INHIBITION OF THE LESIONS OF PRIMARY VACCINIA AND OF DELAYED HYPERSENSITIVITY THROUGH IMMUNOLOGICAL TOLERANCE IN RABBITS*

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Although the concept, that hypersensitivity plays an important role in the development of the lesion of primary local vaccinia, was offered by von Pirquet (1) early in this century, it has been obscured in recent years by greater interest in the lytic abilities of the multiplying virus in host cells, especially as observed in tissue culture (2–4).

However, when opposing hypotheses of lesion formation, *i.e.* the allergic *versus* the cytopathic concepts, have been put to test in intact animals, the experimental results have favored hypersensitivity over cytopathogenicity as basically responsible for the skin lesion. Thus, in children the shortened incubation period of a second, local vaccinial lesion derived from an infection initiated 2 days after a primary inoculation of vaccinia virus, indicated that the second infection was not an independent event, but rather was influenced by allergy already provoked by the first infection in the same host (5). Pincus and Flick (6) also found that a primary vaccinial lesion in the guinea pig was completely suppressed by the local injection of a rabbit anti-guinea pig mononuclear cell serum. This treatment also prevented the occurrence of delayed hypersensitivity to inactivated vaccinia virus as well as other expressions of delayed allergy.

But before the role of developing hypersensitivity in the production of the vesicular lesion of primary vaccinia can be considered as proven, further evidence supporting this concept must be obtained by other, unrelated technics. Accordingly, this report deals with the suppression of the primary vaccinial lesion when delayed hypersensitivity to the vaccinial antigens is inhibited by another method. The method of immunological tolerance was used in newborn rabbits to suppress the development of allergy to the vaccinial antigens. This procedure also accomplished a suppression of the expected local lesion following infection with the active virus.

The use of microbial antigens to induce specific immunological tolerance has lacked the degree of success associated with tolerance directed toward tissue homo-

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grafts or toward heterologous serum proteins. Burnet *et al.* (7) using chickens, and Nossal (8) using mice, were unsuccessful in establishing tolerance to the antigens of influenza virus. Cohn (9), as well as Smith and Bridges (10), failed to induce tolerance to a variety of microbial antigens. Festenstein and Bokkenheuser (11) were unable to induce tolerance in rabbits with *Treponema pallidum*, while Rees and Garbutt (12) likewise failed with *Mycobacterium tuberculosis* in mice.

In contrast to these, there have been a number of reports of successful induction of tolerance, partial or complete, to microbial products. In retrospect, Traub (13) probably encountered the tolerant state with respect to lymphocytic choriomeningitis virus when infection occurred in some colonies of laboratory mice. Buxton (14) observed tolerance with respect to *Salmonella pullorum* infection in chickens. In a small number of calves, Kerr and Robertson (15) observed unresponsiveness (tolerance or paralysis) with respect to *Trichomonas foetus* infection. A dose of about 1 gm of antigen was used in their studies to render the animals tolerant. Weiss (16) was often able to prevent the development of tuberculin skin hypersensitivity by preparative injections into fetal guinea pigs of either tuberculin or killed BCG but not with living BCG. Tolerance here was of relatively short duration. Friedman and Gaby (17) were able to produce partial tolerance in chickens with suspensions of killed *Shigella*. Tolerant animals had significantly lower titers of antibodies after challange than the unprepared controls. However, there was no delay in the antibody response in the tolerant animals like the delay found by Gowland and Oakley (18) as the sole evidence of tolerance in chickens involving diphtheria toxoid as the antigen. They used 10 Lf of toxoid to prepare the animals (about 30 μg, reference 19, of purified toxoid protein) and after challenge, 8 of 10 tolerant animals showed a delay of 4 to 7 days in antitoxin production as compared to the controls. Since no significant depression of antibody level occurred here and a small number of animals was involved, only future experience can decide if this represents true tolerance. Relatively small differences in antitoxin titers suggested to Lindorfer and Subramanyam (20) that they had successfully produced tolerance to staphylococcal toxins in rabbits. Sterzl and Trnka (21) prepared newborn rabbits with low dose (10^8 organisms) and high dose (5×10^9 organisms) suspensions of *Salmonella schottmuelleri*. Both groups of animals developed homologous antibodies shortly thereafter. But, upon challange about 4 months later when these antibodies had disappeared from the blood, only the high dose group failed to show a rise in homologous antibody, suggesting tolerance. This observation, that the preparative antigen exposure immediately after birth produced an antibody response, is unusual. But, in spite of this transitory immune response, a state of tolerance seemed to occur later.

