PATHOGENICITY OF AEROMONAS SP. FOR BALB/C MICE

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Abstract

Clinical strains of Aeromonas hvdrophlla and Aeromonas sobria were used to develop a mouse model for determining virulence potentials of food isolates. lntraperitoneal injection with these strains caused hemorrhaging and death of 10—12 week old male BALB/c mice within 24 h post challenge. Non-supplemented injections of Aeromonas sp. suspensions resulted in a median LD 50 value of 4.9 X 10^7 cells, whereas supplementation with 0.1 ml of 5% iron dextran with the bacterium decreased the LD 50 value to 3.1 X 10^7 cells. Immunosuppression of the mice with cadmium chloride did not substantially alter the lethality for mice. Boiled suspensions 109 cells did not cause more deaths, indicating a lack of pathological contribution by the LPS component when using this approach for virulence assessment. lsolates of Aeromonas sp. from retail chicken, beef and pork products were supplemented with iron dextrart and injected into BALB/c male mice. Twenty-two of 26 mice injected with 26 A. hydrophila food isolates died; 13 of 14 mice injected with A. sobria isolates died; and 10 of 16 mice injected with A. cav!ae isolates died. These results indicate that approximately 80% of foodborne aeromonas are lethal for mice via intraperitoneal challenge. This virulence assay may or may not be an appropriate choice to assess the potentials of foodborne isolates of Aeromonas sp. to cause disease in humans (JPMA 38: 47 1988).

INTRODUCTION

Species of Aeromonas have been associated with human diarrhea in clinical infections[8,15]. Manifestations of the disease include fever grSter than 38°C, abdominal cramps, vomiting and duration of illness greater than 10 days[1]. In Australia, Aeromonas was isolated from 12.3% of children with diarrhea and from 1.9% of the control group[6]. The evidence suggests that the disease caused by these organisms is usually mild, with a selflimiting diarrheal episode occurring in the healthy adult[7]. Aerornonas sp. are inhabitants of aquatic environments, with large numbers of toxigenic A. hydrophila found in estuarine environments yielding human seafoods[9]. Presence of the bacteria on a variety of foods of animal origin has been reported in Sweden, Mexico and the United States. A survey of potential pathogens in meat. 13th International Congres of Microbiology, Boston, MA. PG (B): [13,19] Palumbo and co-workers[13] reported that all foods which they surveyed contained A. hydrophila. The foods they sampled included red meats, chicken, raw milk, fish, shrimp ,scallops, crab and oysters. Previous observations (Dmzek, Stern and Joseph, 1986, Low incidence of Aeromonas sp. in livestock feces. 86th annual meeting of the American Society for Microbiology, Washington, D.C.) have indicated that a relatively small percentage of red meat and poultry animals harbor Aemmonas sp. in their intestinal contents. Food-borne bacterial enteropathogens are often transferred from the intestinal content of the slaughter animals. During processing, fecal materials from these animals may contaminate the meat products either directly or indirectly. Therefore, it seemed curious that all the foods sampled in retail markets contained the potential human pathogen, while relatively few (about 4%) of the livestock sampled carried Aeromonas sp. The purpose of this research was to determine

whether the Aeromonas sp., which can be frequently isolated from foods of animal origin, which are not of aquatic association, had potential health hazard for humans. To assess this question, we optimized a mouse model, which is described in this report.

