Proceedings of the First International Conference (Egyptian British Biological Society, EBB Soc)
Egyptian Journal of Biology, 2001, Vol. 3, pp 96-101 © Printed in Egypt. Modern Press, Cairo.

Immunological evaluation of *Aeromonas* infection in Albino rat.

Moustafa El-Feki*, Ezzat M Awad , Hanan M Amin and Amany M Mahmoud

Zoology Department, Faculty of Science, Minia University, Egypt.

ABSTRACT
Aeromonas hydrophila is the most common bacterial species causing an acute self limiting diarrheal illness in mammals. Albino rats were categorized into treated and control groups. Treated groups were injected daily intraperitoneally with 0.2ml of bacterial suspension containing $6x10^6$ cells/ml of 0.9% NaCl, while the control groups were injected intraperitoneally with 0.2ml of 0.9% NaCl. Both control and treated groups were sacrificed after 3, 6, 9, 12 and 15 days post-injection. The immunological parameters covering innate, cellular and humoral immune responses were estimated. Phagocytic activity showed highly significant differences in all treated animals ($p<0.01$) either *in vivo* or *in vitro* assays. In addition, cell-mediated immune response exhibited highly significant changes in all treated rats in a time-dependent manner. However, humoral immune response showed significant changes in all treated animals, confirming that the bacterial infection affected on all immunological pathways. This study aimed to clarify the imunological changes induced by *A. hydrophila* infection.

KEYWORDS: *Aeromonas,* immunity, mammals

INTRODUCTION

Aeromonas hydrophila is a gram negative, rod-shape aquatic bacteria and a member of the family Vibrionacae. It is commonly isolated from fresh-water (Schubert 1971). It is recognized as a pathogen in many freshwater fish, amphibians and reptiles (Esch *et al.* 1976). *Aeromonas hydrophila* might be considered as a potential pathogen for a variety of mammals and the presence of fish in their water supplies could be a potential reservoir of infection (Lallier & Higgins 1988). It is also recognized as an important human pathogen. It causes extraintestinal diseases such as wound infection, meningitis, endocarditis and osteomylitis (Freij 1986) and can be associated with gastrointestinal (diarrheal) symptoms and septicaemia (Agger & Callister 1987). There was an association between the isolation of *A. hydrophila* from all animal faeces and its presence in drinking water (Gray 1984).
Strains of A. hydrophila have previously been shown to produce a heat labile enterotoxin and two cytolytic toxins, alpha and beta haemolysins. Haemolysins produced hemorrhagic enteritis with leakage of small amounts of hemorrhagic fluid, while Aeromonas enterotoxin may have a common pathway in the elicitation of intestinal fluid accumulation in rat and mice (Ljungh 1982). Gram negative bacteria produce endotoxins, which are lipopolysaccharides in their nature. Lipopolysaccharides isolated from strains of A. hydrophila contained O-polysaccharide chains of homogeneous chain length (Dooley 1985). Bacterial lipopolysaccharide (Endotoxin) is a potent activator of inflammatory genes in blood leucocytes, including interleukin–1 (IL-1) and induces immune responses by direct stimulation of B-cells and macrophages. These endotoxins possess immunomodulatory activities capable of stimulating immune functions both in vitro and in vivo (Estrada et al. 1998). Therefore, this study has been carried out to understand the immunological changes induced by A. hydrophila as a gram-negative bacteria.

MATERIALS AND METHODS

Animals: 50 Male albino rats (*Rattus norvegicus*), mean weight (120 ± 10 gm), were kept in large cages with sawdust bottoms under the controlled laboratory conditions for 3 weeks

* Address for Correspondence

before inoculation and feed daily on commercial pellets.

Antigen (Bacteria): *A. hydrophila* (A-47) strain, was kindly supplied by Department of Zoology, Faculty of Science, Cairo University, Beni Suef Branch. Bacteria were grown on nutrient agar (pH 6.8) and nutrient broth (pH 7.5 – 7.6). The bacterial identification was performed according to the methods of Cruickshank *et al.* (1975), while the concentrations of bacteria were calculated using the matching technique developed by Mc Farland after some modifications.

