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Study the impact of *Pseudomonas aeruginosa* DNA on liver.Tissue

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ABSTRACT

Bacterial DNA and dioligonucleotide containing Cytosine-phosphate-Guanosine (CpG) sequences and thereby mimicking prokaryotic DNA, have recently been shown to exert potent immunostimulatory properties. We speculated that bacterial DNA may induce inflammation in liver. In order to determine the role of bacterial DNA in liver, we intraperitonially injected prokaryotic DNA extracted from *Pseudomonas aeruginosa* in rats and performed histopathological study for liver,48 houres after the exposure. The results showed infiltration of inflammatory cells consisted mainly of macrophages(Kupffur cells) which played a central part of inflammatory responses in the liver, sinusoidal dilation, swelling of central vein which contained neutrophils, in addition presensce of apoptotic cells and necrotic cells. Finally,loss the architecture of liver. In summary, our results revealed that bacterial DNA is an important virulence determinant and inflammatory stimulus during liver infection.

Introduction

Cells of the innate immunity express a wide variety of pattern recognition receptors(PRRs) such as Toll-like receptors(TLRs) and mannose receptors that are activated by components of microbial pathogens such as unmethylated CpG containing DNA,RNA and plasmid DNA(1,2). These microbial components are key players in infectious diseases and implicate TLRs in the activation of inflammation and coagulation(3). It had been recognized early that DNA possesses immunostimulatory potential when Tokunaga *et al.* identified that DNA –containing fractions of mycobacteria to mediate immune modulation(4,5). Later, it was shown that is due to the relative abundance of unmethylated cytosine-guanosine(CG) dinucleotides, a particular base sequence context termed (CpG motifs)(6), while vertebrate DNA shows a supperession of (CG) dinucleotide frequency and characterized by an increased rate of C-5methylation of cytosine residues and they are nonstimulatory(7,8). It was reported that when the microbe degrade or autolysed for any reason such as killing by antibiotics or processing by the antigen presenting cells, the DNA will be release, evoke the inflammatogenic reactions causing serious histopathological changes *in situ*(9).

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Bacterial DNA can bind to the TLR9 of cells of innate immune system and activate them(10),leading to secretion of pro-inflammatory cytokines and mediators such as TNF-α, IL -6, IL-12, nitric oxide , B cell proliferation, resistance to apoptosis, natural killer cells(NK) , secretion of IFNγ , increasing lytic activity and monocyte /macrophage secretion of IFNα/β ,chemokines and TNFα. (11,12).

According to the critical role of DNA in pathogenesis,the goal of this study was to elucidate histopathological changes in liver caused by *P. aeruginosa* DNA.

Materials and Methods

Isolation and identification

P. aeruginosa was isolated from 3 years old child suffering from urinary tract infection, streaked on MacConkey agar plates and citramide agar (all media were purchased from Himedia, India), incubated at 37° C for 24 h., thereafter, the grown colonies were identified according to the 2st edition of Bergey's Manual (13).

Bacterial DNA extraction and purification

Pure DNA was extracted from *P. aeruginosa* following the procedure applied by (14):

An overnight tryptic soy broth culture of *P. aeruginosa* was obtained. One ml of this culture was transferred into a micorcentrifuge tube which was spin for 10 seconds. The supernatant was removed carefully and 600 µl of cell lysate solution (tris-EDTA-SDS) were added, gently pipet up and down to resuspend the bacterial pellet and incubated at 80°C for 5 minutes. Thereafter the sample was slowly cooled at room temperature and 3 µl of RNase solution were added, mixed 25 times by inverting the microcentrifuge tube at 37°C for 30 minutes, and cooled to room temperature. Two handred µl of protein precipitation solution (ammonium acetate) were added, vortexed very gently for 20 seconds. The sample was microcentrifuged for 3 minutes at 14000 rpm to pellet the protein, supernatant was poured into a clean tube. Then, six hundred µl of 100 % isopropanol was added, the tube was capped, mixes very gently by inverting the tube at least 50 times, centrifuged at 14000 rpm for 1 minute to pellet the DNA. The supernatant was poured off and the liquid was drain onto an absorbent towel. Six hundred µl of 70 % ethanol was added and the tube was inverted several times, subsequently, the 70% ethanol was decanted and six hundred µl of absolute ethanol was added and the tube was inverted several times, centrifuged at 14000 rpm for 1 minute then the supernatant was poured off very slowly. The DNA pellet was air dried for at least 15 minutes. Then 100 µl of the hydration solution (tris-EDTA) were added in a water bath at 65°C for 1 hour(14).

