia virus as described earlier (3, 6). VSV was titrated in RK-13 cell cultures under agar overlay consisting of 199 medium with 4% bovine serum, 0.002% neutral red, and 1.2% Noble agar. Plaques were counted 4 days postinfection (p.i.).

Preparation of Vaccinia-Soluble Early Antigens (Es Antigen).—The method was described in a previous report (3). Briefly, HeLa cells were infected with the conditional lethal mutant at an input multiplicity of infection (m.o.i.) of 10 plaque-forming units (PFU)/cell in the presence of cytosine arabinoside at 20 μg/ml to block a leak of the late antigen synthesis that was observed in a few percent of cells when infected at a high m.o.i., and 18 h later cells were scraped off into the medium with a rubber policeman. The cell suspension was clarified by centrifugation, concentrated by acetone precipitation, and served as the soluble early antigen preparation (Es antigen). No infectivity could be found in the Es antigen thus prepared. Mock-infected HeLa cell cultures were treated similarly and served as a control antigen. The protein content of the antigen preparations was determined by Lowry's method (7). Es antigen preparation used for immunization contained 420 μg/ml of protein with CF antigen titer of 1:64 against antivaccinia serum, while host cell antigen preparation contained 680 μg/ml of protein without any cross CF reactivity with vaccinia antiserum.

Complement-Fixation (CF) Test.—The micromethod in a disposable tray (8) was used.

Immunization of Rabbits.—Albino rabbits of about 2 kg of body weight were immunized with antigen preparations with or without Freund's complete adjuvant. Two injections of 2 ml each of the antigen were given subcutaneously or intramuscularly at an interval of 2–3 wk. When used with adjuvant, the antigen was mixed with an equal volume of Freund's complete adjuvant and 4 ml each of the mixture was injected. Sera were obtained from each rabbit before immunization, and before and 4 wk after virus challenge.

Challenge of Rabbits with Vaccinia Virus.—At a specified time interval after immunization each rabbit was inoculated intradermally with 200 PFU each of the Ikeda strain of vaccinia virus in 0.2 ml into four spots on the shaved back. After 5–6 days the skin of two inoculated sites (3 cm² each) was excised to quantificate the amount of virus multiplied.

Interferon Assay.—A portion of the skin of immunized rabbits was excised at a specified interval. A 10% homogenate was prepared and assayed for interferon activity. The homogenate was inoculated onto RK-13 monolayers and incubated at 36°C for 24 h. The cell cultures were then washed and inoculated with VSV. Plaques were counted after 4 days at 36°C and interferon activity was expressed as reciprocals of the highest dilution of the preparation that reduced plaque number to 50% of those of virus control. In each test the titration of the standard rabbit skin interferon preparation diluted to 16 U with a 10% homogenate of the skin of a normal rabbit was set up in parallel to check the dependability of the test. The standard interferon preparation from the rabbit skin was kindly provided by Dr. Y. Kojima, Kitasato Institute, Tokyo.

Production of Migration Inhibitory Factor (MIF) and Macrophage Migration Inhibition Assay.—These were carried out by the method described by Rocklin et al. (9) with minor modification. Cell preparation consisting of more than 95% circulating lymphocytes from an immune rabbit was cultured at 37°C in a CO_2 incubator in the presence or absence of the Es antigen. 24 h later, the cells were removed by a light centrifugation. The supernatants were stored at -70°C until tested in vitro for the migration inhibition of guinea pig macrophages. The Es antigen used for MIF induction had a CF antigen titer of 1:128 against an antivaccinia-soluble early antigen rabbit serum, but less than 1:8 against an anti-HeLa cell antigen rabbit serum.

