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STUDIES ON ANTI-VIRAL MUCOSAL IMMUNITY WITH THE LIPOIDAL AMINE ADJUVANT
AVRIDINE

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INTRODUCTION

Enteric priming is effective in initiating a specific secretory immunoglobulin A (s-IgA) response in non-intestinal as well as local sites. If the concept of the "Common Mucosal Immune System" (1,2) also applies to respiratory mucosal immunity, enteric immunization could be utilized to advantage in vaccination against airborne pathogens which impinge upon the conjunctiva, nasopharynx, respiratory and gastrointestinal tracts. Oral vaccines are also more likely to be accepted by the public than intranasal or aerosol vaccines because of concerns about possible allergic, inflammatory and sclerotic effects of antigenic aerosols in the lungs.

Recent studies showed that parenteral immunization with inactivated viral vaccines was ineffective in protecting rodents from aerosol challenge with virulent virus, although similarly vaccinated animals were completely protected from parenteral challenge with the same dose of virus (3). The immunomodulator, Avridine, enhances uptake and retention of viral antigens in Peyer's patches (4), resulting in enhanced specific s-IgA levels in intestinal secretions (5,6); therefore, we initiated the present studies to test the protective effects of Avridine on aerosol or subcutaneous exposure to the ZH-501 strain of Rift Valley Fever (RVF) virus (7-9).

MATERIALS AND METHODS

Animals. Adult, female, BALB/c Jax and C3H/HeJ mice were used for the radiolabel uptake, immunization and protection studies. All animals were

housed in Biogard laminar flow units and fed conventional mouse chow. After challenge, mice were housed in filter top cages located in ventilated P-3 Biohazard glove boxes.

Antigens. Formalin-inactivated RVF vaccine (NDBR-103) was made from the Entebbe strain of RVF virus as previously described (10). Avridine, previously called CP20,961, was provided by Dr. Ivan Otterness of Pfizer Central Research, Groton, CT. An emulsion (6 mg/ml) was prepared by dissolving 42 mg of Avridine in 0.3 ml of absolute ethanol and 0.03 ml of Tween 80. The mixture was sonicated for ten seconds after adding 6.67 ml of Intralipid (10% soybean oil lipid emulsion, Cutter Laboratories, Berkeley, CA) and 7 ml of hydrated RVF virus vaccine.

Immunization procedures. Mice were anesthetized with methoxyflurane for direct intraduodenal (ID) injection via laparotomy (4). RVF virus vaccine and combinations with Avridine were injected ID or subcutaneously (SC) in aliquots of 0.2 ml. Intranasal and SC boosters were given to the appropriate groups at 14 and 21 days following priming. Monoclonal antibodies (F4-10-10A IgG_{2a}, F4-25-1B IgG₁ and F1-25-6A IgG₁) were obtained from Dr. Clarence J. Peters at USAMRIID.

Challenge studies. Half of each of the above groups were challenged by SC inoculation or aerosol exposure (7,9) to 4700 plaque forming units (PFU) of virulent RVF virus per mouse ($10 \times LD_{50}$). The challenge strain (ZH-501) was originally isolated in Egypt by Dr. James Meegan (8). All procedures were conducted by immunized personnel in a high containment laboratory.

Histopathology. All mice were necropsied as they died. The remaining mice were euthanized and necropsied at the end of 14 days following challenge. The liver, brain, nasal turbinates and lungs were examined for characteristic lesions of hepatitis, encephalitis and mucosal damage.

Radiolabel uptake. Rift Valley Fever Virus was grown in the presence of 10 mCi/ml of ³H-uridine to a specific activity of 300,000 DPM per 10^{10} virions. ³H-Avridine was obtained from Pfizer Central Research (specific activity 11.79 mCi/mg). Labelled virus and/or Avridine were injected ID. At sequential time intervals, mice were euthanized and the intestines, Peyer's patches, mesenteric lymph nodes, liver, spleen and lungs were removed, weighed, dissolved and counted in a scintillation counter.

RESULTS

Inclusion of Avridine with ^3H -virus resulted in preferential accumulation of label in Peyer's patches at all time points studied. The specific activities for labelled virus in the intestines and Peyer's patches were virtually identical when Avridine was omitted. Avridine emulsions with RVF virus, which were processed for transmission electron microscopy, revealed soybean oil droplets contained in unilamellar or multilamellar liposomes. There were $75.2\% \pm 4.1$ SD of the RVF virions associated with the outer membranes of the liposomes. Passage of Avridine through the acid environment of the stomach resulted in fivefold less accumulation of ^3H -Avridine in the Peyer's patches (Table 1). We estimate that each mouse received an oral effective dose of 30-60 μg of Avridine out of 600 μg delivered ID.