Animal injection: Rats were categorized into treated and control groups. Treated groups were injected daily intraperitoneally with 0.2 ml of bacterial suspension containing 6×10^6 cells/ml of 0.9% NaCl, while the control rats were injected intraperitonealy with 0.2 ml of 0.9% NaCl. Both control and treated groups were sacrificed after 3,6, 9,12 and 15days post–injection.

Immunological studies: The phagocytic activity of different leucocytic cells was determined with a phase contrast microscope (*in vivo*) according to El- Feki (1994), while the determination of *in vitro* phagocytic activity of polymorohonuclear (PMN) cells against *Candida albicans* was carried out according to Wilkinson (1981). The cell mediated immune response was determined by carrying the immunocytoadherence (Rosette forming cell, RFC) technique, as according to Hudson & Hay (1980) and Ali (1987) and the migration inhibition factor of leucocytes (MIF), which indicates the acquisition of cellular immunity to an antigen using capillary technique (Abu El–Saad 1996). In addition, the humoral immune response was established using the plaque forming cell (PFC) technique of Cunningham & Szenberg (1968), which was modified by Kanakambica & Muthukkaruppan (1972), the detection of agglutinating antibodies was established according to the haemagglutination technique (Hudson & Hay 1980), the detection of precipitating antibodies was carried out using the double immuno-diffusion technique (Hudson & Hay 1980) and the quantitatin of serum immunoglobulins was carried out the by Zinc sulphate turbidity method (Pfeiffer *et al.* 1977).

Statistical analysis: Results were represented as mean ± standard deviation (S.D.). Student's t-test was applied in the present study, as presented by Sndecor & Cochran (1971).

RESULTS

Innate immunity

Phagocytosis in vivo* and *in vitro: The percentage of the leucocytes phagocytic activity either *in vivo* or *in vitro* increased along all the time intervals when compared with the control percentage (Figs.6,7).

Cell -mediated immune response

Rosette forming cells (RFC): RFC number increased along the period of the experiment in comparison with the control values. The maximum value was achieved on the 12 th day (20.2±1.30), as compared with control one (1±1.41) (Fig.4).

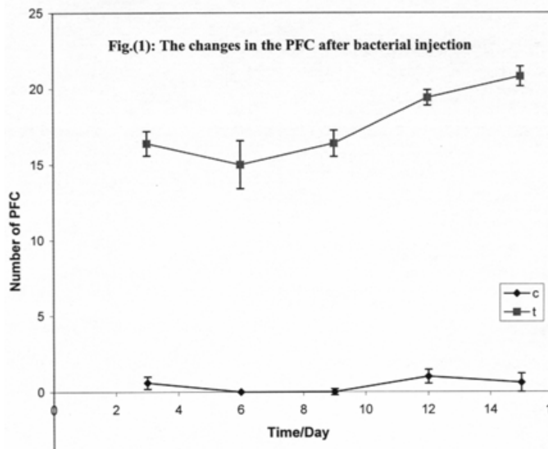
***The inhibition of the leucocytic migration (MIF)**:* The percentage of migration inhibition factor decreased after injection with the bacterial suspension when compared with the control values. The minimum value (1.06±0.59) was obtained after the first time interval (Fig 4).