Peritoneal exudate cells from normal guinea pigs were induced by intraperitoneal injection of Drakeol no. 35. The cells were washed in Eagle's medium and suspended to 8% by volume in Eagle's medium supplemented with 20% calf serum. Capillary tubes were filled with the cell suspension and centrifuged to obtain cell packs. The tubes were cut and the portion containing the cells was placed in micro Petri dishes. The chambers were filled with the supernatants of lymphocyte cultures to be assayed and incubated at 37°C for 48 h in a CO_2 incubator. The area of migration was projected and measured by planimetry, and percent migration inhibition was calculated:

$$\text{percent migration inhibition} = \frac{X - Y}{X} \times 100,$$

where X is the area of migration in the supernatant of lymphocyte cultures without antigen and Y is the area of migration in the supernatant of lymphocyte cultures with antigen.

RESULTS

Antibody Response of Rabbits Immunized with the Es Antigen of Vaccinia-Infected Cells.—Immunization of rabbits with the Es antigen in Freund's complete adjuvant did not induce detectable circulating neutralizing antibody against either intracellular or extracellular vaccinia virus (Table I). These sera,

TABLE I

Response of Rabbits Immunized with Vaccinia-Soluble Early Antigens in Freund's Complete Adjuvant to Intradermal Inoculation of Vaccinia Virus

Antigen used for immunization	Interval between the last shot and virus challenge	Serum obtained before virus challenge		Skin reaction (diameter of erythema)‡ Day(s) p.i.				Virus contents of the skin§ 6 days p.i.	Serum 4 wk p.i. NT‖
		NT*	CF*	1	2	4	6		
	wk				*mm*			*log PFU*	
Es antigen in	2	<4	32	8	12	12	10	<1.7	<4
Freund's com-		<4	16		12	21	13	<1.7	<4
plete adjuvant		<4	128	8	20	16	10	2.3	4
		<4	128	±	13	17	8	<1.7	4
	6	<4	32	7	16	24	10	4.2	16
		<4	64	4	15	16	12	4.5	16
Control HeLa	2	<4	<8	—	—	19	25	6.4	256
cell antigen in		<4	<8	—	±	20	17	6.2	256
Freund's com-		<4	<8	—	±	15	18	7.2	256
plete adjuvant		<4	<8	—	±	15	28	7.3	256
None	—	<4	<8	—	—	16	19	5.7	N.D.
		<4	<8	—	—	13	22	6.3	N.D.

* NT: Neutralizing antibody against extracellular and intracellular vaccinia virus as measured by 50% plaque reduction in CE cultures. CF: Complement-fixing antibody against vaccinia soluble antigen (CAM).

‡ Underline means erythema with induration.

§ About 3 cm² each of the skin around two inoculation sites was excised and a 10% homogenate was made. Virus titer was expressed as log PFU per one inoculation site (3 cm²).

‖ NT: Only neutralizing antibody against CAM virus preparation consisting of mainly intracellular virus was measured.

however, fixed complement when reacted with the soluble antigens of vaccinia-infected cells (CAM). Neither neutralizing antibody nor vaccinia-specific CF antibody were detectable in the sera of nonimmune rabbits or those immunized with the control host cell antigen.

Skin Reaction and Virus Growth in the Skin of Immune Rabbits by Intradermal Inoculation of Vaccinia Virus.—At specified time intervals after the last immunizing shot each rabbit was inoculated intradermally with 200 PFU each of vaccinia virus as described in Materials and Methods. Rabbits immunized with the Es antigen responded to the virus challenge with an earlier appearance of erythema at the inoculation site compared with nonimmune rabbits or those treated with the control antigen. Erythema in the skin of immunized rabbits later became accompanied with induration, which was not so prominent and tended to wane from 5 to 6 days p.i., although faint reddening of the skin still remained in some rabbits up to 6 days p.i. In contrast, the skin reaction in control nonimmune or host cell antigen-treated rabbits took a typical course, showing an appearance of recognizable erythema and induration at the inoculation sites from 3 to 4 days p.i., which increased their intensity up to 6 days p.i. with typical lesions characterized by intense induration accompanied with central necrosis. The virus contents of the skin around the inoculation site were assayed 6 days p.i. Results of one of the replicate experiments are shown in Table I. The virus propagation was greatly inhibited in the skin of rabbits immunized with the Es antigen, compared with those in control rabbits. The neutralizing antibody titers of the sera obtained 4 wk p.i. appeared to reflect the grade of virus replication in the skin of each rabbit. It was thus shown that the immunization of rabbits with the Es antigen in Freund's complete adjuvant induced resistance to intradermal inoculation of vaccinia virus without eliciting the production of detectable circulating neutralizing antibody.