Protective efficacy studies. The design parameters in these studies included ID RVF vaccine with or without Avridine, SC RVF vaccine with or without Avridine, Avridine ID or SC, and an untreated control group. Each of the primed groups were divided into SC and aerosol challenge groups. Three studies of this nature were run, one utilized an $\text{LD}_{75\%}$ challenge dose (400 PFU), and the other two utilized 4700 PFU (10-20 X $\text{LD}_{50\%}$).

In the first study, priming SC with vaccine and Avridine resulted in 100% survival following either SC or aerosol challenge. The survival was 62% for the aerosol-challenged group that had been primed ID with vaccine and Avridine. The untreated controls left 20% to 27% survivors after SC and aerosol challenge, respectively. No animals were primed with vaccine lacking Avridine in this study.

Table 1. Effect of stomach acid on localization of ^3H -Avridine

| Organ | Specific Activity (DPM/mg) | |
|-----------------|----------------------------|------|
| | Intraduodenal | Oral |
| Intestine | 10 | 32 |
| Peyer's patch | 407 | 77 |
| Mesenteric L.N. | 5 | 3 |
| Liver | 6 | 4 |
| Spleen | 3 | 3 |
| Lung | 11 | 7 |

There were 12 treatment groups in the second study which controlled for presence or absence of Avridine, subcutaneous versus intraduodenal vaccination and aerosol versus subcutaneous challenge (Fig. 1). The best survival was obtained for the SC-primed/SC-challenged groups, as expected. In the aerosol-challenged groups, survival was 40% for SC vaccine plus Avridine, 15% for SC vaccine alone, and 0% for the rest. Based on the survival curves, there did not appear to be any benefit to intraduodenal vaccination in producing protection against aerosol exposure. However, we found a slight beneficial effect in the ID-primed/SC-challenged group only when Avridine was present in the inoculum. Indeed, absence of Avridine in the ID vaccine appeared to hasten the demise of treated mice midway in the course of the SC infection.

Histopathology of the mice in the second study revealed interesting alterations of RVF viral pathogenesis which correlated with the immune effects of mucosal versus peripheral priming (Table 2).

As expected, SC vaccination with RVF virus prevented both hepatitis and encephalitis after SC challenge, but a high frequency of encephalitis was encountered following aerosol challenge. In addition, SC vaccination followed by aerosol challenge was the only condition where interstitial pneumonitis was found. Surprisingly, a high frequency of hepatitis was encountered with ID vaccination, despite a complete prevention of encephalitis following aerosol challenge. Following ID vaccination and SC challenge, we found both hepatitis and encephalitis, although encephalitis is usually not a component of systemic RVF in immunized mice, as can be seen in the untreated groups given SC challenge. Encephalitis was only

Table 2. Histopathology of mice challenge with RVF virus

| <u>Vaccine</u> | | <u>Hepatitis (%)</u> | | <u>Encephalitis (%)</u> | | <u>Pneumonitis(%)</u> | |
|----------------|-----------|----------------------|----------------|-------------------------|----------------|-----------------------|----------------|
| | | <u>SC</u> | <u>Aerosol</u> | <u>SC</u> | <u>Aerosol</u> | <u>SC</u> | <u>Aerosol</u> |
| <u>Rt</u> | <u>Ag</u> | | | | | | |
| SC | VA | 0 | 14 | 0 | 71 | 0 | 21 |
| SC | V | 0 | 0 | 0 | 44 | 0 | 0 |
| SC | A | 33 | 50 | 0 | 25 | 0 | 25 |
| ID | VA | 83 | 71 | 17 | 0 | 0 | 0 |
| ID | V | 50 | 38 | 25 | 0 | 0 | 0 |
| ID | A | ND | 100 | ND | 20 | ND | 0 |
| UNTX | | 100 | 100 | 0 | 86 | 0 | 0 |

