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Development of Delayed Dermal Hypersensitivity in Guinea Pigs Immunized with Inactivated Respiratory Syncytial Virus Vaccines* (33423)

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Respiratory syncytial (RS) virus is an RNA containing virus which has been classified as a member of the myxovirus family on the basis of its nucleic acid composition, ether sensitivity, and ultrastructure (1). Many clinical studies have demonstrated that this virus is a major cause of serious respiratory disease in infants and young children (2). In addition, several investigators have indicated that RS virus may be a cause of significant respiratory disease in adults (3). The development of a safe and effective vaccine to prevent infection by this agent would be of great public health importance.

The recent epidemiologic studies by Chanock and associates have emphasized the fact that RS virus causes serious lower respiratory tract illness predominently in young infants under 6 months of age (4). During this period of time, almost all of the infected infants possess passively acquired maternal serum neutralizing antibody against RS virus. This temporal association of passive maternal RS neutralizing antibody and severe disease caused by RS virus suggested to these workers that there was immunologic enhancement of RS disease in young infants by the maternally transmitted antibody. The recent experience by several groups demonstrating that infants who received an antigenic, inactivated concentrated RS virus vaccine developed more extensive respiratory disease following natural RS infection than did comparable control groups has provided additional impetus for the investigation of the role of immunological reactions in the pathogenesis of RS virus infections (4).

Although delayed hypersensitivity has not been implicated by these investigations as a cause of these adverse vaccine reactions, this mechanism has been suggested as the etiology of the adverse reactions seen in individuals who received another inactivated myxovirus vaccine, measles vaccine (5). The present study was undertaken in an attempt to determine if delayed hypersensitivity could be demonstrated in animals which had received inactivated RS virus vaccines and to define the nature of the antigen or antigens responsible for such reactions.

Methods. Vaccines[1]. (1) *RS-monkey kidney-alum (RS-MK-A) vaccine.* The Burnett strain of RS virus was grown in vervet monkey kidney cells. The vaccine was inactivated with 1:4000 formalin, filtered, concentrated 25 times by Sharples centrifugation and precipitated and concentrated 4-fold with 4

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[1] The RS vaccines were made available by the Vaccine Development Branch, NIAID, NIH. The RS-MK-A vaccine was prepared by Chas. Pfizer & Co., Inc. The RS-BK and RS-BK-A vaccines were prepared by Abbott Laboratories, Inc. The P3-BK and INF-BK vaccines were kindly provided by Dr. J. Holper, Abbott Laboratories, Inc.

mg/ml of alum. The final vaccine contained polymyxin B, neomycin, and streptomycin (200 mg/ml of each) and 1:40,000 benzethonium chloride. The final vaccine represented a 100-fold concentration of the original material.

(2) *RS-Bovine kidney-aqueous (RS-BK) vaccine.* The Simon TR strain of RS virus was grown in bovine kidney cells. The vaccine was concentrated approximately 200-fold by centrifugation and then was inactivated with 1:4000 formalin. The final vaccine contained 1.11 mg/ml of protein prior to the addition of 0.5% human serum albumen as a stablizer. The final vaccine also contained 1:10,000 thiomerisol.

(3) *RS-Bovine kidney-alum (RS-BK-A) vaccine.* This vaccine was identical to the RS-BK vaccine except that it also contained 15 mg/ml of alum.

(4) *Parainfluenza type 3—bovine kidney (P3-BK) vaccine.* The C243 strain of parainfluenza type 3 virus was grown in bovine kidney cells. The preparation of the vaccine was the same as that described for the RS-BK vaccine. No protein could be detected prior to adding the stabilizer.

(5) *Influenza type A2-bovine kidney vaccine (INF-BK).* The influenza A2/Japan/170/62E8 strain of influenza was grown in bovine kidney cells. The vaccine was concentrated 40-fold by centrifugation and inactivated with formaldehyde. The vaccine had a protein concentration of 0.22 mg/ml.

(6) *Diphtheria toxoid–tetanus toxoid–pertussis (DPT-A) vaccine.* Commercial alum adsorbed DPT vaccine (Tri Solgen, AP-Lilly) was used.

Skin test antigens. (1) The Long strain of RS virus (RS-H) was grown in HEp-2 tissue cultures as previously described (6). This material had a titer of $10^{5.0}$ TCD_{50} /ml of infectious virus when assayed in roller tube cultures of HEp-2 cells and had a complement fixing (CF) titer of 1:16 when tested with 4 units of human postinfection reference antiserum by methods previously described (6).