Replicate experiments indicated that the resistance of immunized animals could be observable from 1 to 6 wk (the longest interval examined) after the last immunizing shot, reaching its height 2–3 wk and declining thereafter. Induction of skin resistance in rabbits was not influenced by the route (subcutaneous or intramuscular) or site (lower portion of the flank or thigh) of immunization.

Absence of Detectable Soluble Inhibitory Substance of Vaccinia Virus in the Skin of Immunized Rabbits.—Experiments were carried out to examine a possibility that interferon or any inhibitory substance of vaccinia virus existed in the skin of immunized animals that might account for the restricted multiplication of vaccinia virus inoculated intradermally. A 10% homogenate of the skin of two rabbits immunized with the Es antigen in Freund's complete adjuvant as well as of those treated with the host cell antigen was prepared. The supernate of the homogenate from either group of rabbits did not reduce the number of plaques produced by vaccinia virus in CE cultures nor contained any interferon activity when assayed in RK-13 cell cultures by use of VSV. The standard

interferon preparation diluted to 16 U with a 10% homogenate of the skin of a normal rabbit was titrated in parallel and it was confirmed that the skin homogenate did not reduce the interferon activity. Another experiment was designed to examine a possibility that intradermal inoculation of vaccinia virus might induce interferon earlier in immunized rabbits than in control animals. Two rabbits immunized with the Es antigen in Freund's complete adjuvant were inoculated with 200 PFU each of vaccinia virus intradermally into four spots on the back 2 wk after the last immunizing shot. The skin of immunized animals around the site of vaccinia inoculation was excised 24 h p.i. and a 10% homogenate was prepared. The homogenate clarified by a light centrifugation was acid treated at 4°C for 3 days, neutralized with N NaOH, and diluted 1:10 with Eagle's medium containing antivaccinia serum. This rather long treatment of the homogenate with acid followed by dilution with the medium containing antiserum was necessary, because vaccinia virus in a 10% rabbit skin homogenate was more stable to acid treatment than in a less proteinous suspending medium. The standard rabbit skin interferon preparation diluted to 16 U with a 10% normal rabbit skin homogenate was treated in the same manner. They were assayed for interferon activity in RK-13 cell cultures against plaque formation by VSV. The skin homogenates from immunized animals showed no interferon activity, while the standard interferon preparation treated in the same manner showed no significant loss of activity.

Inability of Antisoluble Early Antigen Rabbit Sera to Passively Immunize Normal Rabbits against Intradermal Inoculation of Vaccinia Virus.—Although sera from rabbits immunized with the Es antigen in Freund's complete adjuvant did not contain detectable neutralizing antibody against either intracellular or extracellular vaccinia virus, a possibility should be considered that the serum may be effective in vivo in conjunction with complement or any other cofactor to inhibit the multiplication of vaccinia virus. The serum from three rabbits immunized with the Es antigen was transferred to four normal rabbits subcutaneously and intradermally. Each rabbit received 4 ml of the serum, 1 ml each subcutaneously on the lower portion of each flank, and 0.2 ml each intradermally in 10 spots around the four sites to be inoculated with vaccinia virus. As controls two other rabbits received the same amount of rabbit serum against host cell antigen and four rabbits received the same amount of hyperimmune serum from two rabbits obtained by dermal infection with vaccinia virus. 6 h after the serum transfer each rabbit was inoculated intradermally into four spots with 200 PFU each of vaccinia virus. Rabbits that received immune serum against either the Es antigen or HeLa cell antigen showed the typical primary skin reaction of vaccinia infection. Although there were some variations in the size of induration and local virus contents 5 days p.i., they were within the range of reactivities of normal rabbits. On the contrary those that received antivaccinia immune serum showed complete resistance to intradermal inocula tion of vaccinia virus (Table II).