Thus, even with those reports of successful induction of tolerance toward microbial antigens, there is considerable variation in reliability of the effect; partly because of the small animal sample used in some of the experiments and also partly because of some of the unduplicated, unique results interpretated as tolerance in other experiments. Although several investigators have suggested that the degree of "foreignness" of an antigen plays an important role in its inability to induce tolerance in a given species of animal (8, 22, 23), in view of the relatively high probability of success with some microbial antigens, better explanations of the difficulty in tolerance induction might include the dosage effect. The work of Smith and Bridges (10), dealing with

tolerance against bovine serum albumin as the antigen, emphasizes the importance of large doses of antigen in inducing complete tolerance and bringing about a prolonged state of immunological failure. With microbial suspensions especially, a small total mass of organisms represents a much smaller amount of each component antigen of which there are usually many.

Following this lead, a relatively large dose of inactive vaccinia virus was used to prepare our immature animals; 0.5 to 1.0 mg of partially purified virus per rabbit. In addition, since the duration of the tolerance state seems to be dose-dependent (10), it seemed highly desirable to infect the tolerant-prepared, newborn rabbits as soon as possible with active vaccinia virus. These and possibly other factors to be considered later, may have increased the chances of successfully inducing a high level of tolerance to vaccinia virus for a period of sufficient duration to allow the study of the local disease.

Materials and Methods

Active vaccinia virus, in the form of a large batch of commercial calf lymph virus, was obtained through the generosity of Dr. M. Z. Bierly of Wyeth Laboratories, Inc., Marietta, Pennsylvania. It was inoculated always by the multiple pressure technic into a depilated area of skin. The preparation had an activity of 10^8 $TCIU_{50}$/ml in monkey kidney cell culture (24).

Inactive virus for skin testing was prepared by heating the calf lymph vaccine at 60°C for 1 hour. This was tested for the lack of infectivity in tissue culture before use. It was inoculated into the skin of the animals in the same manner as the active virus.

Inactive, purified virus for tolerance preparation was grown in calf embryo[1] epidermal cell cultures (25). Approximately 4 days after inoculating the sheet of epidermal cells in each large bottle with about 10^6 infectious units of virus, the cells were mostly lysed and the fluid was harvested. A batch of about 4.6 liters of culture medium was processed to purify the vaccinia virus therein. The fluid was first centrifuged at 800 RPM (50 *G*) for 20 minutes to remove large cellular debris, then at 4500 RPM (1600 *G*) for 2 hours to sediment the virus. This cycle of differential centrifugation (26) was repeated twice, using Hanks' solution to resuspend the virus. The purified virus in final suspension was found to contain $10^{11.2}$ $TCIU_{50}$/ml. 20 ml of this suspension was lyophilized, weighed, and the weight of the solids from the Hanks' solution subtracted. The resulting 18 mg of virus was then resuspended in water to yield a suspension containing 1.0 mg of virus/ml. This weight of the virus must also include some fine cellular particles comparable to the virus in size.

The virus suspension was divided in half, and each half inactivated by a different method; one part by means of 60°C for 1 hour and the other by use of 0.2 per cent beta propiolactone at 37°C for 1 hour (27). The 2 aliquotes were recombined. Following inactivation, the virus was found to be non-infectious in calf embryo epidermis culture, both undiluted and diluted 1:1000 in order to reduce the possibility of the interference phenomenon effect masking active virus. As further evidence of inactivity of the virus was the absence of evidence of generalized vaccinia following the injection intramuscularly of about 1.0 mg of the virus preparation into each of about 40 rabbit fetuses. The use of 2 different methods of inactivation was to improve the probability that all native antigens would be present in the final product.

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[1] We wish to express our thanks and appreciation to Dr. Eugene Rosenoff for permitting us to prepare several large quantites of virus in the research laboratories of the Wyeth Laboratories, Inc.

In order to improve the chance of having some antigen in a soluble (12) rather than particulate form, half of the bulk of inactivated virus was treated in a 10 kc sonic oscillator for ½ hour and then recombined. Solubilization of viral antigens is certainly not complete under these circumstances since such treatment does not appreciably reduce the infectivity of vaccinia virus (28, 29).

