European Journal of Biology and Medical Science Research

Vol. 1, No. 2, pp. 31-39, June 2013

Published by European Centre for Research Training and Development UK (www.ea-journals .org)

MECHANISMS OF IMMUNITY TO *ESCHERICHIA COLI* O157:H7 IN ALBINO RATS: ROLE OF HOMOLOGOUS ANTIBODIES

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Abstract: *The role of antibodies in conferring protection against infection caused by Escherichia coli O157:H7 in albino rats was carried out in this study. Antibody against E. coli O157:H7 of known titre was raised actively and used to passively protect some sets of rats intraperitoneally. Antibody titre was determined using tube agglutination test. At different intervals, starting from 30 minutes to 168 hours, the liver, spleen and ileum of actively, passively immunized and unimmunized rats were dissected out and the number of trapped E. coli O157:H7 was counted in these selected organs using standard microbiological techniques. The highest recovery of the organism was seen in unimmunized rats followed by the actively immunized rats. The least recovery was seen in the passively immunized rats. The spleen trapped more of the organism compared to the liver and ileum throughout the experiment. From this investigation therefore, passively raised antibodies to E. coli O157:H7 play a significant role by causing a reduction in the number of trapped E. coli O157:H7 in the organs examined. It is conceivable therefore that in case of an outbreak of infection caused by this organism, administration of preformed antibodies against E. coli O157:H7 to infected individuals, that is, passive immunity will reduce the severity of the infection and also the spread of the infection in the community.*

Keywords: Immunization, Antibody, Liver, Spleen, Ileum, *E. coli* O157:H7

INTRODUCTION

Enterohaemorrhagic *Escherichia coli* or EHEC, is a group of pathogenic *Escherichia coli* comprising *Escherichia coli* O157:H7 which causes a condition known as heamorrhagic colitis (HC) or bloody diarrhoea due to their ability to colonize the gastrointestinal tract (Karch *et al*., 2005). Human infections caused by *E. coli* serotype O157:H7 have been shown to result in a host of immune responses as evident by the raising of antibodies to the organism (Karmali *et al*., 1986).

Although it has been nearly 30 years since the discovery of *E. coli* O157:H7 as an enteric pathogen, no effective treatment exists because the organism is resistant to almost all antibiotics. Moreover, treatment with antibiotics may promote the expression of toxins from the lysogenised phage that carries shiga-like toxins genes inherent in this organism (Fox *et al*., 2009).

Additionally, antimotility agents are also able to promote and prolong the expression of this toxin in the gastrointestinal tract (Moxley *et al*., 2009). Therefore, attention is now being shifted to the use of toxin receptor analogs, antibody therapy and vaccines to protect humans against the effect of the toxins produced by this organism (Thomson *et al*., 2009). Although there are two vaccines currently being used for cattle, they have not been licensed to be used for humans (Treor, 2008). Therefore for now, minimizing exposure to this pathogen is a key step in curtailing the infection caused by *E. coli* O157:H7 (Smith *et al*., 2009). This study therefore was designed to investigate the effectiveness and the possible role antibodies to *E. coli* O157: H7 play against infections caused by this organism at the cellular level.

MATERIAL AND METHODS

Experimental animals

Seven to eight weeks old, adult male albino rats, with average weight of 65g were used for this work. They were housed in cages with adequate ventilation and fed with growers mash and clean water for 2 weeks during which period they were observed for any sign of illness.

The organism used

The *Escherichia coli* O157:H7 (single plasmid, molecular weight 24,980bp) used was collected from the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos

Preparation of cells of *Escherichia coli* O157:H7

The organism was transferred from an agar slant into 10ml Nutrient broth using a sterile inoculating loop, mixed well and incubated at 37^0C for 24 hours. After incubation, the content was centrifuged at a speed of 3000 revolution per minute (rpm) for 10 minutes to harvest the cells. The supernatant was discarded and the harvested cells were washed with sterile distilled water and re-centrifuged, the cells were then re-suspended in 10ml sterile distilled water which served as the stock cell solution for serial dilution. The number of cells per ml was then determined using pour plate technique.