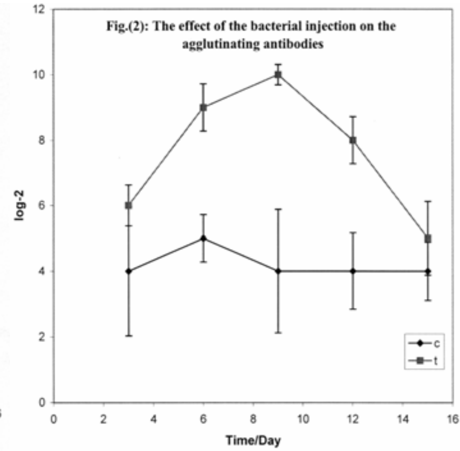
Humoral immunity

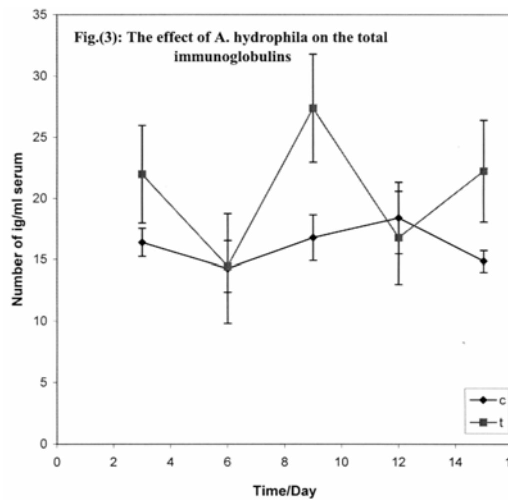
Plaque forming cells (PFC): Plaque forming cells showed a remarkable increase in number during the period of injection, where it became much higher than the control value. The highest value (20.8 ±1.48) was recorded after 15 days of injection, as compared with the control value of 0.6±1.34 as shown in figure 1.

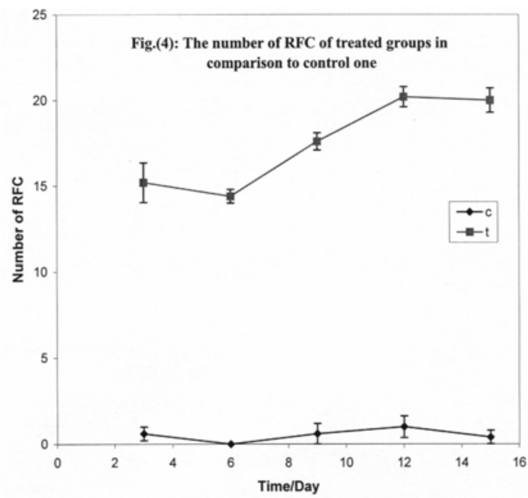
Haemagglutination test: The antibody titre production with the bacterial antigen increased throughout the period of injection. The maximum antibody production was recorded on the 9th day (10±0.7) (Fig. 2)

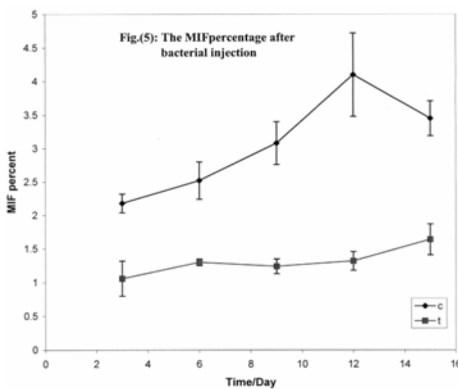
Precipitation technique: The precipitating arc appeared in all treated rats, but it was much higher after the third time interval.
Total immunoglobulins (Ig): The number of immunoglobulins per ml serum increased in treated groups over the control values. The maximum value (27.37±9.81) was obtained after the third time interval (Fig. 3).