Animals and Preparation.—Rabbit fetuses on the 20th to 24th day of gestation were injected with 1.0 mg of inactivated, purified virus, through the intact uterine wall. Those surviving the 3rd day postpartum were then inoculated with active vaccinia virus.

In an attempt to produce tolerance after birth, rabbits were injected intramuscularly with 0.5 mg of inactive, purified virus within 24 hours of birth. A period of 2 days was allowed to elapse in order to observe for evidence of acute toxicity of the virus preparation. No adverse effects were observed. At the end of this observation period the animals were vaccinated with active virus.

Control newborn rabbits were those which were not prepared by the injection of inactive virus. They were vaccinated with active virus at the same age as the test animals.

RESULTS

With all of the animals the timing of the various observations is designated as days after the first application of active vaccinia virus, which occurred, then, on day zero.

In order to obtain a normal baseline of reaction, 15 newborn rabbits were inoculated with active vaccinia virus 4 days after birth. Two of the animals failed to develop local vaccinial lesions and subsequently failed to give a reaction of local hypersensitivity to inactive virus. These rabbits were not investigated further. But 13 of the rabbits developed typical, local lesions of vaccinia. Papules or vesicles appeared by day 2 and all had vesicular lesions by day 3. These became pustular by the 4th day and gradually healed with a small scar remaining. On day 7, they were skin-tested with inactive virus for hypersensitivity. By the following day, 11 of the 13 rabbits with lesions had reactions of allergy consisting of papules surrounded by erythema. None of the control animals showed gross evidence of systemic vaccinia.

In an attempt to produce immunological tolerance, 22 newborn rabbits derived from 6 does were given 0.5 mg of inactive, purified vaccinia virus on the day following birth by intramuscular injection. Over the next several days of observation, these animals appeared normal. Three of the animals were not treated further but were observed for gross evidence of acute or chronic toxicity resulting from the injection of inactive virus. These remained normal in appearance and behavior.

The 19 remaining, tolerant-prepared rabbits were vaccinated with active virus at 4 days of age (now day zero). On day 1, slight erythema appeared at the inoculation sites of 2 rabbits. By day 2, these and 6 more showed a flat small area of erythema. There was no evidence of edema at these sites of inoculation in any of the rabbits. By day 3 all redness had disappeared, but now 7 rabbits had a small, flat, brown scab covering the site of vaccination. Six of these rabbits

were among those with early erythema, but 1 had not shown redness. Subsequently, as long as the scab persisted, hair failed to grow at the site; although it grew profusely elsewhere. The scabs persisted for over 7 days in surviving animals but disappeared without scar by the 14th day. At no time did these rabbits with scabs have papules, vesicles, or diffuse edema at the inoculation sites.

The remaining 12 tolerant-prepared, vaccinated rabbits developed no visible lesion at the inoculation site at any time during the period of survival. Also, none of the test or control animals developed obvious vaccinial lesions of the skin at sites distant from the area of inoculation of virus.

Although the control animals survived the postinfection period without loss, such was not the case with the tolerant-prepared rabbits. A high mortality rate occurred in this group. All these rabbits, both with and without scabs survived through postvaccination day 3, at which time the controls had vesicles. Starting on day 4, deaths occurred in both groups of tolerant-prepared rabbits. By the 7th day, 11 of the 19 rabbits had died. One other rabbit died on the 10th day, and another on the 14th day, leaving 6 survivors for late study (see Fig. 1).

Evidence of Successful Infection.—Because failure of lesion formation in the tolerant-prepared rabbits could be due to a failure of infection, it was necessary to prove that the vaccinia virus had actually infected the test animals. Virus isolations were attempted on the organs of 4 of the 5 rabbits dying on day 4. At autopsy, the liver and lungs of each rabbit were pooled for studies in tissue culture. Virus was isolated from all 4 pools, and was proven to be vaccinia by specific neutralization with rabbit antivaccinia serum in the second serial passage. In addition, the 5th rabbit dying on the 4th day and 2 rabbits dying on the 5th day were found to have typical vaccinia inclusion bodies in many cells of the lungs, liver, and of the epidermis at the site of inoculation. These microscopic findings may be interpreted as presumptive evidence of vaccinia infection; since in themselves, they are not completely diagnostic of vaccinia rather than some other viral infection.