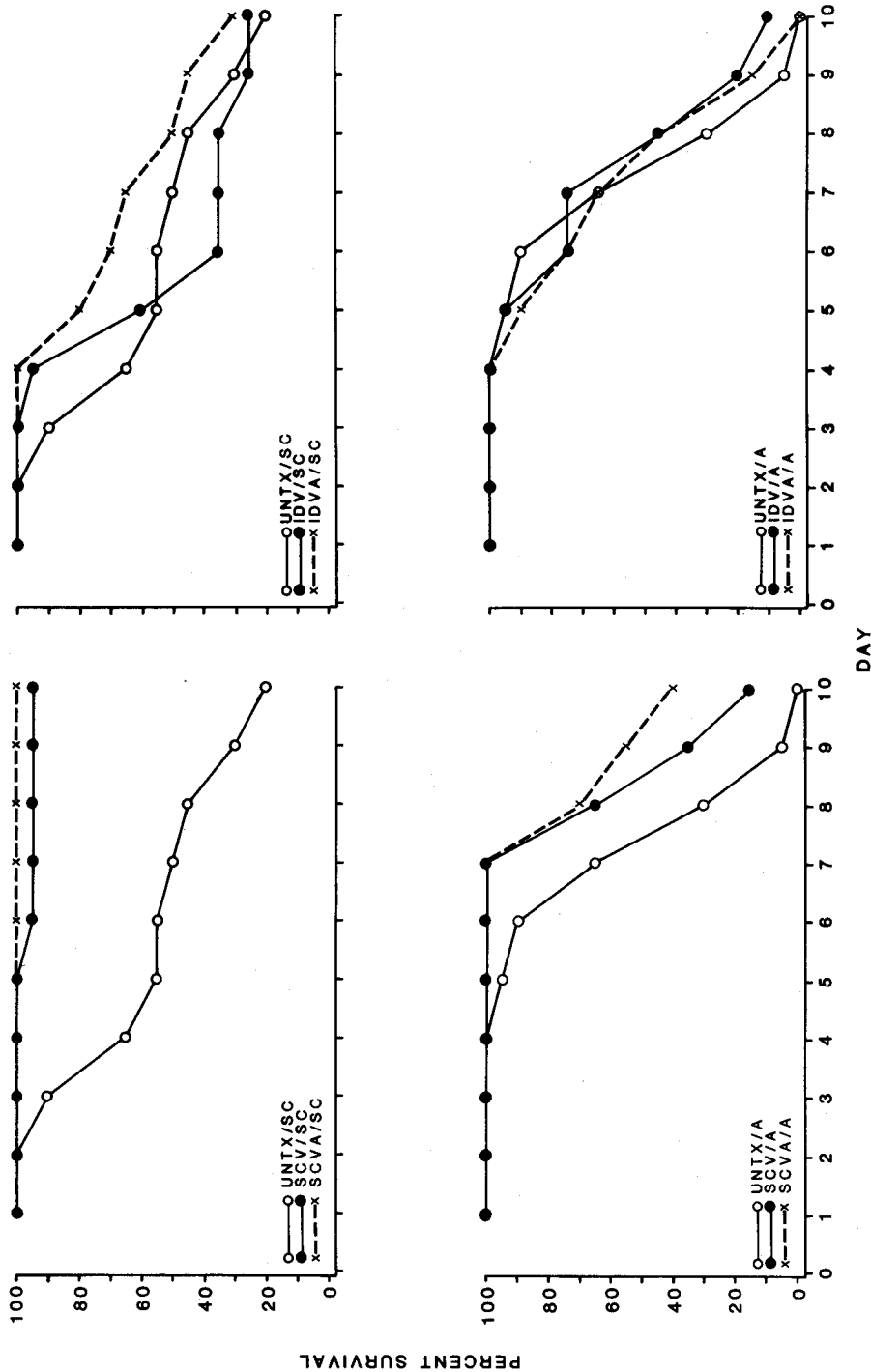


Fig. 1. These are survival curves for groups of 40 mice which were vaccinated either subcutaneously (SC) or intra-duodenally (ID) with RVF virus (V), with Avridine (A), or without. The letter after the / indicates the mode of challenge which was either aerosol /A or subcutaneous /SC. Interpretation is in the text.

found in the untreated group receiving aerosol challenge. Thus, the effect of immunization upon expression of characteristic pathological lesions is reflective of the relative role mucosal versus peripheral immunity has in preventing infection of the brain and liver. In addition, these data reveal some potential risks of mucosal immunization. The appearance of encephalitis in ID-vaccinated, SC-challenged mice is unusual and might have some relationship to conveyance of RVF virus into the brain by mucosally-committed, RVF-specific lymphoid cells.

