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MUCOSAL PRIMING ALTERS PATHOGENESIS OF RIFT VALLEY FEVER

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INTRODUCTION

The concept of a common Mucosal Immune System implies that enteric priming would be effective in initiating secretory immunity in local and non-intestinal mucosal sites. If this[1,2] tenet also applies to protective immunity, enteric immunization could be utilized to advantage in vaccination against viruses that may impinge upon the conjunctiva, nasopharynx, respiratory, and gastrointestinal tracts. Recent studies showed that parenteral immunization with non-replicating viral antigens 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]. Live attenuated viral vaccines elicited protective immunity for either route of challenge regardless of the route of immunization. The present studies were conducted in order to ascertain the optimum route of immunization for respiratory immunity and the effects of mucosal VS peripheral immunization on Rift Valley fever Virus (RVFV) pathogenesis in mice.

MATERIALS AND METHODS

Adult female C3H/HeJ mice were immunized with 0.2 ml formalin-inactivated RVFV vaccine (NDBR-103)[10] by direct intraduodenal (ID) injection via laparotomy on anesthetized mice[4], intraperitoneally (IP) or subcutaneously (SC). Intranasal and SC boosters were given to the appropriate groups at 14 and 21 days following priming. On day 28 the 3 vaccinated groups and a control group were halved and distributed either into SC or aerosol challenge groups. They were challenged with a 10 x LD_{50} dose[7,9] of virus strain ZH-501[8] (SC dose of 600 plaque forming units (PFU)

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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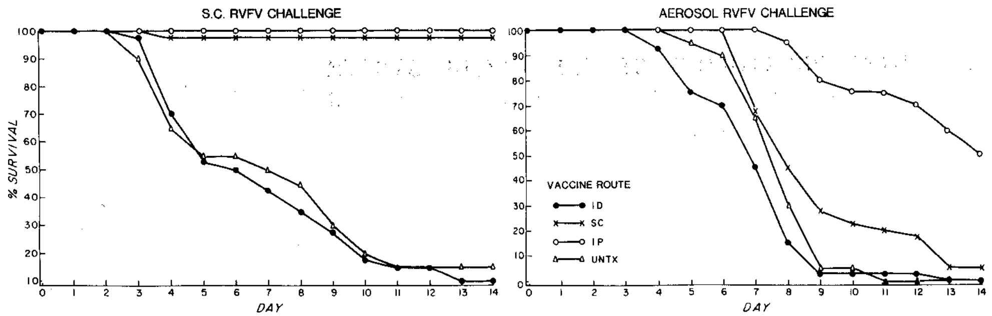

Fig. 1. Above are cumulative survival curves of untreated controls (UNTX) or mice vaccinated with RVFV by intraduodenal (ID), subcutaneous (SC) or intraperitoneal (IP) routes and then challenged with 10 x LD_{50} doses of RVFV (ZH-501) as indicated above.

Table 1. HISTOPATHOLOGY OF RVFV NON-SURVIVORS

Vaccine Route	SC Challenge			Aerosol Challenge		
	%HEP	%OBE	%ENC	%HEP	%OBE	%ENC
S.C.	3	0	0	68	61	29
I.D.	100	0	15	100	18	5
IP	0	0	0	0	67	100
Untx	81	19	19	74	58	26

Data represents percent of mice who died with the following lesions: HEP = hepatitis, OBE = olfactory bulb encephalitis (perineural), ENC = multifocal encephalitis (angiocentric). S.C. = subcutaneous, ID = intraduodenal, IP = intraperitoneal, UNTX = untreated.

and an aerosol dose of 4700 PFU of virulent RVFV per mouse). All procedures were conducted by immunized personnel in a biohazard containment laboratory. After challenge, mice were housed in filter top cages located in ventilated P-3 Biohazard glove boxes.

All mice were necropsied as they died. Fourteen days following challenge the surviving mice were euthanized and necropsied. Serum and Bile samples were taken for plaque reduction/neutralization titers against RVFV (ZH-501 strain) using standard techniques[8]. The liver, brain, nasal turbinates, and lungs were prepared for histopathology and examined for characteristic lesions of hepatitis, encephalitis, and mucosal damage. Selected specimens were examined by ABC-peroxidase immuno-histochemistry with biotinylated RVFV-specific monoclonal antibodies[11].