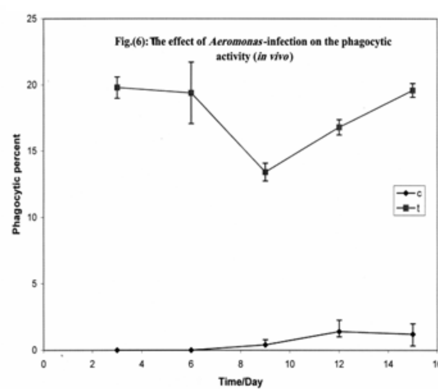


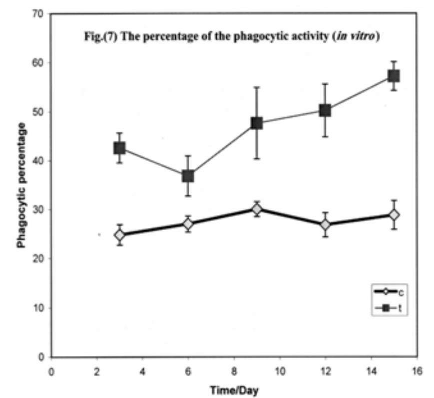












DISCUSSION

The immune response kinetics of rats against the bacterial infection was established using the innate, cell-mediated and humoral responses. The CD14 molecule expressed on monocytes and macrophages is a high-affinity receptor for bacterial lipopolysaccharide (LPS) and hence an important component of the innate immune system (Fan 1999).

Phagocytosis is the main mechanism for destruction of extracellular bacterial pathogens as well as several viral and fungal organisms in mammals (Cohn 1978). This study showed increased phagocytic activity in all treated groups, which might be because the intraperitoneal administration of endotoxin in mice resulted in the accumulation of leucocytes in the peritoneal cavity and in the increase of IL-1, IL-6, TNF- alpha and IFN- gamma concentration in serum. Endotoxin possesses immunomodulatory activities capable of stimulating immune function both *in vitro* and *in vivo* (Estrada *et al.* 1998). The increase in the phagocytic activity either *in vivo* or *in vitro* may be attributed to (1) the presence of the optimal conditions required for efficient phagocytosis, (2) the increase in the haemopoietic activity to produce new phagocytic cells instead of the dead ones, because the natural killer (NK) cells regulate MHC expression via interferon (INFγ), (3) the increase of LPS as a bacterial endotoxin affects bone marrow and spleen cells (Saito-Taki & Nakano 1982), inducing the phagocytic and killing activity of macrophages and neutrophils (Staber *et al.* 1978), (4) the production of C-reactive protein (CRP) activates macrophages, and has an opsonising effect on the phagocytosis (Kodama *et al.*. 1989), or (5) the increase of the neutrophils released from marginated vascular endothelium as a result of early defense against antigen infection (Steele *et al.* 1987). In addition, opsonization with serum antibodies may be another reason for phagocytosis, because antigen antibody complexes would be formed and removed early by macrophages and neutrophils (Shoemaker & Klesius 1997).

Results of phagocytosis *in vitro* indicated that the membrane CD14 (mCD14) is the most important factor for the elimination of LPS that hyperactivates the phagocytic cells and induces high levels of proinflammatory cytokines in monocytic cells that ingest and kill bacteria. Therefore, LPS plays a role in the innate immune response to gram negative infections (Kanangat *et al.* 1999).

The present data demonstrated that the RFC gave higher values than the PFC. The RFC measurements detect any immune cells that have the appropriate surface receptors for the antigen and including T- equivalent lymphocytes as well as B – cells that have not yet begun to secrete antibodies. Therefore, RFC values give a more complete picture of cellular reactivity in lymphoid organs than the PFC assay (Ali 1987). The increase in most of the immune assays, (PFC, total immunoglobulins, agglutiniating antibodies, RFC and precipitating antibodies) may be due to the antigenic challenge (El- Feki *et al.* 1999), which may in turn be due to the secretion of interferon (IL – II) " The T– cell growth factor, T-CGF " prompting T- cell clone proliferation, or to the secretion of interleukin (IL-1) " the lymphocyte activating factor, LAF" by macrophages resulting in increased interforon killer (INFγ) production by natural killer (NK) cells. These activate T- helper cells which promote multiplication and activation of B- cells. Alternatively may be due to the secretion of B- cell growth factor by macrophages which synergies with IL- I in B – cell activation (Roitt *et al.* 1996).