Determination of Infective dose of *E. coli* O157:H7

This was carried out according to the method of Willey *et al*. (2008) with slight modifications. Sterile test tubes were arranged serially, 9ml sterile distilled water was dispensed into 6 set of test tubes, and 1ml of the stock (cell) solution was introduced into the first tube making 1:10 dilution. This procedure was repeated for the remaining 5 tubes. One ml of each dilution was pour plated on Sorbitol MacConkey agar. The plates were incubated at 37^0C for 18 - 24 hours. Visible colonies were counted and estimated according to the dilution factor. The rats, 4 groups

of 3 each were challenged orogastrically with 1ml of the different corresponding dilutions. They were observed for any clinical symptom of infection. The dose that was able to produce clinical effects such as unformed stool, weakness and loss of appetite on the animal was calculated and used as the infective dose of the organism.

Active immunization

Thirty rats were immunized with a dose lower than the infectivity dose of the organism. After 7 days, their blood was collected through cardiopuncture, allowed to clot and the serum (Anti-*Escherichia coli* O157:H7 antibodies) was purified by centrifuging at 2,500 rpm for 30 minutes. The antibody titre of the serum was determined using tube agglutination test according to the method of Willey *et al*. (2008). A booster dose was administered to the rats and the titre was also determined after 7 days.

Passive Immunization

This was done according to the method of Mullan *et al*. (1974) with slight modifications. Antiserum (0.25ml) obtained from the actively immunized rats (agglutination titre 1:1280) was injected intraperitoneally into a new set of rats. After 4 hours, they were challenged with the infective dose of the organism, 2.7×10^2 cfu/ml orogastrically.

Time course growth of *E. coli* O157:H7 in selected organs in immunized and control rats

The liver and spleen of the infected rats were dissected and homogenized while the ileum (10cm) was washed in 10ml sterile distilled water, serial dilution was done and 1ml of each dilution was pour-plated on Sorbitol MacConkey Agar and incubated at 37^0C for 18-24 hours. The number of viable bacteria was counted and expressed as $\log_{10}$ (bacterial count). This procedure was repeated at different intervals, that is, 30 minutes, 1 hour, 6, 24, 48, 72, 96, 120, 144, and 168 hours.

For the control experiment, normal rats were administered with the infective dose of the organism without immunization and their organs were dissected and analysed at different intervals as done for the immunized rats.

Statistical analysis

Statistical Analysis of data was carried out using analysis of variance (ANOVA) and Duncan's Multiple Range Test for the estimation of means. The't' value was tested at 95% confidence interval.

RESULTS

Estimated Antibody titre for passive immunization

The titre of the antibody raised against *Escherichia coli* O157:H7 in albino rats was 1:320 when the rats were administered with the first dose of the organism. This however increased to 1:1280 after the administration of the booster dose (Table 1).

Table 1. Antibody titre of rats after the administration of first and booster dose of *Escherichia coli* O157: H7

Dilutions (Titre)	Agglutination	
	First dose	Booster dose
1:10	+	+
1:20	+	+
1:40	+	+
1:80	+	+
1:160	+	+
1:320	+	+
1:640	_	+
1:1280	_	+
1:2560	_	_
1:5120	_	_

Key: Agglutination (+), No Agglutination (-)

Time Course Growth of *Escherichia coli* O157:H7 in the Liver, Spleen, and Ileum of immunized rats compared with the unimmunized rats

In the immunized rats, there was an increase in the number of bacterial count in the organs examined at 1 hour, followed by a reduction at the 6th hour, after which the bacterial count started to increase to reach maximum level at 72 hours in all the organs. This was followed by a gradual decrease till 168 hours. However, in the unimmunized rats, after the initial decrease by 6 hours, the bacterial growth reached its peak at 96 hours in the liver and ileum but at 144 hours in the spleen. The results in figures 1, 2, and 3 show the growth of *E. coli* O157:H7 in the liver, spleen, and ileum of the immunized rats as compared with the control rats respectively. The percentage recovery and mean value of $\log_{10}$ bacterial count (cfu/ml) of *E. coli* O157:H7 that was recovered from the immunized rats however was lower than that of the control rats throughout the experiment, but the number of *E. coli* O157:H7 recovered from the passively immunized rats was lower than that of the actively immunized rats.