Hypersensitivity and Serology.—On the 7th day, the 7 surviving tolerant-prepared rabbits were tested for hypersensitivity with inactive virus. None of these rabbits showed reactions of allergy on the next and subsequent days at the inoculation site.

Blood was drawn on the 11th day from the 6 surviving test rabbits and from 6 control rabbits. None of the tolerant-prepared rabbits had detectable hemagglutination-inhibition antibodies. On the other hand, 3 of the 6 newborn control rabbits had detectable antibodies with titers of 1:2 to 1:4.

Pathological Examinations.—All tolerant-prepared rabbits were autopsied shortly after death. Certain gross features were common to all. The lungs appeared congested with multiple small subpleural hemorrhages. Visceral pox, which often accompanies generalized vaccinia in animals (30), did not occur in the gross in the organs of these animals.

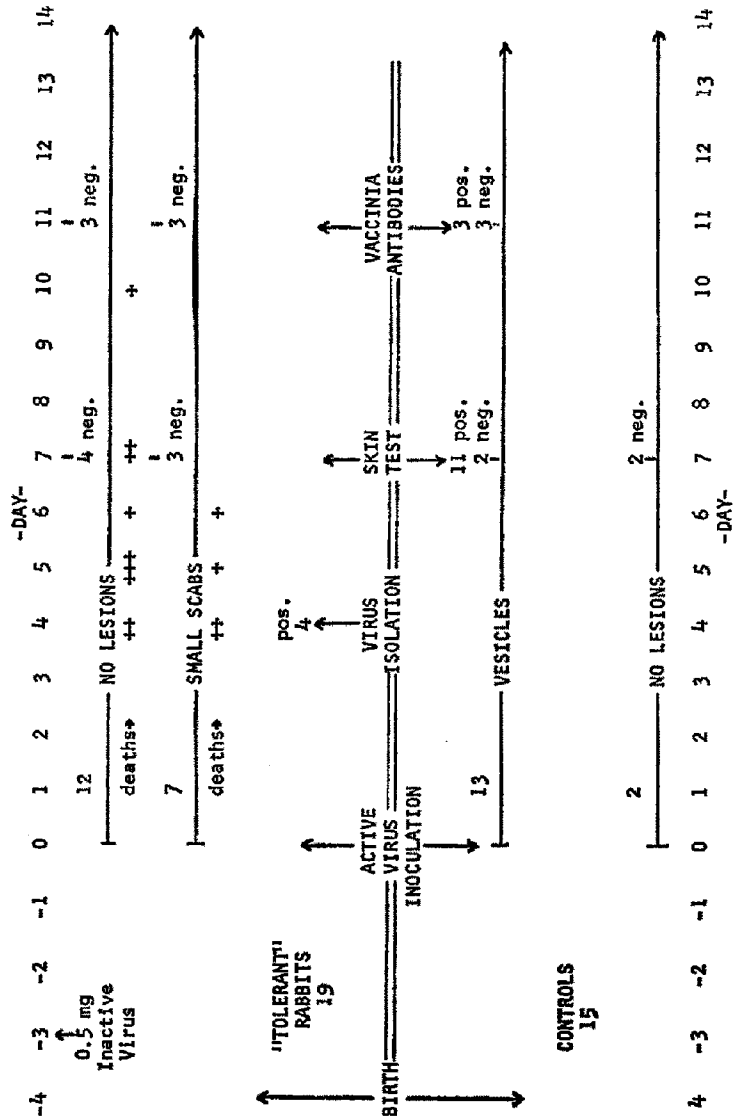

FIG. 1. Summary of events in the postnatal "tolerance" experiment. Each + represents the death of a single animal on the day indicated. Each number represents the number of animals studied.

Histological sections were prepared from lung, liver, and skin at inoculation site of rabbits dying on the 4th and 5th day. The lung sections presented thickened alveolar walls, some infiltration of septa by polymorphonuclears and by round cells, and minute areas of hemorrhage. The liver contained microscopic areas of necrosis of the parenchymal cells without appreciable inflammatory reaction or loss of cellular architecture. The single skin section was through a scab. There was loss of a small area of epidermis and the dermis below was infiltrated with inflammatory cells. In all sections, the cells containing inclusion bodies (Hotchkis-McMannus stain), did not appear necrotic. Also the necrotic areas of liver did not appear to be closely adjacent to cells containing viral inclusions. Sections were not obtained of inoculation sites which failed to produce a scab or other lesion.