Our results showed decreased MIF accompanied by increased PFC, which may be because the elaboration of MIF (a lymphokine) by T-cells suppresses the macrophage movement, because the latter acted as an antigen presenting cells (APC) that offer the antigen to B&T-cells and consequently suppress PFC response (Hung *et al.* 1996). MIF was decreased against the control values post infection following the *in vivo* sensitization with the antigen, because the migration of leucocytes was reduced after the *in vitro* challenge with the specific sensitizing

antigen (Howell 1987). The elevated agglutinating titres following the bacterial infection were recorded as a result of the continuous stimulation of the antibodies by the T-independent nature of certain components found in *A. hydrophila* (Lamers *et al.* 1985).
The weak results of the precipitation test might be due to the fact that the antigen used is a molecular antigen not a soluble one and it would be expected to have lower titre of precipitating factors in the infected sera when compared to the control one (Manning & Tatner 1985).

In conclusion, this study may suggest that the complications associated with septicemia may be due to the immunological alterations caused by the bacteria. Therefore, immunomodulators and immunopotentiators should be used besides the antiseptic drugs to improve the immune response against the bacterial infection.

REFERENCES

Ali HM (1987) The Histogensis of lymphoid organs in Tilapia mossanbica and antibody responses in adults and fry. Ph.D. University of Aston, Birmingham, England.

Abu El- Saad ASA (1996) Pathogenicity of *Aeromonas hydrophila* against common carp *Cyprinus carpio*. M.SC. Thesis, Fac. Sci., Minia Univ., Egypt.

Agger WA & Callister SM (1987) Intestinal infections with *Aeromonas*. *Intern. Med*.106: 497.

Cohn Z (1978) The activation of mononuclear phagocytes: fact fancy and future. *J. Immunol*. 121: 813-816.

Cruichshank R; Duguid J; Maanino B & Swain R (1975) Medical Microbiology. The practice of medical Microbiology. 10th Ed. Churchil livingstone. London and New York.

Cunninjham AJ & Szenberg A (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunoiogy*. 14: 599.

Dooley JS, Lallier R, Shaw DH & Trust TJ (1985) Electrophoretic and immunochemical analysis of the lipopolysaccharides from various strains of *Aeromonas hydrophila. J. Bacteriol.* 164(1): 263-269.

EL-Feki MA (1994) The role of chemo-attractants in phagocytosis of catfish (*Clarias lazera*). *Bull. Fac. Sci*. 23 (2): 185-200.

El-Feki MA, Radwan ZA & El-Fayoumi HM (1999) Fish immune response against heterogeneous antigens. *Egypt. J. Aquat .Biol. & Fish* 3(4): 473-491.

Esch GW, Hazen TC, Mock JR & Gibbons JN (1976) Thermal effluents and the epizootiology of cilliates Epistylis and the bacterium *Aeromonas* in association with centrar-chid fish. *Trannsaction of the Americans in association Microbiological Society* 95: 687-693.

Estrada A, Van Kessel A &Yunch L (1998) Effect of endotoxin on cytokine production and cell dynamics in mice. *Immunopharmacol. Immuntoxicol*. 20(2): 217-310.

Fan X, Stelter F, Menzel R, Jack R, Spreitzer I, Hartuing T & Schutt C (1999) Structures in *Bacillus subtithis* are recoginzed by CD14 in a lipopolysaccharide binding protein dependent reaction. Infect. *Immun.* 67(6): 2964-8.

Freij BJ (1986) Human diseases other than gastroenteritis cuasd by *Aeromonas plesiomonas*, P.19-20 in 1st International Workshop on *Aeromonas Plesiomonas* 5106

Gray S J (1984) *Aeromonas hydrophila* in livestock: incidence, biochemical characteristics and antibiotic susceptibility. *J. Hyg. Camb*. 92: 365-375.

Howell C J (1987) A chemokinetic factor in the carp *Cyprinus carpio*. Develop. *Comp. Immunol*. 11: 139-164.