For instance, the load (expressed as log_{10}cfu/ml $x10^3$) and percentage of *E. coli* O157:H7 that was recovered from the liver (3.99, 1.48%) in fig. 1, spleen (3.63, 1.34%) in fig. 2, ileum (4.47, 1.65%) in fig. 3 of passively immunized rats was lower than that of the liver (4.04, 1.50%) in fig.1, spleen (3.80, 1.41%) in fig 2, ileum (4.47, 1.65%) in fig. 3 of actively immunized rats and that of the liver (4.28, 1.59%) in fig. 1, spleen (4.00, 1.48%) in fig. 2, and ileum (4.48, 1.66%) in fig. 3 of non immunized rats after 30 minutes of infection. However, after 1 hour of infection, the bacterial count rose to 4.31 (1.60%), 4.27 (1.58%), 4.70 (1.74%) in the liver (fig. 1), spleen (fig. 2), and ileum (fig. 3) of passively immunized rats respectively, 4.42 (1.64%), 4.35 (1.61%), 4.70 (1.74%) in the liver (fig. 1), spleen (fig. 2), and ileum (fig. 3) of actively immunized rats respectively, and 4.69 (1.74%), 4.37 (1.62%), 4.73 (1.75%) in the liver (fig. 1), spleen (fig. 2), and ileum (fig. 3) of the control rats respectively. At 6 hours of infection, it reduced to 4.10 (1.52%), 4.03 (1.49%), and 4.67 (1.73%) in the liver, spleen, and ileum of the passively immunized rats respectively, 4.23 (1.57%), 4.03 (1.49%), and 4.67 (1.73%) in the liver, spleen, and ileum of actively immunized rats respectively, and 4.40 (1.63%), 4.04 (1.50%), 4.69 (1.74%) in the liver, spleen, and ileum of the unimmunized rats respectively. This was however followed by an increase in the bacterial growth till it reached maximum level before decreasing till the last day.

At 24 hours of infection, the bacterial count increased significantly ($P \leq 0.05$) in the immunized and unimmunized rats, while there was insignificant difference in the ileum of passively and actively immunized rats ($P \geq 0.05$) as compared to the non immunized rats.

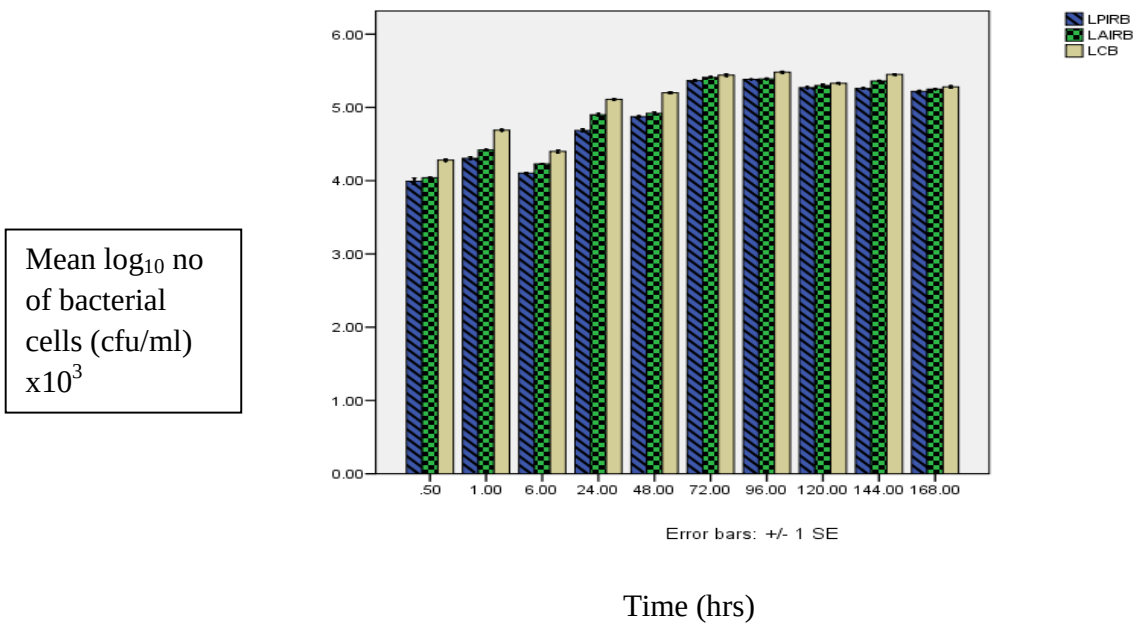

Fig. 1: Time course growth of *E. coli* O157:H7 in the Liver of passively and actively immunized rats after challenge with the infective dose of the organism as compared with the control rats