Revaccination Studies.—About 1 month after the initial vaccination with active virus, some of the control, and all the surviving test rabbits were reinoculated with virus to determine their immunological status and responsiveness. With active virus reinfection, 4 control rabbits developed "immune" responses, consisting of papule formation by 24 hours. Inactive virus inoculation in these animals produced a similar reaction. Vaccinial antibodies 4 days after revaccination varied in titer from 1:2 to 1:8.

The 6 surviving tolerant-prepared rabbits behaved differently upon rechallenge. Three rabbits died: 1 on the 3rd day and 2 on the 5th day postrevaccination. The former rabbit failed to react to either active or inactive virus before death. One of the latter animals developed a typical "immune" response at both the active and inactive virus sites within the first 24 hour period. The other developed a typical "primary" reaction in timing and morphology at the active virus site, but no reaction at the inactive virus site prior to its death. The cause of death in these animals is unknown. The remaining 3 rabbits survived beyond the 5 day period of observation without developing evidence of inflammation at either inoculation sites. Thus, 4 of 6 tolerant-prepared rabbits failed to react locally to the virus upon reexposure.

In Utero Tolerant-Prepared Rabbits.—Relatively little information was obtained from this group because of short survival times. Of 40 rabbit fetuses injected with 1.0 mg of inactive purified vaccinia virus 1 to 2 weeks before birth, only 8 survived the 3rd postnatal day, at which time they were vaccinated with active virus. At 24 hours, 4 rabbits had slight erythema without edema at the inoculation site. This redness persisted to the 2nd day in 3 of the 4 rabbits and appeared in a 5th rabbit on this day for the first time. On the 3rd day, 7 animals were found dead. The 8th rabbit was dead on the 4th day. None of the animals had developed evidence of edema or vesiculation at the inoculation sites before death whereas the controls usually had typical lesions by this day. Virus isolation was attempted on 4 of the 7 animals dead by the 3rd day. Again, pools were made of the liver and lungs from each animal; but in addition, in 2 cases, the

skin of the inoculation site was included in the pool. Vaccinia virus, as proven by specific neutralization, was isolated in tissue culture from 3 of the 4 animals.

The other 3 animals of this group were studied histologically. The gross and microscopic findings were like those described for the postnatal, tolerant prepared animals. Again, inclusion bodies compatible in appearance with vaccinial inclusions were observed in the organ sections.

DISCUSSION

Those rabbits, which were tolerant-prepared postnatally, lived sufficiently long after active virus inoculation to insure a failure of development of the typical local vaccinial lesion. Those rabbits, which were prenatally tolerant-prepared, did not survive sufficiently long after viral infection to lend certainty to lesion failure, but were suggestive of the same trend. In contrast the control animals developed vaccinial lesions within 2 days after infection. Therefore, tolerance preparation seemed to be responsible for the local lesion failure. In addition, tolerance preparation was associated with a failure of the animals to develop delayed hypersensitivity to the vaccinia viral antigens. This was most evident after the first challenge of the postnatally prepared animals, but was still evident in 66 per cent of the survivors when rechallenged with active and inactive virus 1 month later. The lack of an "immune" response to active virus in many of these animals especially emphasizes the failure of hypersensitivity production against vaccinial antigens. The ability to isolate vaccinia virus from the organs of the test animals, and to a lesser degree, the finding of viral inclusions, presumably vaccinia, in organ sections of some animals, indicates that viral multiplication occurred in these rabbits. Therefore, the lack of a typical local vaccinia lesion is not due to failure to infect the animals. Rather, the evidence points to the inability to produce vaccinial hypersensitivity as the probable cause of lesion failure. The evidence incriminating hypersensitivity does not depend upon the mechanism of immunological tolerance for the effect obtained. Rather, the method of immunological tolerance was used in an attempt to suppress hypersensitivity formation to vaccinia antigens. The mechanism of such suppression is speculative at the present time. It is the association of lesion failure with immunological failure that points to the mechanism of pathogenesis of the lesion here.

