Advances in Mucosal Immunology

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C3H mouse model of genetic predisposition to vaccine induced allergy

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ABSTRACT. Allergic reactions in recipients of Rift Valley fever vaccine (RVFV) are extremely rare. When vaccine was given by various routes to selected mouse strains, intraperitoneal (IP) priming of C3H/HeJ mice with RVFV resulted in acute respiratory arrest of all mice upon secondary IP inoculation on day 14. All C3H/HeJ mice given intranasal (IN) boost had distress but only 67% died. In contrast, no C3H/HeN mice died following re-exposure to RVFV vaccine. C3H/HeJ mice have high splenic IgA responses as shown by Kiyono et al. [1]. We examined commitment of local B-cells to IgA and IgE expression by Northern blot analysis of extracted RNA from tissues of control and RVFV immunized mice. Using the α660 cDNA probe we found increased α mRNA in lungs and spleens of C3H/HeJ, while there was decreased α mRNA in the same organs of C3H/HeN mice 14 days after IP immunization. Northern blots were also probed for ε mRNA with the cDNA chimera, $C^{2}30$. Low levels of ε mRNA were found in all tissues of control C3H/HeN mice. Immunization resulted in differences in ε mRNA levels consistent with association of locally-produced IgE with respiratory allergy in C3H/HeJ mice.

INTRODUCTION.

The formalin-inactivated Rift Valley fever vaccine has been tested in over 2173 human subjects with only one individual exhibiting an allergic reaction. Such low frequency of allergy is consistent with host genetic predisposition rather than intrinsic reactogenicity of the vaccine. This vaccine has been evaluated in experimental animals for efficacy against exposure to virulent virus (ZH-501, [2]). Routes of vaccination, doses of vaccine or challenge virus, and routes of challenge were studied in mouse models of Rift Valley fever infection [3]. Earlier studies were primarily conducted in C3H/HeN, Balb/c and A/J mice and vaccination by either subcutaneous (SC) or intraduodenal (ID) routes followed by SC or IN boost did not induce acute hypersensitivity reactions. Because of mucosal and parenchymal tropisms of the virus, isolated mucosal or peripheral priming were not sufficient to yield complete protection from either SC or aerosol challenge [3]. C3H/HeJ mice were added to these protocols because of

the observation of Kiyono et al. [1] that C3H/HeJ mice had enhanced IgA responses in Jerne plaque assays of spleen cells. We attempted to boost IP primed C3H/HeJ mice by IP or IN inoculation and encountered dramatic acute hypersensitivity reactions that had not been seen with C3H/HeN and other mouse strains. The present study is concerned with the analysis of the model and genetic elements in vaccine induced allergy. The results show that formalin inactivation of viral antigen, IP priming and differences in organ-specific expression of ε mRNA underlie the allergic predisposition of C3H/HeJ mice.

MATERIALS AND METHODS.

Animals. Adult female C3H/HeJ, C3H/HeN (OuJ), Balb/c ByJ, BDF_1, A/J and Swiss Webster mice were obtained from Charles River and the Jackson Laboratory. Vaccine inoculations were given IP and SC via 30 gauge needle and tuberculin syringe. IN inoculations were given via micropipet dispenser. Euthanasia was via CO_2 exposure. Blood IgA and IgE levels were determined by ELISA.

Vaccine. Rift Valley fever virus vaccine (IND 365) was prepared from Entebbe strain virus grown on fetal rhesus lung cells and formalin inactivated (0.5%). The formalin was inactivated with sodium bisulfite. The vaccine also contains Eagles basal medium, 0.5% (W/V) human serum albumin, Neomycin sulfate equivalent 50 µg/ml and Streptomycin 50 µg/ml.

RNA isolation - mouse tissues. Peripheral, mediastinal and mesenteric lymph nodes (LN), spleens, intestines and lungs were removed and immediately frozen in liquid nitrogen (organs from each group were pooled). RNA was isolated from frozen mouse tissue by the method of Chirgwin et al. [4]. Purified RNA was stored in sterile water at -70°C. RNA concentrations 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 elctrophoresed at 18-20V for 18 hr.

cDNA probes. The chimeric plasmid C^230 containing 1300 bp cDNA for the mouse IgE heavy chain. This plasmid was the kind gift of Dr. Fu-Tong Liu [5]. The α 660 plasmid [6] was the kind gift of Dr. John Cebra and Peter Weinstein. This construct is a Gemini plasmid (Promega, Madison, WI) containing α 660 bp cDNA for mouse IgA.