(2) The Gilchrist strain of rubella virus (RV-MK) was grown in vervet monkey kidney cells as previously described (7). This material had a titer of $10^{4.5}$ $TCID_{50}$ /ml.

(3) Purified RS antigens A and B (RSA-BK, RSB-BK) were obtained from the Long strain of RS virus propagated in bovine embryonic kidney cells by methods previously described (6). These materials had a CF titer of 1:8 when tested with a standard human reference antiserum containing 4 units of antibody for each of the antigens.

(4) Uninfected HEp-2 (N-H) and vervet monkey kidney cells (N-MK) grown and maintained as indicated above, were frozen and thawed three times. This crude material was used as a skin test antigen.

Immunization and skin test procedure. Guinea pigs, weighing between 500 and 800 g were used. The animals were immunized with 1 ml of the indicated material at 0, 14, 21, and 35 days. Control animals were immunized with Hanks' balanced salt solution. Three to 5 days after the last immunizing injection, the animal's back was shaved and 0.05 ml of the indicated skin test antigen was injected intradermally with a disposable turberculin syringe and 25-gauge needle. The animals were examined daily and the induration surrounding each injection was measured. The animals were observed for 5–7 days. The maximal induration occurred at 48–72 hr and was graded as follows: grade I: 0–3 mm induration, grade II: 4–7 mm induration, grade III: 8 mm or greater induration. All of the grade III skin reactions developed central necrosis and scabbing during the period of observation. Serum specimens were obtained prior to immunization and 3–5 days following the skin tests.

Results. All of the animals immunized with the RS-MK-A, RS-BK, and RS-BK-A inactivated RS virus vaccines demonstrated dermal hypersensitivity when skin tested with these vaccines (Table 1). The animals immunized with the concentrated RS-MK-A vaccine developed the most severe reactions and these were the only animals that developed any dermal hypersensitivity to the RS-H skin test antigen. The RS-MK-A vaccine was also a more potent skin test antigen for detecting the development of delayed hypersensitivity in animals immunized with the RS-

TABLE I. Delayed Dermal Reaction of Animals Immunized with Various RS Virus Vaccines.

Immunizing material	No. of animals immunized	Number of animals with indicated grade of delayed dermal reactions when tested with indicated skin test antigens[a]											
		RS-MK-A			RS-BK-A			RS-BK			RS-H		
		I	II	III	I	II	III	I	II	III	I	II	III
RS-MK-A	4	0	0	4	NT[b]	NT	NT	NT	NT	NT	2	2	0
RS-BK-A	6	0	6	0	0	1	5	2	3	1	6	0	0
RS-BK	6	0	6	0	0	6	0	3	3	0	6	0	0
RS-H	5	NT	NT	NT	0	5	0	3	2	0	5	0	0
None	5	5	0	0	5	0	0	5	0	0	5	0	0

[a] All animals had negative skin test reactions when tested with RV-MK, N-H, N-MK, RSA-BK, and RSB-BK.

[b] NT = Not tested.

BK and RS-BK-A vaccines. Animals immunized with live unconcentrated Long strain RS virus (RS-H) did not demonstrate delayed dermal hypersensitivity when tested with the homologous material but did show increased dermal reactivity when skin tested with RS-BK and RS-BK-A. None of the immunized animals showed any dermal hypersensitivity when tested with RV-MK, N-MK, N-H, RSA-BK, and RSB-BK antigens. None of the control animals had reactions greater than 2 mm of induration when tested with any of skin test materials.

These results indicated that dermal delayed hypersensitivity developed in guinea pigs following immunization with inactivated RS virus vaccines. These results suggest also that the antigens producing this reaction were components of the virus since animals immunized with RS virus grown in one tissue culture system showed delayed hypersensitivity when tested with RS virus antigens derived from a different tissue culture system. In addition, tests with control tissue culture antigens were negative. In an attempt to define more precisely the possible role of host cell antigens in producing the observed reactions, guinea pigs were immunized with bovine kidney parainfluenza type 3 virus vaccine (P3-BK), bovine kidney influenza vaccine (INF-BK) and live Long strain RS virus (RS-H) and were subsequently skin tested (Table II). Although these animals did not develop delayed dermal hypersensitivity to the homologous immunizing material, two of four animals immunized with P3-BK vaccine demonstrated delayed dermal hypersensitivity when skin tested with RS-BK.

It has been suggested that alum used as an adjuvant may potentiate the delayed hypersensitivity state (5). In addition, the combination of alum adjuvanted DPT vaccine with viral antigens has been used in the past in an attempt to reduce the number of immu-

TABLE II. Delayed Dermal Reaction of Animals Immunized with Bovine Kidney Grown Virus Vaccines.