Support for the allergic hypothesis of formation of the primary local vaccinial lesion comes not only from the evidence presented here, the evidence previously cited in the introduction concerning humans (5) and guinea pigs (6) investigated by different technics, but also from other types of experiments. Thus, Pincus and Flick (31) have found that x-irradiation of guinea pigs in doses as high as 1000 roentgen failed to prevent the occurrence of the primary vaccinial lesion and also to prevent the rapid development of hypersensitivity to vaccinial antigens. This too supports the hypersensitivity concept in association with other evidence cited, in that even high doses

(32) of x-ray failed to inhibit both the allergy development and the appearance of the vaccinia lesion. In still another direction Ledingham (33) found, and Widelock (34) confirmed that the local injection of India ink before or during vaccinia virus inoculation inhibited the appearance of the local vaccinia lesion. Ledingham also found that local India ink injection inhibited the reaction of allergy to vaccinia virus in infected animals. However, the ink injection did not interfere with local virus multiplication at the site of injection (34).

On the other hand, Baron *et al.* (35) found that guinea pigs treated with 300 r of x-irradiation and the leukocyte suppressive drug, methotrexate, developed local primary vaccinia lesions but failed to show evidence of delayed hypersensitivity. This evidence better supports the cytopathic concept of lesion development, or, some non-allergic hypothesis. Our observations with vaccinia suggest that, under certain conditions, allergy will develop, but is difficult to demonstrate using inactive virus. But, by reinfection with active virus, provided the immunity is not great enough to inhibit viral multiplication completely, an accelerated lesion suggestive of a Koch phenomenon appears. Thus, the problem here may well be one of attempting to detect low levels of hypersensitivity or even local but not generalized hypersensitivity.

The development of flat scabs without edema at the site of inoculation of active virus in 7 of 19 tolerant-prepared animals is perplexing. These are certainly not typical vaccinial lesions. Nor is this scab lesion produced by a similar needling of the skin through a drop of normal serum or saline. The scab seems to have covered a small area of epidermal cell loss and beneath this, there is some infiltration of the dermis with inflammatory cells. Although virus inclusions occurred in some cells in this region, degenerate or necrotic cells were not obviously present at 4 days after infection. Animals with scabs otherwise reacted like animals without scabs in this group. One of several mechanisms might account for the scabs. This lesion could represent a degree of epidermal cell necrosis due to the cytopathic properties of the virus. If it does, then the majority of tolerant-prepared animals fail to show gross evidence of such cytopathogenicity, and also other technics (6, 31) useful in suppressing the vaccinia lesion are not associated with scab formation. Rather, it would seem more likely that scab formation is associated with the particular technic reported here and used to suppress the immunological responses.

Another possible mechanism of scabbing might involve a partial escape of the immunological apparatus from the suppressive effects of the preparative virus antigen injection. Enough allergy may have developed to produce the scab without being detected by the skin-testing procedure in use. This explanation also seems unlikely to us at the moment because of the lack of a vesicular component, the lack of detectable edema, and failure of persistence or complete lack of erythema, all so characteristic of even weak hypersensitivity reactions.

A third possible mechanism of formation of the scab could be connected with the toxic properties of vaccinia virus (36, 37). This reaction could be akin to the endotoxin type of damage caused by Gram-negative bacteria or their active fractions and not associated with the act of viral multiplication. Sufficient virus "toxin" (associated with the virus particle) might be produced locally in some animals as a result of unrestrained local multiplication when the specific defense mechanisms are completely suppressed as in these tolerant-prepared animals. On the other hand, the suppression

of immunological responses locally as with the use of an antimononuclear cell serum (6) or India ink (32) would not prevent some specific immunological forces (antibodies, etc.) from entering the area to neutralize the toxic properties. Although we favor this third concept, only further detailed investigations can determine the true mechanism.