RESULTS

The survival for the SC and IP vaccinated groups challenged SC (Fig. 1) was 97.5% and 100% respectively. The ID vaccinated mice exhibited mortality to SC challenge identical to that of untreated controls. In the aerosol-challenged groups, survival at 10 days was 75% for i.p. vaccinated,

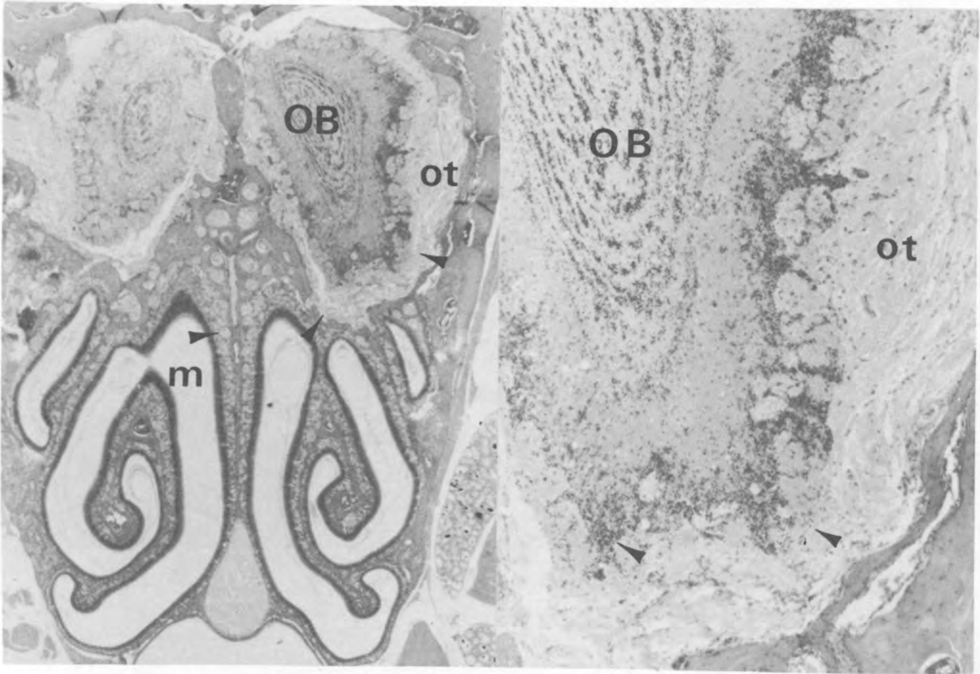

Fig. 2. Aerosol exposure to RVFV often results in olfactory bulb encephalitis. On the left is frontal section through a mouse head showing the anatomical relationships between nasal mucosa (m) olfactory tracts (arrows) and olfactory bulb (OB). Note that necrosis and leukocyte infiltration is greatest in the lateral olfactory tracts (ot).

Table 2. CROSS REGULATION IN ANTIBODY TO RVFV

Vaccine Route	Serum Titer			Bile Titer: PRN80 at 1/200	
	PRN80(x10-1)	IgA	IgM	% Pos.	# Pos./Total
S.C.	306	67	147	36	4/11
I.D.	40	190	140	60	6/10
UNTX	0	0	0	0	0/5
I.P.	N	N	N	N	N

N = Not done

22% for SC vaccinated, 1% for ID vaccinated and 5% for the untreated controls. Based on the survival curves, there did not appear to be any benefit to intraduodenal vaccination in producing protection against either SC or aerosol exposure.

Histopathology of the mice revealed interesting alterations of RVF viral pathogenesis which correlated with the immune effects of mucosal versus peripheral priming (Table 1).

Subcutaneous vaccination with RVFV vaccine prevented both hepatitis and encephalitis after SC challenge but not after aerosol challenge. In addition to increased incidence of hepatitis in the aerosol challenge group there was also an appreciable occurrence of olfactory bulb encephalitis (Fig. 2) that was more than double that of multifocal encephalitis which was primarily angiocentric in pattern. In contrast, ID vaccination

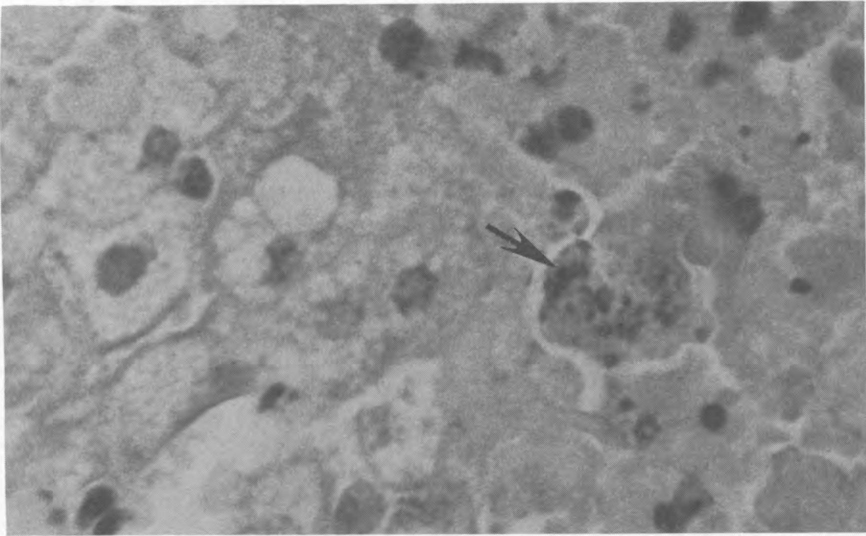

Fig. 3. Viral antigens localized in necrotic hepatocytes (arrow) during fulminant RVFV hepatitis.