Immunizing material	No. of animals immunized	Number of animals with indicated grade of delayed dermal reactions when tested with indicated skin test antigens											
		P3-BK			INF-BK			RS-BK			RS-H		
		I	II	III	I	II	III	I	II	III	I	II	III
P3-BK	4	4	0	0	4	0	0	2	2	0	4	0	0
INF-BK	3	3	0	0	3	0	0	3	0	0	3	0	0
RS-H	4	4	0	0	4	0	0	3	1	0	4	0	0
None	4	4	0	0	4	0	0	4	0	0	4	0	0

TABLE III. Delayed Dermal Reactions of Animals Immunized with Both Live RS Virus and DPT Vaccine.

Immunizing material	No. of animals immunized	Number of animals with indicated grade of delayed dermal reactions when tested with indicated skin test antigens														
		RS-H			RS-BK-A			RS-BK			DPT-A			N-H		
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
RS-H + DPT-A; not mixed; injected in separate sites	3	3	0	0	0	3	0	3	0	0	3	0	0	3	0	0
RS-H + DPT-A; mixed and injected in same site	3	3	0	0	0	0	3	0	3	0	3	0	0	3	0	0
None	3	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0

nizing injections needed for the pediatric age group (8). For these reasons, the effect of the combination of DPT-A and RS-H on the development of delayed hypersensitivity in the guinea pigs was determined (Table III). In animals immunized with 1 ml of DPT-A and 1 ml of RS-H administered in two separate sites, the development of delayed dermal hypersensitivity to RS-BK-A and RS-BK was similar to that seen in animals immunized with RS-H alone. When animals were immunized with equal amounts of a mixture of equal parts of DPT-A and RS-H, increased delayed dermal hypersensitivity was present when the animals were skin tested with RS-BK-A and RS-BK. Neither group of animals showed any delayed hypersensitivity to RS-H, N-H, or DPT-A.

Discussion. The present study demonstrated that guinea pigs immunized with inactivated RS virus vaccines derived from different tissue culture systems develop delayed dermal hypersensitivity when skin tested with both homologous and heterologous RS virus vaccines. The alum containing vaccines appeared to be more potent inducers of this reaction and were also the most sensitive skin test antigens for detecting hypersensitivity in the immunized animals. The animals immunized with the highly concentrated RS-MK-A had the most severe hypersensitivity reactions when skin tested with a variety of antigens. These animals were the only ones which demonstrated delayed hypersensitivity when tested with the unconcentrated tissue culture RS-H antigen. These results suggest that these potential RS virus vaccines contain a viral antigen (s) which is capable of inducing delayed dermal hypersensitivity. Although there is no definite evidence of delayed hypersensitivity occurring after natural RS virus infections, these findings are consistent with the observations that delayed dermal hypersensitivity develops in certain individuals naturally infected with other myxoviruses such as influenza, measles, and mumps (9).

Since there are no known serological cross-reactions between RS and parainfluenza type 3 virus, the results of the experiment in which the animals immunized with parainfluenza virus vaccine developed delayed dermal hypersensitivity to RS virus vaccine cannot readily be explained on the basis of shared viral antigens. These observations suggest that host tissue culture cell antigens may also be involved in producing delayed dermal hypersensitivity in the immunized animals. Myxoviruses are known to incorporate or to be closely associated with host cell antigens during their release from infected cells (10). Thus, it is possible that these antigens may be concentrated with the virus and perhaps altered during the preparation of the vaccine and then induce delayed dermal hypersensitivity in the immunized animals. This mechanism would explain the development of delayed hypersensitivity to RS bovine kidney material in the animals immunized with the parainfluenza virus–bovine kidney vaccine. All of the vaccines used in the study had been concentrated 100- to 200-fold in an attempt to increase their antigenicity. It is therefore, likely that there was a significant

increase of host cell material during concentration. The RS vaccines prepared in bovine kidney had significantly more protein present in the final vaccine than did comparable influenza and parainfluenza vaccines. This increased protein, representing host cell material, may have been responsible for observed dermal hypersensitivity.

Although the presence of common viral and host cell antigens in the various vaccine preparations will account for the hypersensitivity reactions observed, the exact nature of the sensitizing antigens cannot be determined on the basis of the data presented. It is possible that the vaccines contained other antigens which were responsible for sensitizing the animal. In addition, it is possible that the different tissue culture cells contained a common antigen that induced hypersensitivity in the immunized animals. However, a cross-reacting sensitizing antigen does not appear to explain the reactions observed, for the sensitized animals did not demonstrate delayed hypersensitivity when skin tested with various materials derived from noninfected tissue culture cells.