Although this study was concerned mainly with the local vaccinial skin lesion, the high mortality rate in both pre- and postnatal, tolerant-prepared rabbits after vaccinia infection cannot be ignored. The observations suggest that these animals died from generalized vaccinia and perhaps, as a result of the toxic properties of the virus producing an endotoxin-type of disease. Thus, other infectious diseases do not account for these deaths. There was little evidence of other infectious diseases in the other rabbits housed in the unit. Also control rabbits not prepared for tolerance usually remained healthy aside from the local vaccinial lesion. The isolation of vaccinia virus from the organs of some of the animals and the finding of inclusions typical of vaccinia virus in the organs of others in the experimental group indicate that a spreading type of vaccinial infection had occurred from the site of skin inoculation in the tolerant-prepared animals. These two lines of evidence suggest that the deaths were due to systemic vaccinia infection. The gross and microscopic tissue reactions of small hemorrhages in the lungs, and of scattered small areas of necrosis seen in the liver are at least compatible with the concept of a toxic disease and vaccinia virus has the toxic potentials (36, 37) for producing such *in vivo* damage. The fact that the virus spread readily to distant tissues from the local site of infection suggests that an early, probably specific, immune response normally develops to prevent this spread in non-tolerant inoculated animals. A similar inhibition of this hypothetical immune response probably occurred when adult guinea pigs (31) were infected locally with vaccinia virus following a single, lethal (death at 10 to 14 days from the drug alone) dose of the antileukocyte drug, vincaleukoblastin[2] (38). The vaccinated animals died at 3 to 4 days and presented the same type of gross and microscopic pathological lesions, including viral inclusions in the organs, as the infected tolerant-prepared neonatal rabbits.

This suggestion of an immunity arising within 2 to 3 days of local vaccinial infection at the level of the regional lymph node or even at the site of original infection itself is consistent with the observations of McMaster and Kidd (39) that an antivaccinia factor (probably either antibody or interferon) can be obtained from the draining lymph node as early as 3 days' postinfection. It is also consistent with the early occurrence of delayed hypersensitivity by skin test to vaccinia antigens as observed in other studies (5, 6). Although we are not attempting to equate here the cellular allergic response with a mechanism of viral immunity there is nothing in the vaccinial infection observations that is incompatible with this view.

The methods of immunological tolerance production were used here as a tool rather than to elucidate the production of tolerance to a microbial agent. That some form of immunological unresponsiveness was produced by the procedure used seems highly likely from the lack of immunological responses to vaccinia by the animals. Whether this state is best described by the term, tolerance, with its implications of being inducible only in the immature period of the young animal or by immunological paralysis which seems to be independent of age (40), cannot be resolved now. Tolerance does not require excess antigen to linger in the tissue fluids outside of immunological

[2] Generously supplied by Eli Lilly & Co., Indianapolis.

cells during its existence according to the cell transfer studies of Smith and Bridges (10). Paralysis (probably a misnomer) may require antigen to be in the tissue fluids or adsorbed to cells in order that antibody may combine with an excess of antigen outside of the cell as fast as it is formed (41). Whatever the detailed mechanism, it is evident in our study that newborn rabbits have been made tolerant immunologically toward the antigens of vaccinia virus for at least a relatively brief period. Perhaps tolerance also explains the early observations of Olitsky and Long (42) made on a single pregnant rabbit and its 3 offspring. The mother was infected with vaccinia virus during pregnancy. The 3 offspring failed to develop lesions when vaccinated with active virus at 32 days (1 rabbit) or 64 days (2 rabbits) of age. One of the latter animals had vaccinia virus (derived from the mother before birth) isolated from its testicles 1 month before the challenge vaccination.

SUMMARY

In order to gain insight into the pathogenesis of the vesicular lesion of local primary vaccinia infection, newborn rabbits were injected with 0.5 mg of purified inactivated vaccinia virus in an attempt to render them immunologically tolerant. Within a few days these, and control normal rabbits of the same age, were infected on the skin with active vaccinia virus. Most of the tolerant-prepared rabbits failed to develop a local lesion of vaccinia but some developed a very atypical lesion. Successful virus isolation from some, and the presence of inclusions in the tissues of others, indicated successful infection with the virus. Skin allergy to the active virus failed to develop in the test animals but did in the controls. Thus, there was a high degree of correlation between inability to produce delayed hypersensitivity to the viral antigens and failure to develop a vaccinial skin lesion, indicating the probable allergic nature of the primary lesion.

There was also a high mortality rate in the group of tolerant-prepared, infected animals. It was associated with a spreading of the virus from the site of infection to the organs, suggesting that generalized vaccinial infection was the cause of death. The observations were compatible with the hypothesis that death was due to viral toxicity.

The observations also suggest that, in the animal possessing normal immunological function, active immunity develops rapidly, perhaps at the level of the draining lymph node, to prevent appreciable virus from leaving the site of infection.

The absence of detectable immunological activity toward vaccinia virus early in the tolerant-prepared animals and even after 1 month in some of the survivors, indicates that a high degree of immunological tolerance was produced against these microbial antigens.

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