increased the proportion of mice dying with hepatitis in both challenge groups compared to the untreated controls. Fulminant hepatitis occurred on days 4-12 in the ID vaccinated group compared to days 2-6 in the untreated controls. The frequency of olfactory bulb and multifocal encephalitis was significantly reduced in both challenge groups compared to the untreated controls and the SC challenged mice that had been vaccinated SC. There were no mortalities in the IP vaccinated SC challenged group, but of the 25% mortalities that occurred in the aerosol challenged group none had fulminant hepatitis. All those that died had extensive multifocal encephalitis which also involved the olfactory bulbs. Although it was difficult to distinguish between the angiocentric and perineural patterns of encephalitis in these mice, there appeared to be fewer of the latter. Immunohistochemistry with monoclonal antibodies specific for RVFV confirmed that the hepatitis and encephalitis were both associated with significant deposition of viral antigen. In the liver of RVFV infected mice, viral antigen is frequently found extracellularly because of the extensive liver cell necrosis (Fig. 3).

There were two patterns of viral antigen deposition in the brain lesions. Aerosol exposure with RVFV resulted in olfactory bulb encephalitis with neuronal cell necrosis occurring in nuclei supplied by myelinated nerve tracts, principally the lateral olfactory tracts. RVFV antigen was found in neuronal cells and in the axons of myelinated tracts of these sites (Fig. 4a). The multifocal encephalitis which occurred after either SC or aerosol exposure to RVFV was centered around blood vessels whose endothelium was frequently positive for RVFV antigen (Fig. 4b). The geometric mean 80% plaque neutralizing titers in serum samples were 1/40 (n=10) for the ID vaccinated group versus 1/306 (n=11) for the SC vaccinated group. Six of ten (60%) and four of eleven (36%) bile samples (approximate dilution 1/200) were positive for neutralizing antibody in the ID and SC vaccinated groups, respectively (Table 2).

DISCUSSION

The initial observations by Chase in the 1940's that mucosal priming with skin sensitizing agents diminishes peripheral cell-mediated responses