Although the nature or origin of the sensitizing antigen has not been determined, it is apparent that the administration of inactivated RS vaccines to guinea pigs did result in the development of delayed dermal hypersensitivity to both viral and host cell components of the vaccine. The development of delayed hypersensitivity following immunization with RS virus vaccines is not necessarily an adverse or undesirable reaction. If, however, similar delayed hypersensitivity did develop in infants immunized with an inactivated RS virus vaccine, it is possible that this altered immunologic response could be involved in producing the enhanced clinical respiratory disease seen in vaccinees following natural RS infection. It is of some interest that the only animals that displayed delayed dermal hypersensitivity to the unconcentrated live RS virus preparation (RS-H) were the guinea pigs immunized with the same vaccine (RS-MK-A) used in the clinical trials that demonstrated the adverse vaccine effect in vaccinees following natural RS virus infection.

Similar delayed dermal hypersensitivity to both viral and host cell vaccine components has been demonstrated in man in the recipients of another inactivated myxovirus vaccine, measles virus vaccine (5). In these investigations, it has been postulated that the adverse reactions observed in individuals immunized with inactivated measles virus vaccine following either exposure to natural measles or live measles virus vaccine may be a manifestation of delayed hypersensitivity to vaccine components. The present study may be a demonstration in guinea pigs immunized with RS vaccine of the same immunologic reaction observed in the human measle virus vaccinees.

The combination of DPT vaccine with viral antigens has been suggested as a method of reducing the number of immunizing injections required by young infants (8). In addition, the components of the DPT vaccine appear, in some instances, to increase the immunogenicity of the viral antigens (9). However, the results of this study indicate that DPT may enhance the development of delayed hypersensitivity to the components of RS vaccines and therefore the use of combined DPT–myxovirus vaccines in man should be approached with caution.

Although the nature of the sensitizing antigens in the vaccine material have not been determined, it should be noted that no delayed dermal hypersensitivity was detected when animals immunized with concentrated inactivated RS vaccines were skin tested with partially purified RS antigens A and B. Since one of these antigens, RS antigen A, has been previously demonstrated to induce neutralizing antibody in guinea pigs, it is possible that a purified inactivated RS virus vaccine can be developed which will stimulate the formation of neutralizing and protective antibody without inducing potentially hazardous delayed hypersensitivity to the vaccine components (6). We intend to extend these observations to animals immunized with the purified RS viral antigens in an attempt to prepare and define an efficacious and safe RS viral immunogen.

Summary. Guinea pigs immunized with various potential inactivated RS virus vac-

cines grown in different tissue cultures systems developed delayed dermal hypersensitivity when skin tested with vaccine materials. Animals immunized with alum containing vaccines developed more severe reactions than did animals immunized with aqueous vaccines. The addition of DPT vaccine to RS virus preparations also appeared to intensify the observed reactions. In addition, animals immunized with bovine kidney parainfluenza vaccine developed delayed dermal hypersensitivity when skin tested with bovine kidney RS vaccine. It appeared that both viral and host cell components of these vaccines were responsible for the observed reactions. Partially purified RS antigen A and B did not elicit delayed dermal hypersensitivity in animals immunized with these vaccines.

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Effect of Gamma Irradiation and Radioprotectors on Alkaline Phosphatase and ATPase* (33424)

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Doses of ionizing radiation produce biochemically important changes in tissues resulting in metabolic derangement which, in time, may lead to cellular damage or even death of the cells. A dose of ionizing radiation lethal to mammals involves an infinitesimal energy absorption and a corresponding low number of biochemical changes per cell. Hollaender (1) estimated that a radiation dose of 0.1 krad deposits within the nucleus (assumed to be 1 μ^3) 6×10^3 eV of energy. Since most radiochemical reactions involving organic materials require 10–20 eV for bond rupture, it is reasonable to estimate that chemical changes will occur in only some 600 molecules out of the hundreds of millions of molecules present in the nucleus. The extent to which radiation-induced chemical transformations in cells and tissues are associated with specific chemical groupings is largely unsolved. Comparatively few radiochemical changes can result in a severe biological damage suggesting that ionizing radiation may exert a considerable degree of specificity on metabolism and on the complex high polymer substances of living organisms. One possibility is that the "target" molecules are part of the catalytic system of the cells (2).

Dale (3, 4) made one of the earliest attempts to determine if the effects of X-rays

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