Keys: LPIRB – Liver of passively immunized rats, LAIRB – Liver of actively immunized rats, LCB – Liver of control rats

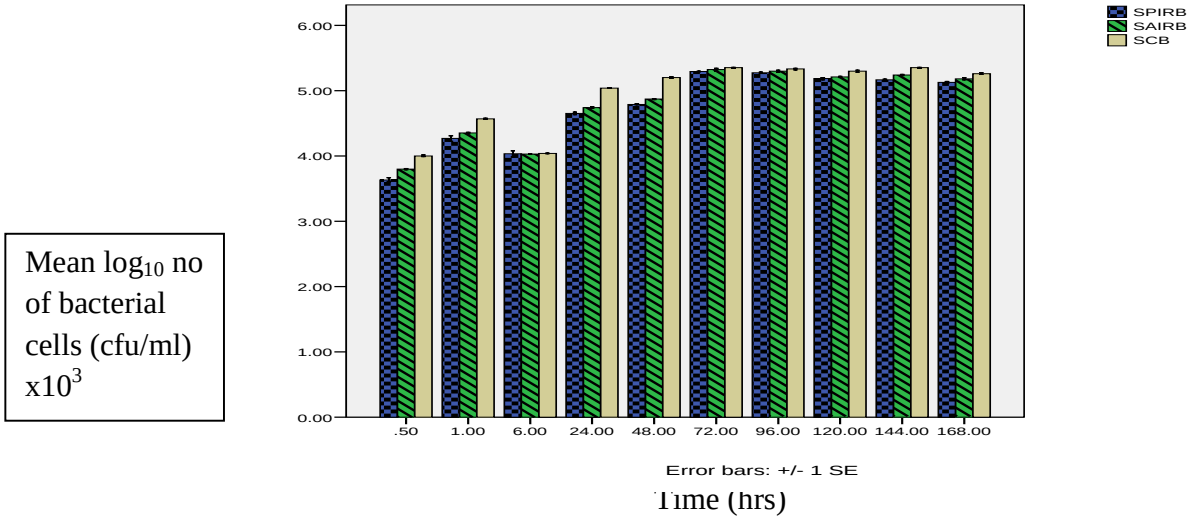

Fig. 2: Time course growth of *E. coli* O157:H7 in the Spleen of passively and actively immunized rats after challenge with the infective dose of the organism as compared with the control rats

Keys: SPIRB – Spleen of passively immunized rats, SAIRB – Spleen of actively immunized rats, SCB – Spleen of control rats

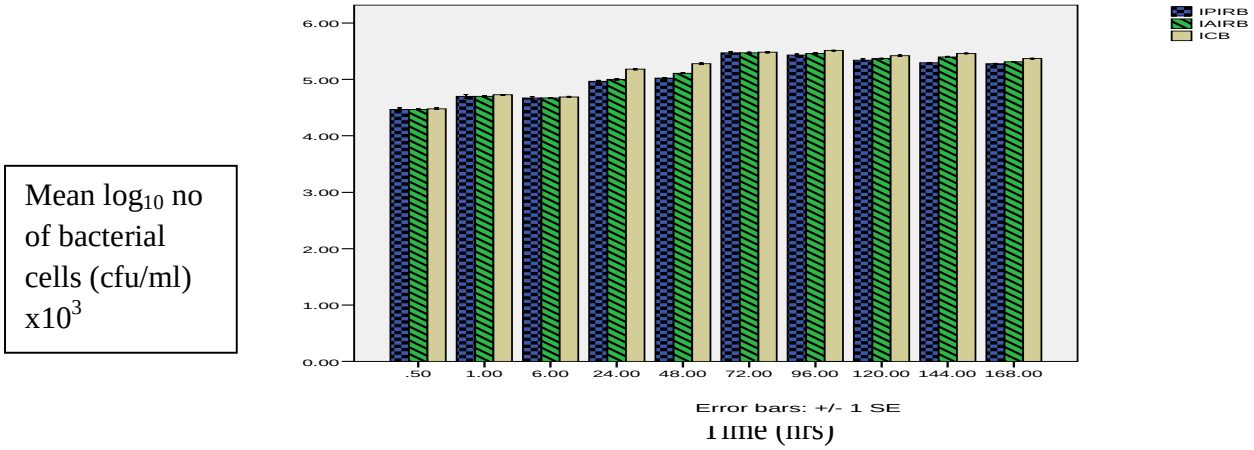

Fig. 3: Time course growth of *E. coli* O157:H7 in the Ileum of passively and actively immunized rats after challenge with the infective dose of the organism as compared with the control rats