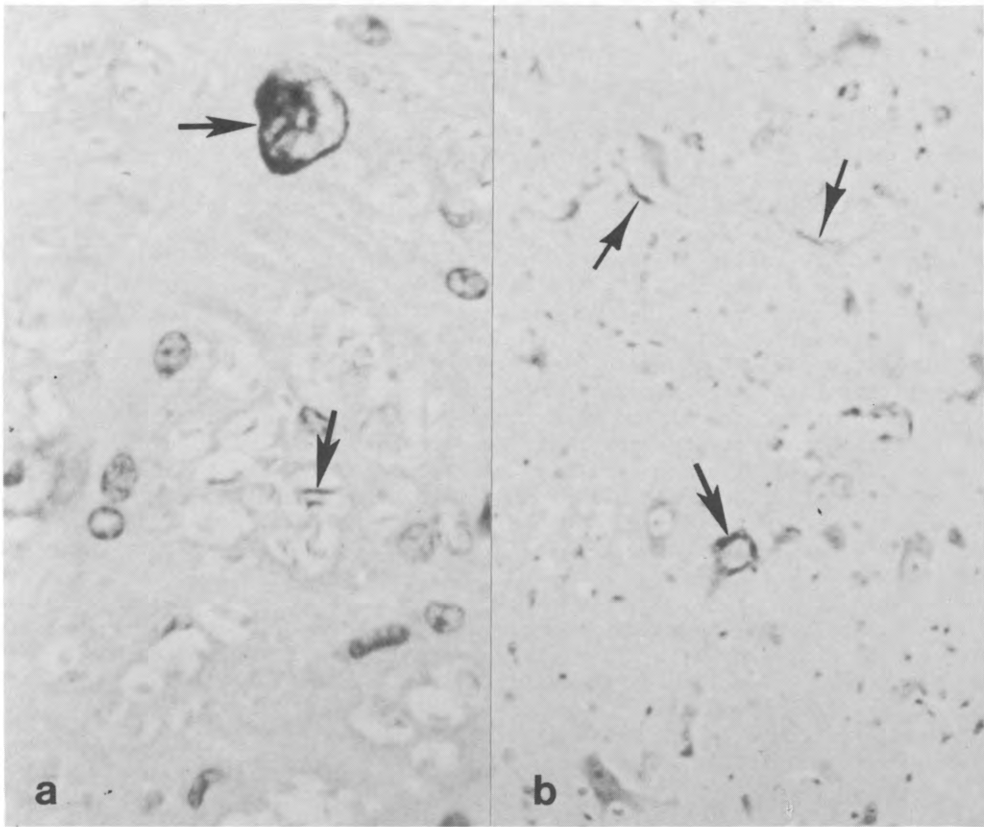

Fig. (4 a & b) a. RVFV antigen in neurons and axons of intracerebral myelinated nerve tracts (arrows) after aerosol exposure. b. Late encephalitis in RVFV infected mice often shows an angiocentric pattern. Visual antigen is located in neurons (arrows) and some endothelial cells (arrows).

in guinea pigs[12] and recent studies by Pierce and Koster where mucosal priming resulted in suppression of peripheral immunity and vice versa[13,14] has led to a better appreciation of cross regulation between the mucosal and peripheral immune systems. Furthermore, this cross regulation seems to be linked to phenomena that control the committment of specific B-Cells to IgA or IgG isotypes[15], where antigen presentation in mucosal-associated lymphatic tissues[16] and mucosally committed, immunoregulatory T-cells play obligatory roles in the selection of IgA B-cell precursors[17].

It is customary to test vaccine efficacy by 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 viral 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 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" against induction of secretory

immunity by parenteral priming, which left no immunological barrier that would prevent encephalitis 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 in mice followed by death between 4 and 10 days following challenge. Early deaths (before day 6) are usually due to massive hepatic necrosis, while encephalitis is encountered late in the disease. Encephalitis in rodents occurs frequently after 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 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. Intraduodenal priming with RVF viral vaccine prevented the occurrence of olfactory bulb encephalitis after aerosol challenge, presumably due to increased commitment to specific IgA and neutralizing antibodies in secretions.

In our system, the best mode of vaccination for protection against SC or aerosol challenge with RVF virus appears to be intraperitoneal vaccination. This route of immunization resulted in effective priming for secretory antibody in bile as well as neutralizing antibody in serum whereas commitment to IgA expression might otherwise be suppressed by parenteral immunization.

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, for instance, organisms that have never been shown to penetrate beyond the mucosa. In the present study, we have uncovered complications, e.g. altered pathogenesis rather than protection, when using a strict mucosal or parenteral approach to protection against an organism that exhibits divergent tropisms. The observation that i.p. vaccination primes both mucosal and peripheral systems may be of value in respiratory vaccine development.

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REFERENCES

1. S. W. Craig, and J. J. Cebra, J. Exp. Med. 134:188, (1971).
2. M. R. McDermott, and J. Bienenstock, J. Immunol. 122:1892, (1979).
3. P. B. Jahrling, and E. H. Stephenson, J. Clin. Microbiol. 19:429, (1984).
4. A. O. Anderson, T. T. MacDonald, and D. H. Rubin, Int. J. Immunother. 1:107, (1985).
5. D. H. Rubin, A. O. Anderson, and D. Lucis, Ann. N.Y. Acad. Sci. 409:866, (1983).
6. N. Pierce, and J. B. J. Sacci, Infect. Immun. 44:469, (1984).

7. W. S. Miller, P. Demchak, C. R. Rosenberger, J. W. Dominik, and J. L. Bradshaw, Amer. J. Hygiene. 77:114, (1963).
8. J.M. Meegan, Trans. Roy. Soc. Trop. Med. and Hygiene, 73:618, (1979).
9. J. L. Brown, J. W. Dominik, and R. L. Morrissey, Infect. Immun. 33:848, (1981).
10. R. Randall, L. N. Binn, and V. R. Harrison, J. Immunol. 93:923, (1964).
11. H. I. Tappert, J. M. Meegan, J. M. Dalrymple, and C. J. Peters, (Submitted).
12. M. W. Chase, Proc. Soc. Exp. Biol. Med. 61:257, (1946).
13. N. F. Pierce, J. Exp. Med. 148:195, (1978).
14. F. T. Koster, and N. F. Pierce, J. Immunol. 131:115, (1983).
15. J. A. Fuhrman, and J. J. Cebra, J. Exp. Med. 153:534, (1984).
16. M. L. Dunkley, and A. J. Husband, Immunol. 57:379, (1986).
17. S. C. Gautom, and J. R. Battisto, J. Immunol. 135:2975, (1985).
18. J. Bienenstock, (ed.), IMMUNOLOGY OF THE LUNG AND UPPER RESPIRATORY TRACTS, McGraw-Hill Book Co., (1984).