MATERIALS AND METHODS

Preliminary animal handling. Nine to ten week old male BALB/c mice were purchased from Jackson Laboratory (Bar Harbor, Maine) and maintained on antibiotic-free food and tftp water. The animals were housed, five per cage, for at least one week before the experiment. Fecal specimen from each group were taken on two consecutive days, suspended in sterile phosphate buffered! saline (PBS) and cultured, ensuring that the mice were not carriers of Aeromonas sp. Mice were immunocompromised with cadmium chloride to determine the effects on susceptibility to Aeromonas sp. infection[10] - The animals were injected with 10 ug cadmium chloride at day 0, 2 and 4. The ten to twelve week old mice were then rested for an additional two days prior to bacterial challenge. Bacterial strains. Aeromonas hydrophila (MSH 11) and Aeromonas sobria (MSH 24) were kindly provided by S. Joseph of the Department of Microbiology, University of Maryland. These strains were clinical isolates from diarrheal stool specimens, and were used in the development of the below described mouse model. The 56 food isolates tested were generously provided by B. Rose of the Food Safety Inspection Service USDA. All the isolates were held on slants of brain heart infusion at room temperature, transferred on a monthly basis and incubated at room temperature. Each culture had several passages on artificial media before use in the mouse model. Prior to the animal challenge, inocula were prepared by suspending a loopful of the culture in sterile PBS and swabbing the suspension onto a single Mueller Hinton plate. The overnight growth was harvested and washed in PBS. Challenge studies, using the mean LD 50 values obtained in developing the model, were standardized by comparison of culture turbidity with McFarland standards. The cultures were diluted and numbers in the inocula were confirmed by plating decimal dilutions onto Mueller Hinton agar plates and enumerating the resultant colony growth. Intraperitoneal challenge. Mice were subjected to intraperitoneal challenge with the above described Aeromonas sp. cultures. Contained in the inocula were 0.4 ml of culture, with 0.1 ml of 5% iron dextran (Sigma, St. Louis, MO) or 0.1 ml lysed sheep blood (Otisville Biotechnology Inc., Otisville, NY) or 0.1 ml PBS controls. Non-specific deaths which occurred within one-hour after injection were not included in the data reported. The challenged animals were observed for 24 h. During the development of the model, exudate from the peritoneal cavity of the dead mice was aseptically removed and streaked onto Starch-Ampicillin plates[13] for re-isolation of the Aeromonas sp. culture.

RUSULTS

Data from our initial set of experiments developing a mouse model for describing pathogenesis of Aeromonas sp. is shown in Table 1.

TABLE I

Deaths among 10–12 week old BALB/c male mice challenged by intraperitoneal (IP) injection of *Aeromonas hydrophila* (MSH 11) as influenced by the presence or absence of supplemental Iron Dextran.

CFU/IP challenge[1]	Supplementation	
	Iron Dextran[2]	None[3]
2.3×10^8	5/5 [4]	5/5
2.3×10^7	3/5	3/5
2.3×10^6	2/5	0/5
none	0/3	0/3
2.3×10^9 boiled *A. hydrophila*	0/8	ND 5

1. IP injection contained the indicated numbers of CFU in 0.4 ml challenge suspension.
2. IP injection supplemented with 0.1 ml of 5% iron dextran.
3. IP injection supplemented with 0.1 ml of phosphate buffered saline (pH 7.2).
4. No. of deaths/No. of mice challenged as observed at 18 h post injection.
5. not determined.

The median LD 50 values for BALB/c mice injected with the organism in the presence of supplemental iron dextran was calculated as 3.1×10^7. Using the same cultures without any supplementation, the LD 50 values were calculated as 4.9×10^7. The control experiments, injecting either only iron dextran or only PBS, without Aeromonas sp., indicated that these supplements did not cause deaths of the mice. When ten-fold the LD 50 value of heat killed cells were injected into the mice, no deaths occurred. Whenever deaths did occur, the IP-infected mice manifested ruffled fur with a moist appearance, and a large amount of hemorrhaging in the peritoneal cavity at the site of injection. Aeromonas sp. were consistently re-isolated in large numbers from the peritoneal exudate of the dead animals. Further assessing the value of other strategies in the development of the Aeromonas mouse model included supplementation of the organism with lysed sheep blood or, immunocompromising the animals with cadmium chloride injection before bacterial challenge[10]. Results of these experiments are presented in

Table II,

TABLE II

Deaths among 10–12 week old BALB/c male mice challenged by intraperitoneal (IP) injection of either Aeromonas hydrophila (MSH 11) or *Aeromonas sobria* (MSH 24) as influenced by pre-treatment with immuno-suppressive levels of cadmium chloride, supplemental Iron Dextran or lysed sheep Blood.

Species injected–– CFU/IP challenge[1]	Treatment or Supplement None[2]	Cd[3]	ID[4]	LSB[5]
A. hydrophila				
1.8×10^8	5/5[6]	1/5	5/5	5/5
1.8×10^7	0/5	0/5	0/5	0/5
A. Sobria				
1.9×10^8	5/5	4/5	5/5	4/5
1.9×10^7	0/5	0/5	1/5	0/5

and indicate that no virulence enhancement occurred by supplementing with lysed sheep blood or by immunocompromising the animals with cadmium chloride. Only 5 of 10 mice treated with cadmium chloride died as compared with approximately twice as many dying when using the other treatments employed. As numbers of boiled cells greater than ten-fold the LD 50 values were injected without any signs of pathology, we conclude that there is limited influence of the LPS in this mouse model. We decided to use the model employing hon dextran, because it did provide slightly lower LD 50 values. We assessed 56 food isolates of We decided to use the model employing hon dextran, because it did provide slightly lower LD 50 values. We assessed 56 food isolates of Aeromonas sp. for lethality to mice.