In the third experiment, we controlled for down-regulation of peripheral immunity resulting from mucosal priming by passively protecting mice with monoclonal antibodies which had previously been shown to protect against parenteral challenge and neutralize virus in vitro (11). The results are depicted in Table 3.

The monoclonal anti-RVFPV antibodies are a pool of two IgG₁ and one IgG_{2a} isotypes that are specific for the G2 capsular polypeptide recognized by neutralizing antibodies. These data confirmed the protective efficacy of the antibodies against SC challenge with 4700 PFU (10-20 LD₅₀%) and demonstrated a beneficial effect against aerosol challenge. Because only a single pre-challenge dose was given, it is likely that the decrease in survival between day 6 and day 10 was due to exhaustion of antibody by viremia. We expected continuous 100% survival for the Intraduodenal vaccine plus Avridine with IP Monoclonal Antibody group, but the attrition rate from this group became more rapid than that of the monoclonal antibody treated group after day 7 post challenge. The necropsy results of this study may be useful in interpreting the result but they were not available in time for this preliminary report.

Table 3. Active mucosal and passive peripheral protection against RVF virus

| <u>Vaccination</u> | <u>IP MAB^a</u> | <u>Challenge</u> | <u>Survival d 6</u> | <u>Survival d 10</u> |
|--------------------|---------------------------|------------------|---------------------|----------------------|
| none | yes | SC | 100% | 98% |
| none | yes | Aero | 100% | 62% |
| ID VA ^b | yes | Aero | 100% | 40% |
| ID VA | no | Aero | 66% | 4% |
| none | no | Aero | 64% | 0% |

^aIP MAB - Intraperitoneal monoclonal antibodies.

^bIDVA - Intraduodenal vaccine plus adjuvant.

DISCUSSION

Cross regulation between the mucosal and peripheral immune systems is becoming better appreciated following the initial observations by Chase in the 1940's that mucosal priming with skin sensitizing agents diminishes peripheral cell-mediated responses in guinea pigs (12). More recently, studies by Pierce and Koster, who used cholera toxin as antigen, showed similar suppression of peripheral immunity by mucosal priming and suppression of mucosal immunity by parenteral priming (13,14). Furthermore, this cross regulation seems to be linked to phenomena that control the commitment of specific B-cells to IgA or specific IgG isotypes (15), where antigen presentation in mucosal-associated lymphatic tissues (16) and mucosally committed, immunoregulatory T-cells play obligatory roles in the generation of IgA B-precursors (17).

It is customary to test vaccine efficacy by using relatively simple procedures such as IP or SC vaccination followed by IP or SC challenge. When aerosol challenge was introduced as a routine test of vaccine efficacy (3; E.H. Stephenson, unpublished), surprising results were noted in animal models. Not only did parenteral vaccination with non-replicating antigens fail to protect mice, rats and hamsters from aerosol challenge, but SC-primed animals appeared less resistant to lethal infection than untreated controls. The cause of death was usually viral encephalitis. Fortunately, this phenomenon only occurred when the antigen was a non-replicating protein or peptide. Live, attenuated vaccines exhibited complete protection against both parenteral and aerosol challenge (3). These phenomena suggested that the mucosa of the conjunctiva and the nasopharynx had been "tolerized" by parenteral priming, which left no immunological barrier that would prevent encephalitic viruses from infecting mucosal cells and penetrating ophthalmic or olfactory cranial nerves lying beneath mucosal surfaces.

We conducted our studies in order to better understand these phenomena and to devise prophylactic regimens to resolve them. Rift Valley Fever virus rapidly produces high viremia followed by death of mice between 4 and 10 days following challenge. Early deaths (before d 6) are usually due to massive hepatic necrosis, while encephalitis is encountered late in the disease (after d 6). Encephalitis in rodents is more frequent following aerosol exposure, and death due to encephalitis occurs in parenterally vaccinated mice given aerosol exposure, regardless of their state

of total protection from parenteral challenge (9). The results confirm our impression that encephalitis following aerosol infection with RVF virus was due to loss of the ability to acquire mucosal protection because of immunoregulatory influences of parenteral immunization (14). This is especially illustrated by the histopathological observation that the olfactory bulbs were involved with encephalitis more frequently than the cerebrum in groups of mice that had been primed SC. Furthermore, neuronal cell bodies and axons in intracerebral, myelinated nerve tracts of these animals were stained with peroxidase-labelled monoclonal antibodies specific for RVF virus (King and Anderson, unpublished observations). Intraduodenal priming with RVF viral vaccine prevented the occurrence of olfactory bulb encephalitis following aerosol challenge. A potential risk factor is the observation that ID priming also appears to initiate cellular events that favor development of encephalitis in parenterally challenged mice by another mechanism which possibly involves transmission via migrating lymphoid cells.