TABLE II

Inability of Rabbit Sera against the Es Antigen to Passively Immunize Normal Rabbits against Intradermal Inoculation of Vaccinia Virus

Nature	Code of donor rabbit*	Neutralizing antibody titer‡	Induration 5 days p.i.	Virus contents 5 days p.i.
			mm	*log PFU*
Anti-Es antigen (with	A09	<4	16	7.3
Freund's complete	A10	<4	13	5.7
adjuvant)	A24	<4	13	6.0
	A24	<4	11	5.4
Anti-HeLa cell antigen (with	A15	<4	20	7.1
Freund's complete adjuvant)	A16	<4	19	6.9
Antivaccinia	S-22	4,096	0	<1.7
	S-22	4,096	0	<1.7
	S-18	1,024	0	<1.7
	S-18	1,024	0	<1.7

* Rabbits nos. A09, A10, A15, and A16 correspond to the first two rabbits each in Es antigen and HeLa cell groups, respectively, in Table I. Rabbit A24 was immunized with Es antigen for another experiment.

‡ Measured with the conventional-infected CAM homogenate.

Induction of MIF by Lymphocytes from Es Antigen-Immunized Rabbits.—The results of the preceding experiments suggested that the resistance against intradermal inoculation of vaccinia virus induced in rabbits by the Es antigen in Freund's complete adjuvant may not be due to the humoral antibody but of cell-mediated type. Two rabbits each were immunized with two intramuscular injections at an interval of 2 wk either with the Es antigen or with the control HeLa cell antigen in combination with Freund's complete adjuvant. Blood was withdrawn from each rabbit 2 wk after the last injection, and lymphocytes were cultured with or without the Es antigen as described in the Materials and Methods. The supernate of the culture fluids obtained 24 h later was assayed for the migration inhibition of guinea pig peritoneal macrophages. As shown in Table III, lymphocytes from rabbits immunized with the Es antigen produced MIF in the presence of the Es antigen, whereas those from control rabbits immunized with the host cell antigen did not.

Response of Rabbits Immunized with the Es Antigen without Adjuvant to Intradermal Inoculation of Vaccinia Virus.—The immunizing effect of the Es antigen without adjuvant was then examined. Rabbits were given two subcutaneous or intramuscular injections of the antigen. At specified intervals after the last injection the animals were inoculated intradermally with 200 PFU each of vaccinia virus in four spots on the shaved back. The results of one of the typical experiments are shown in Table IV. The circulating antibody

TABLE III

Induction of MIF by Lymphocytes of Rabbits Immunized with the Es Antigen

Source of lymphocytes	Code of donor rabbit	Antigen for MIF induction	Area of migration of macrophages	Percent migration inhibition
				%
Rabbit immunized with the Es antigen in Freund's complete adjuvant	7121	Yes	919	23.4
		No	1,148	
	7122	Yes	826	31.6
		No	1,211	
Rabbit immunized with control HeLa cell antigen in Freund's complete adjuvant	7123	Yes	1,465	−11.5
		No	1,313	
	7124	Yes	1,457	−8.2
		No	1,348	

TABLE IV

Response of Rabbits Immunized with Vaccinia-Soluble Early Antigens without Adjuvant to Intradermal Inoculation of Vaccinia Virus

Immunization	Interval between the last shot and virus challenge	Serum withdrawn before virus challenge		Skin reaction (diameter of erythema)‡ at day				Virus contents of the skin§ 6 days p.i.	Serum 4 wk after challenge NT‖
		NT‖	CF*	1	2	4	6		
	wk					mm			
Two subcutaneous injections of the Es antigen at an interval of 2 wk	3	<4	16	20	30	cfl¶	cfl	8.1	1,024
		<4	8	19	32	cfl	cfl	8.4	4,096
	1	<4	32	29	38	39	cfl	6.3	256
		<4	8	26	40	cfl	cfl	8.0	ND**
Two intramuscular injections of the Es antigen at an interval of 3 wk	1	<4	8	19	30	36	18	5.4	64
		<4	8	10	21	16	12	4.9	256

*, ‡, §, ‖ See footnotes in Table I.