TABLE III

Mouse deaths caused by *Aeromonas* sp. injected via the intraperitoneal (IP) route of challenge in the presence of Iron Dextran supplementation. Aeromonas used for injection were isolated from retail chicken, beef or pork products.[1]

Deaths	Animals Challenged	Challenge Identification or source
22	26	*A. hydrophila* food isolates
13	14	*A. sobria* food isolates
10	16	*A. caviae* food isolates
15	22	*Aeromonas sp.* from chicken product
16	20	*Aeromonas sp.* from beef product
14	14	*Aeromonas sp.* from pork product

Table III illustrates the summarized results of the survey, and indicates that 45 of the 56 (80%) isolates were lethal by the model described. Of the Aeromonas species injected, A. cavae caused the lowest percentage of deaths (63%) as compared to A. hydrophila (8 5%) and A. sobria (93%). Aeromonas sp. isolates from pork meat products were uniformly lethal for mice (100%), while the isolates from chicken products caused the lowest percentage of deaths (68%) among the isolates from the three meat products assessed.

DISCUSSION

Intraperitoneal injection of Aerornonas spin BALB/c mice provided a model for assessing the virulence potentials of food isolates. Employing iron dextran supplement only slightly enhanced the lethality of the organism in this animal model. We decided to continue using this source of iron, as our data indicated its potential advantage, and because of other reports in the literature observing the production

of iron binding siderophores in Aeromonas sp[4]. (Liles, D., It. Byers, P. Byers, J. Arceneaux and C. Lobb, 1985. Production of a siderophore and utilization of transferrin-iron by Aeromonas hydrophila. 85th annual meeting of the American Society for Microbiology, Las Vegas, NV. K 179). The physiological "war" over the possession of hon has been well documented[17]. The immunological response by the host includes sequestering this mineral by producing transferrin and lactoferrin proteins, which deprives the infectious agent of its nutritional requirement[11,18]. Thus, the term "nutritional immunity" has been used[20]. In turn, certain pathogenic bacteria elaborate siderophores, which "wrestle" with the iron-binding proteins of the host. Success in attaining iron allows for bacterial growth and, subsequently, pathogenesis in the host. Thus, our approach in providing iron by way of the iron dextran supplement, saturated the mouse iron-binding proteins and allowed for the physiological requirements in the pathogenesis of Aeromonas sp. The importance of iron to Aeromonas sp. was further supported by our observation of the dramatic hemorrhaging within the peritoneal cavity of the challenged mice - Hemolysins have been reported as a virulence-associated factor found in Aeromonas[3,5], and is likely to be contributing to the pathology observed in our mouse model. The invasiveness of Aeromonas sp. is an in-vitro, virulence-associated factor that corresponds well with both the mouse model and with the occasional dysenteric clinical symptoms produced by some isolates[12,16] Other potentially important virulence determinants, such as production of cholera-like enterotoxin by A. hydrophila have been reportedv[14]. Ambiguous reports disagree on the correlations of cytotoxicity, hemolytic activity, mouse lethality and human enteropathogenicity[12] (Clarke, R.B., E. J. Boftone, and MJ. Janda, 1985. Differential virulence of Aerornonas species as assessed through mouse lethality. 85th annual meeting of the American Society for Microbiology, Las Vegas, NV. B 191; Morgan, D., P.C. Johnson, Hi. DuPont, T.K. Satterwhite, and L.V. Wood, 1985. Lack of correlation between virulence properties of Aeromonas hydrophila and enteropathogenicity for humans. 85th annual meeting of the American Society for Microbiology, Las Vegas, NV. B 190). Determination of the key virulence factors will only be accomplished after the appropriate animal model, resembling the human enteric pathology, is described, and used to illustrate the roles of the various determinants in the pathology. These observations will also need to correlate closely with the clinical evidence, Although the mouse model described herein was useful in manifesting the virulence of Aeromonas sp., we are still unable to answer whether this assay is appropriate to assess potential virulence for humans. Because these bacteria are almost always associated with a variety of refrigerated foods of animal origin, it is probable that the inevitable and frequent consumption of the organism is made without demonstrable symptoms of human disease. We have shown that 20% of the food isolates were not lethal for mice via intraperitoneal injection, but considering the large numbers and the presence of this organism, this does not correspond well with the relatively low rate of human infection. Still lacking are the means to discriminate the innocuous and infectious Aerornonas sp. isolated from human foods.

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