In this study, the best mode of vaccination for protection against SC or aerosol challenge with RVF virus appears to be parenteral vaccination with RVF virus and Avridine. The contribution of Avridine to the vaccine appears to be more than a quantitative increase in the immune response. The quality of the response was changed as well. Some of the cross-regulatory effects of mucosal versus parenteral priming were abolished by introduction of this adjuvant (19) and the resulting response exhibited increased antibody diversity. Where commitment to IgA expression might otherwise be suppressed by parenteral immunization, use of Avridine permitted protective levels of specific s-IgA to be produced, in addition to other immunoglobulin isotypes.

CONCLUSIONS

The relative roles of s-IgA and IgG isotypes in protecting the respiratory tract from infectious diseases is a topic of interest in many laboratories. The consensus is that s-IgA is important for protection of upper respiratory tract structures and IgG isotypes become more important deeper into the lungs (18). Therefore, it would be undesirable to develop a vaccine that inhibited the production of either isotype, except for agents that exhibit very limited tropisms, like for example, organisms that have never been shown to penetrate beyond the mucosa. In the present study, we uncovered some of the complications that could be expected when using a

strict mucosal or parenteral approach to an organism that exhibits divergent tropisms, that is, vaccination resulting in altered pathogenesis rather than protection.

Despite the negative conclusion of this study, Avridine did enhance the uptake and accumulation of RVF virus in Peyer's patches, which resulted in protection of the mucosa. Use of Avridine in parenteral vaccine gave acceptable, general, protective efficacy to a large challenge. Use of alternative methods (IgG MAB) to support the suppressed Ig isotype improved the realization of the beneficial effect of mucosal protection on survival.

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REFERENCES

1. Craig, S.W. and Cebra, J.J., *J. Exp. Med.* 134, 188, 1971.
2. McDermott, M.R. and Bienenstock, J., *J. Immunol.* 122, 1892, 1979.
3. Jahrling, P.B. and Stephenson, E.H., *J. Clin. Microbiol.* 19, 429, 1984.
4. Anderson, A.O., MacDonald, T.T. and Rubin, D.H., *Int. J. Immunother.* 1, 107, 1985.
5. Rubin, D.H., Anderson, A.O. and Lucis, D., *Ann. N.Y. Acad. Sci.* 409 866, 1983.
6. Pierce, N. and Sacci, J.B.J., *Infect. Immun.* 44, 469, 1984.
7. Miller, W.S., et. al., *Amer. J. Hyg.* 77, 114, 1963.
8. Meegan, J.M., *Trans. Roy. Soc. Trop. Med. Hyg.* 73, 618, 1979.
9. Brown, J.L., Dominik, J.W. and Morrissey, R.L., *Infect. Immun.* 33 848, 1981.
10. Randall, R., Binn, L.N. and Harrison, V.R., *J. Immunol.* 93, 923, 1964.
11. Tappert, H.I., Meegan, J.M., Dalrymple, J.M. and Peters, C.J., (submitted).
12. Chase, M.W., *Proc. Soc. Exp. Biol. Med.* 61, 257, 1946.
13. Pierce, N.F., *J. Exp. Med.* 148, 195, 1978.
14. Koster, F.T. and Pierce, N.F., *J. Immunol.* 131, 115, 1983.
15. Fuhrman, J.A. and Cebra, J.J., *J. Exp. Med.* 153, 534, 1984.

16. Dunkley, M.L. and Husband, A.J., *Immunol.* 57, 379, 1986.
17. Gautom, S.C. and Battisto, J.R., *J. Immunol.* 135, 2975, 1985.
18. Bienenstock, J. (ed.), *Immunology of the Lung and Upper Respiratory Tracts*, McGraw-Hill Book Co., 1984.
19. Mowat, A.McI. and Parrott, D.M.V., *Immunol.* 50, 547, 1983.