¶ cfl: Confluent lesion with inflammation of hemorrhagic nature.

** Not done because serum was not available due to an accidental death of the rabbit before 4 wk p.i.

response was not so different from those immunized with Freund's complete adjuvant. The local reaction, however, was characterized by a heavier edema and erythema around the inoculation site, which was very marked even 24 h p.i. The lesion progressed gradually to become confluent in some rabbits with those of neighboring inoculation sites and covered a broad area of the back accompanied by hemorrhage and partial necrosis 3 days p.i. and thereafter. The virus contents of the skin were rather higher than those of nonimmunized rabbits in the preceding experiments. Those immunized intramuscularly showed

a milder local reaction, which tended to wane from 5 days p.i. The virus con
tents of the skin were also not so different from those of nonimmune or host cell
antigen-immunized control rabbits in the preceding experiments. Neutralizing
antibody in the sera withdrawn 4 wk p.i. again appeared to reflect the grade of
local reactions or virus contents of the skin.

DISCUSSION

Evidence has been accumulated both clinically (10, 11) and experimentally
(12–14) that cell-mediated immune responses play an important role in the
resolution of vaccinial lesions, while neutralizing antibody only is not always
fully effective on the recovery of animals from vaccinia virus infection. Various
attempts have been made in assessing the relative contributions of humoral
and cellular type of immunological mechanisms to resistance to vaccinial in-
fections (12–19). Hirsch et al. (14) demonstrated that antimouse thymocyte
rabbit serum suppressed in mice host cell-mediated responsiveness to intra-
venously administered vaccinia virus, thereby augmenting the morbidity and
mortality of this infection, while it did not affect either humoral antibody or
interferon production in response to vaccinia virus. Turk et al. (12) produced
a typical delayed hypersensitivity without demonstrable circulating antibody
to vaccinia virus in guinea pigs by injection of virus-antiserum mixtures, and
found that small reduction in the multiplication of vaccinia virus occurred in
the presence of delayed hypersensitivity reactions, whether these were virus-
specific or produced by an unrelated antigen. They concluded that this slight
effect on virus multiplication was unlikely to play a significant part in spon-
taneous remission of vaccinia virus infection. Recently Blanden (17–19) stressed
again the important role of cell-mediated immunity in the mechanism of re-
covery of mice from generalized mousepox virus infection. A contradictory
report, however, was also published by Worthington et al. (20) on the effects
of cyclophosphamide in systemic vaccinia virus infection of mice, suggesting an
essential role for humoral antibody, but not for cellular immunity, in recovery
from primary vaccinia virus infection in the mouse.

Vaccinia virus infection induces a number of virion antigens as well as soluble
antigens, and it has not been elucidated which antigen(s) might be responsible
for cellular immunity essential for the resolution of vaccinial lesions. Ueda and
Nozima (21) reported that the LS antigen was responsible for developing de-
layed hypersensitivity, but it remains still unknown whether or not this antigen
is effective in vivo as an inducer of cellular immunity effective against vaccinial
infections. McNeil (22) described skin resistance and hypersensitivity in rabbits
immunized with inactivated vaccinia virus, especially when combined with
polyvinylpyrrolidone, although variable neutralizing antibody responses were
also observed, and he considered that skin resistance was related to the titer
of circulating antibody rather than to the degree of delayed hypersensitivity.
Boulter et al. (23) suggested that inactivated intracellular virus may induce a

cellular type of immunity, which is effective in protection of rabbits against a lethal disease by rabbitpox infection.