Keys: IPIRB – Ileum of passively immunized rats, IAIRB – Ileum of actively immunized rats, ICB – Ileum of control rats

DISCUSSION AND CONCLUSION

The role played by antibodies to *E. coli* O157:H7 in conferring immunity against *E. coli* O157:H7 in albino rats was studied.

The antibody titre of the serum raised against *E. coli* O157:H7 before the booster dose was administered is in line with the findings of Karmali *et al*. (1985), in which shiga-like toxin-neutralizing antibody produced different titres during *Escherichia coli* O157:H7 infection. Reciprocal antibody titre, ranging from 4 to 80 was observed in acute serum specimens collected between days 4 and 18 after the onset of illness and ranged from 32 to 1280 in convalescent serum specimens collected between days 13 and 43.

The higher rate of trapping and killing of the organism in passively immunized rats might be as a result of the presence of preformed antibodies in the system of the rats that was introduced to neutralize the antigens on the organism. This work agrees with the findings of Krystle *et al*. (2010), on a similar organism where it was observed that passively administered toxin-neutralizing antibody provide protection against wild-type *Escherichia coli* O157:H7 strain 86-24NalR. In their work, however mice were used. The mice were administered anti-Stx2 twice by intraperitoneal administration prior to infection.

The low bacterial count seen in actively immunized rats compared to the unimmunized rats on the other hand might be due to the antibodies that were actively raised following vaccination. From the studies of Zhu *et al*. (2008), active immunization with a Stx toxoid by parenteral or oral routes protects animals from the systemic manifestations of STEC disease. In their study immunized animals demonstrated systemic protection in the form of enhanced weight gain and a reduction in the histopathological effects of intoxication and also showed a statistically significant decrease in enteroadherent bacteria on the surface of their cecal tissues when compared to both naïve and adjuvant-alone-treated controls. There was also a decrease in the levels of *Escherichia coli* O157:H7 colonization of mice after infection when compared to mock-vaccinated control. The result of these findings is also in line with the findings of Wen *et al*. (2006), in which active immunization with a Stx toxoid by oral routes protects animals from the systemic manifestations of STEC disease.

The highest rate of trapping and killing of *E. coli* O157:H7 seen in the spleen might be due to the immune cells in the spleen which include B-cells, littoral cells, T-cells, macrophages, dendritic

cells, and natural killer cells, and as such referred to as the immunological conference centre (Robert and Freitas, 2003). The spleen is the largest lymphoid organ of the body and its interaction with blood can stimulate the production and action of macrophages which helps to scavenge spent red blood cells and recycle hemoglobin. It also produces the lymphocytes, which are geared towards individual recognition of foreign substances.

The ileum recorded the highest bacterial count in the control experiment which could be attributed to the fact that this part of the gastrointestinal tract is the principal site of localization of the organism (Treor, 2008). The decrease in the bacterial count at 6 hours might be due to the collaborative activities of the other cells of the immune system such as the macrophages, T-lymphocytes and the Polymorphonucleocytes. The sharp increase at 24 hours might be due to the virulence factors possessed by this organism as those that were able to escape the initial trapping at 6 hours began to proliferate. This is similar to the findings of Hideki *et al*. (1981) in the synergistic contribution of macrophages and antibody to protection against *Salmonella typhimurium* during the early phase of infection; where *Salmonella typhimurium* was trapped in the liver and spleen within 10 to 60 minutes and killed within 6 hours, as surviving organisms began to multiply after 24 hours and reached a maximum at 5 to 7 days.

Conclusively, since passively administered antibodies caused a significant reduction in the number of the *E. coli* O157:H7 recovered from the selected organs, passive immunization thus has a promising effect in conferring immunity against the infection caused by this organism in case of epidemiological outbreak.

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