Hudson L & Hay FC (1980) Practical Immunology , 2nd Edition. Blackwell Scientific Publications, Oxford.

Hung HW, Lo CF, Tseng CC & Kou GH (1996) Humoral immune response of Japanese eel, *Anguillia japonica* to *Pleislophora anguillarum* (Microspora). *J. Fish Dis.* 19: 243-250.

Kanakambika P & Muthukkaruppan V (1972) Immunological competence in the Newly Hatched Lizard, *Calotes versicolar. Proc. Soc. Exp. Biol. Med.* 140: 21-23.

Kanangat S; Meduri Ch U; Pak Ch; Griffin J P; Bronze M S & Schaberg D R (1999) Effect of Cytokines and Endotoxin on the Intracellular Growth of Bacteria. *Infection and Immunity* 67(3): 1353-1358.

Kodama H, Yamada F, Murai T, Nakanishi Y, Mikami T & Izawa H (1989) Activation of trout macrophages and production of CRP after immunization with *Vibrio anguillarum. Develop. Comp. Immunol.* 13:123-132.

Lallier R & Higgins R (1988) Biochemical and toxigenic characteristics of *Aeromonas spp*. Isolated from diseased mammals, Moribund and healthy fish. *Vet. Microbiol.* 18: 63-71.

Lamers CHJ, De Haas MJH & Van Muiswinkel WB (1985) Humoral respose and memery formation in carp after injection of *Aeromonas hydrophila* bacterin. *Develop. Comp. Immunol.* 9: 65-75.

Ljungh A & Kronevi T (1982) *Aeromonas hydrophila* toxins- intestinal fluid accunmulation and mucosal injury in animal models. *Toxicon* 20(2): 397-407.

Manning M J & Tanter MF (1985) Fish Immunology. Academic Press.

Pfeiffer NE, McGuire TC, Bendel RB & Weikel JM (1977) Quantitation of bovine Immuno-globulins: Comparison of single radial immuno-diffusion, Zinc sulphate turbidity, Serum Electrophoresis, and Refractomter Methods. *Am. J. Vet. Res*. 38 (S): 693-698.

Saito-Taki T & Nakano M (1982) Activation of bone marrow (B.M) cells by bacterial lipopolysaccharides (LPS) and microorganisms. In: " Immuno-modulation by microbial products and related synthetic compounds. " Yamamura Y, Kotani S, Azuma L, Koda A & Shba T (Eds.): Exp. Med. 299-302.

Schubert L (1971) *Aermonas.* In Bergy's manual of determinative bacteriology, 8th Ed. William and Wilkins, Baltimore, Maryland, 1268 pp.

Shoemaeker CA & Klesius PH (1997) Protective immunity against enteric septicaemia in channel cat fish, *Lctalurus punctatus*, following controlled exposure to *Edwerdsiell iclaluri*. *J. fish Dis.* 20: 361-368.

Snedecor G W & Cochran (1971) Statistical methods, 14th ED. Iowa State Univ. Press.

Staber FG, Gisler RH, Schumann G, Tarsay L, Schlafli E & Dukor P (1978) Modulation of myelopoiessis by different bacterial cell wall components : induction of colony stimulating activity (by pure preparation, low molecular weight degradation products, and a synthetic low molecular analogy of bacterial cell wall components) *in vitro*. *Cell. Immunol.* 37: 174- 187.

Steele RW, Steed CR, Pillington NS & Charlton RK (1987) Functional Capacity of migrated and bone marrow reserve granulocytes. *Infect. Immunol.* 55: 2359-2363.

Wilkinson PC (1981) Techniques in Clinical Immunology. 2nd Ed., Blackwell. Scientific Publication, London .287-288.

– – –

- - –

.

6×10^{6} (%0.9) 0.2

-: . 15 12 9 6 3

(1) . PFC RFC

(2) .

(3) .

(4) .

(5) .

(6) .

.