In the present report we presented an evidence that the immunization of rabbits with the vaccinia-soluble early antigens obtained from mutant-infected HeLa cells (Es antigen) induced resistance in the rabbit against intradermal inoculation of vaccinia virus without eliciting demonstrable neutralizing antibody production. Inhibition of the multiplication of virus in immune rabbits was evidenced by reduced virus contents in the skin of inoculation site as well as a lower neutralizing antibody response compared with those in control animals. It seems unlikely that interferon played more favorable role to immunized animals in restricting the multiplication of virus in the skin than to control animals. The resistance may be considered as of cell-mediated type, because serum from immune rabbits could not transfer the resistance to normal rabbits. Preliminary experiments on an attempt to transfer immunity to normal rabbits by lymphocytes from lymph nodes and the spleen of immunized rabbits were not successful. Lymphocytes of immune rabbits, however, induced MIF in the presence of the Es antigen. Earlier appearance of skin reaction in immunized animals than in control animals after intradermal inoculation of virus seems to suggest that delayed hypersensitivity reaction took place against early antigens produced by inoculated virus. Our previous data indicated that vaccinia-specific soluble early antigens appear on the surface of infected cells before the commencement of viral DNA synthesis. Thus, infected cells could be recognized and attacked before progeny virions are produced within them, if cell-mediated immunity is strong enough. When rabbits were immunized with the same antigen without adjuvant, an enhanced reaction of animals was observed against intradermal injection of vaccinia virus. The skin lesion was far more enhanced, being accompanied by heavy induration, hemorrhage, and partial necrosis, and virus multiplication was also much exalted. This type of abnormal skin lesion has also been reported in some rabbits immunized with inactivated vaccinia virus (22). The reason why immunization without adjuvant resulted in an adverse effect against intradermal vaccinia infection remained to be elucidated, but a possibility might be that immunization without adjuvant may be insufficient to induced cellular immunity strong enough to inhibit the multiplication of inoculated virus, whereas enhancing antibody was induced that interfered with the induction and/or manifestation of cellular immunity. The existence of nonspecific antigen-antibody reaction, which may affect the permeability of peripheral blood vessels in tissues near the inoculation site, should also be taken into consideration.

Recently Ito and Barron (24) reported that a strain of vaccinia virus was defective in surface antigen production. Their observation was confirmed by us and it was further revealed that a few strains of variola-vaccinia group were defective in synthesizing the Es antigen (Ueda, Y., I. Tagaya, and H. Amano, data to be published). It was also found that the Es antigen is a portion of

vaccinia early antigens and that the strains of variola-vaccinia group examined including those defective in synthesizing the Es antigen shared the other early antigens, which seemed, however, to be less abundant on the surface of infected cells (Tagaya, I., H. Amano, and Y. Ueda, unpublished observations). In the later stage of infection late antigens also become observable on the surface of infected cells. All these antigens together with the virion antigens may be responsible for the induction of immunity against vaccinia virus infection, and further studies are required to elucidate the immunological roles played by each of these antigens. The present report may be the first step for the studies of complicated vaccinial immunity from the point of the antigens concerned.

SUMMARY

The immunological role of vaccinia-soluble early antigen was examined in rabbits. The antigens were prepared from HeLa cells infected with a conditional lethal mutant of vaccinia virus, which induces in these cells early antigens including those responsible for surface immunofluorescence of infected cells, but not viral DNA and late protein syntheses. Immunization of rabbits with the antigens in Freund's complete adjuvant induced complement-fixing antibody but neither detectable circulating neutralizing antibody nor any detectable level of inhibitory substance or interferon in the skin of the animals. When immunized animals were inoculated intradermally with vaccinia virus, multiplication of virus in the skin was greatly inhibited, being accompanied by an earlier appearance as well as an accelerated wane of the local reactions. The resistance could not be transferred passively by the serum of immunized animals to normal rabbits. Immunization of rabbits with the antigens without the adjuvant not only failed to inhibit but, contrariwise, enhanced the multiplication of intradermally inoculated vaccinia virus, inducing heavy skin lesions and exalted virus multiplication.

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