Northern Blots. RNA 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, 7mM Tris pH 7.4 and 25 µg Salmon sperm DNA. Hybridization was carried out in the same buffer at 47°C for 20 hours. Radiolabeled probe was added to a final concentration of $1x10^8$ dpm. Post hybridization washes were done for 30 min each in 2x,1x,.1x SSC, 0,1%SDS and for 5 min at 68°C.

RESULTS AND DISCUSSION.

CeH/HeJ mice that died within 30 min of IP or IN secondary

inoculation 14 days after intraperitoneal immunization with RVF vaccine exhibited signs of acute respiratory distress. The lungs were hyperinflated, remaining inflated when the thoracic cavity was opened. There was congestion of the abdominal viscera and hyperperistalsis. Formalin-inactivated RVFV virions in the vaccine triggered this phenomenon. C3H/HeJ that were primed IP with whole vaccine did not show hypersensitivity or acute death after IP challenge with human serum albumin (HSA), Eagles basal medium with Neomycin sulfate and Streptomycin. Exposure to live RVF virus or β-propriolactone inactivated virions did not induce acute hypersensitivity. C3H/HeN, Balb/cByJ, BDF_1 and A/J mice were completely resistant to this phenomenon, while 20% of Swiss Webster mice died. C3H/HeN and C3H/HeJ mice were studied for acute hypersensitivity after SC, ID, or IP primary inoculation followed 14 days later by SC, IN or IP secondary inoculation with RVF vaccine. Of 100 C3H/HeN and 106 C3H/HeJ mice studied the only deaths were among C3H/HeJ mice that were IP primed and IP (100%) or IN (67%) boosted.

The above data indicated that IP priming and formalin modified RVFV antigen(s) initiated the allergic phenomenon but there was a very strong genetic component. Although mouse models of hypersensitivity are exceedingly complex [7,8], with networks of immunoregulatory cells, cytokines (IL-4 vs γ-IF), differences in mediator responsiveness [9], levels of IgE Fc receptor expression, and IgE binding factors. We examined messenger RNA expression for ε and α heavy chain in total RNA extracted from lymph nodes, spleen, lung and intestines of untreated and IP vaccinated mice (Table 1).

TABLE 1. Effect of IP vaccination with RVFV on ε and α mRNA levels

Mouse strain	Intestine		Lung		Med. LN		Mes. LN		Per. LN		Spleen	
	E	A	E	A	E	A	E	A	E	A	E	A
C3H/HeJ	D	D	I	I	I	=	I	=	D	D	I	I
C3H/HeN	D	D	D	=	D	D	D	D	D	=	I	I
Balb/cByJ	D	D	=	D	D	I	I	I	D	I	D	D
BDF_1	I	I	D	=	D	=	I	D	D	D	=	D
Swiss Webster	D	I	I	D	I	D	I	D	D	=	I	D

Comparison between control and immunized mice using densitometry of Northern blots. E = ε mRNA, A = α mRNA, D = decreased, I = increased, Med. = mediastinal, Mes. = mesenteric, Per. = peripheral, and LN = lymph node.

Low levels of ε mRNA were found in all tissues of control C3H/HeJ, BDF_1 and Swiss Webster mice and higher levels in the C3H/HeN and Balb/c mice. However, we found increased ε and α mRNA levels in lungs and spleen of C3H/HeJ, while there was decreased or unchanged ε and α mRNA levels all organs except the spleens of C3H/HeN mice 14 days after immunization with RVFV. The ε mRNA changes for BDF_1 resembled that of C3H/HeJ.

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. The highest control levels of IgE were found in Balb/cByJ (22.7 ± 4.8) and BDF_1 (15.6 ± 11). All strains showed at least double the control IgE levels after immunization. The IgA levels are reported in the meeting paper by Pitt et al.

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 responses [7], there are organ-specific differences in IgA and IgE commitment following IP immunization that correlate with differences in expression of mucosal immunity and/or allergy in the mouse strains tested. C3H/HeJ and A/J mice are susceptible and resistant to antigen-triggered bronchospasm, respectively. They hold opposite relationships with regard to acetylcholine mediated hyperreactivity [9]. Could there be upregulation of IgE receptors to compensate for alterations in neurotransmitter binding? In the case of the C3H/HeJ and C3H/HeN mice the complexity of converging etiologies [8,9] should be minimized because there are limited genetic differences. We have not determined what role the genes controlling IL-4 and γ interferon play in this system.

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