Lymphatic Tissues and In Vivo Immune Responses

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Tissue-Specific Expression of ε and α Messenger Ribonucleic Acid in Allergy-Prone C3H Mice

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INTRODUCTION

Intraperitoneal inoculation of Rift Valley fever virus (RVFV) in C3H substrains of mice resulted in acute respiratory distress of all C3H/HeJ mice upon repeat exposure on day 14. In contrast, no C3H/HeN mice were affected by reexposure to RVFV. This phenomenon was never observed until C3H/HeJ mice were substituted for other strains because of increased immunoglobulin A (IgA) responses (1,2). We examined immunoglobulin heavy chain gene expression by in situ hybridization in sections and by Northern blot analysis of extracted tissue ribonucleic acid (RNA) from control and RVFV-immunized mice.

MATERIALS AND METHODS

Animals

Adult female C3H/HeJ, C3H/HeN (OuJ), mice were vaccinated intraperitoneally (i.p.), subcutaneously (s.c.), and by intranasal (i.n.) instillation. Mice were killed via CO_2 exposure. Blood IgA and IgE levels were determined by ELISA.

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 Animals Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author(s) do not purport to reflect the positions of the U.S. Department of Defense.

Vaccine

Rift Valley fever virus vaccine (IND 365) was prepared from Entebbe strain virus grown on fetal rhesus lung cells and formalin inactivated (2).

RNA Isolation—Mouse Tissues

Peripheral, mediastinal, and mesenteric lymph nodes (LNs), spleens, intestines, and lungs were removed and immediately frozen in liquid nitrogen (organs from each group were pooled). Ribonucleic acid was isolated from frozen mouse tissue by the method of Chirgwin et al. (4). Concentrations of RNA were adjusted to 10 μg per lane. Samples were heated to 65°C for 2 min and loaded onto 1.5% agarose gels containing 0.66 M formaldehyde, 20 mM MOPS, 5 mM EDTA, 6.6 μg ethidium bromide. Gels were electrophoresed at 18–20 V for 18 hr.

Cyclic Deoxyribonucleic Acid (DNA) Probes

The chimeric plasmid $C^{3}30$ containing 1300 bp cDNA for the mouse IgE heavy chain was the kind gift of Dr. Fu-Tong Liu (5). The α660 plasmid (6) was the generous gift of Dr. John Cebra and Peter Weinstein. This construct is a Gemini plasmid (Promega, Madison, Wisconsin) containing α660 bp cDNA for mouse IgA.

Northern Blots

Ribonucleic acid gels were blotted overnight onto nitrocellulose. Filters were baked under vacuum at 80°C for 2 hr. Prehybridization was carried out for 20 min at 47°C in 40% formamide, 4X SSC, 1X Denhardt's solution, 7 mM Tris pH 7.4, and 25 μg salmon sperm DNA. Hybridization was carried out in the same buffer at 47°C for 20 hr. Radiolabeled probe was added to a final concentration of 1×10^{8} dpm. Posthybridization washes were done for 30 min each in 2.0x, 1.0x, 0.1x SSC; 0.1% SDS; and for 5 min at 68°C.

In Situ Hybridization

Lung tissue sections from each strain/treatment group were exposed to the above probes according to the method of Singer et al. (7) using the Genius kit.

RESULTS AND DISCUSSION

C3H/HeJ mice that died within 30 min of i.p. or i.n. secondary inoculation 14 days after i.p. immunization with RVFV exhibited antimortem and postmortem signs of acute respiratory distress. C3H/HeN mice were completely resistant to this phenomenon. Mouse models of hypersensitivity are exceedingly complex (8), with networks of immunoregulatory cells, cytokines (interleukin-4 [IL-4] vs interferon-γ [IFN-γ]) differences in mediator responsiveness (9), IgE Fc receptor expression, and IgE binding factors. We examined messenger RNA (mRNA) expression for α and ε heavy chains in total RNA extracted from lymph nodes, spleen, lung, and intestines of untreated and i.p. vaccinated mice (Table 1).

Low levels of mRNA were found in all tissues of control C3H/HeJ and higher levels in the C3H/HeN mice. However, we found increased α and ε mRNA levels in lungs and spleen of C3H/HeJ, whereas there was decreased or unchanged α and ε mRNA levels in all organs except the spleens of C3H/HeN mice 14 days after i.p. immunization with RVFV. In situ hybridization of lungs from C3H/HeJ and C3H/HeN mice with the same cDNA

Table 1 Effect of I .P. Vaccination with RVFV on α and ε mRNA Levels

	Intestine		Lung		Med LN		Mes LN		Per LN		Spleen	
Mouse strain	α	ε	α	ε	α	ε	α	ε	α	ε	α	ε
CeH/HeJ	D	D	I	I	=	I	=	I	D	D	I	I
C3H/HeN	D	D	=	D	D	D	D	D	=	D	I	I

Comparison between control and immunized mice by using densitometry of Northern blots. Abbreviations: D, decreased; I, increased; =, is unchanged; Med, mesiastinal, Mes, mesenteric; Per, peripheral; LN, lymph node.

probes revealed peribronchial distribution of cells bearing α and ε mRNA, whereas ε mRNA was distributed throughout the lung interstitium only in C3H/HeJ mice (Table 2).

There were no significant differences among total serum IgE levels (ng/ml) of control or immunized C3H/HeJ (9.8 ± 1.4) and C3H/HeN (5.1 ± 1.2) mice. Both strains showed at least double the control IgE levels after immunization.

The concentration of total IgA in serum increased from 73 μg/ml in control C3H/HeJ mice to 142 μg/ml in the immunized mice. The concentration of IgA in bronchial lavages also increased dramatically in C3H/HeJ mice after immunization, from 71 ng/ml to 90 ng/ml. There was no significant change in the concentration of IgA in bronchial lavage from C3H/HeN mice.

We have shown that substrains of the C3H mouse have potential as a model of vaccine-induced acute hypersensitivity. Although this strain is intermediary between SJL and Balb/c mice with regard to IgE antibody levels (7), there are organ-specific differences in IgA and IgE commitment after i.p. immunization which correlate with differences in expression of mucosal immunity and/or allergy in the mouse strains tested. Furthermore, in situ hybridization suggests that interstitial B cells are responsive for the increased IgE expression in the lungs of C3H/HeJ mice which had been vaccinated i.p. We have recently observed significant transdiaphragmatic traffic of peritoneal LY-1 B cells into the lungs interstitium (Pitt and Kroese, unpublished data), and suggest that they may be the target population for i.p. stimulation.

Table 2 In Situ Hybridization of α and ε mRNA[a] in Lungs from C3H/HeN and C3H/HeJ Mice

	C3H/HeN				C3H/HeJ			
	Untreated		S/P RVFV[b]		Untreated		S/P RVFV[b]	
Tissue pattern	α	ε	α	ε	α	ε	α	ε
Peribronchial	+	–	++	+	++	+	+++	+
Interstitial	–	–	+	–	–	+	+	++

[a]Mouse IgA heavy chain αmRNA was probed with α660 cDNA, and Weinstein. IgE heavy chain εmRNA was probed with $C^2$30 cDNA.
[b]14 days after i.p. RVFV.

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