In vivo study

Animals

Six female white rats (Rattus norvegicus) weighing 295-302g from the inbreed colony of Department of Biology, College of Science, University of Baghdad, each weighing from 27 to 30 g, were used in this study. Animals were housed in plastic cages and fed with a conventional diet.

Inoculation procedure

Animals were divided into two groups; the first one was administrated with 20 µl of 10 µg/ml bacterial DNA as follows:

Each rat was pentobarbiton anesthetized and held inverted with nose up, thereafter, the inoculum was injected intraperitonially, while the other group was administrated with phosphate buffered saline, consequently, it considered as a control group. All animals kept in their cages for 24 hours. After 2 days of injection they were sacrificed, liver was aseptically removed, fixed with 10% formalin for 24 hours at room temperature, and then embedded in paraffin according to standard histological methods.

Results

Histopathology

No histological changes were observed in liver of control rat as shown in figure 1 .

Histopathological examination of liver from rat challenged with 20 µl of 10 µg/ml of *P. aeruginosa* DNA revealed the loss of architecture of liver, sinusoidal dilation and proliferation of kupffur cells as shown in figure 2 .

In addition, histological section of rat liver treated with 20 µl of 10 µg/ml of *P. aeruginosa* DNAelicit swelling of the central vein which contained neutrophils, dilation of sinusoids, proliferation of apoptotic cells and necrotic cells as shown in figure 3 .

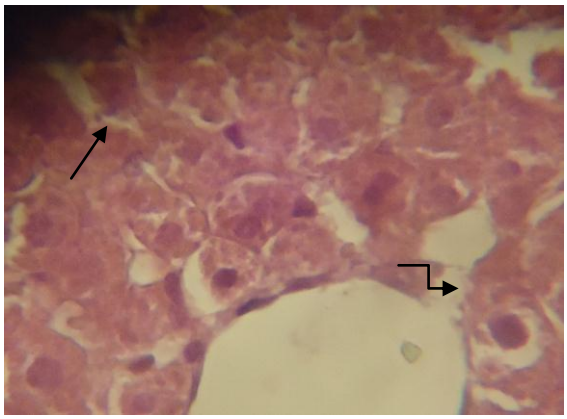

Figure 1: A cross section in liver of control rat 2days after treated with PBS showing normal hepatocytes, hepatic blood vessel , and sinusoids ,, H&E. X400.

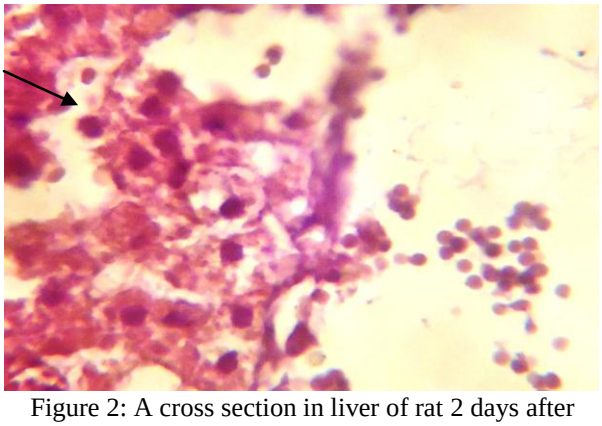

Figure 2: A cross section in liver of rat 2 days after challenged with 20 µl of 10 µg/ml of *P. aeruginosa* DNA showing sinusoidal dilatation, loss architecture of liver and light proliferation of kupffer cell. H&E. X400.

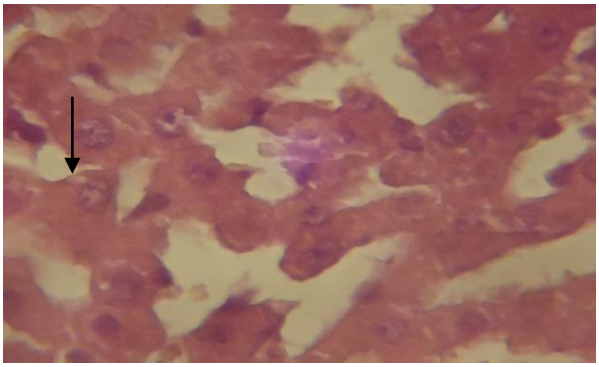

Figure 3:A cross section in liver of rat 2 days after challenged with 20 µl of 10 µg/ml of *P. aeruginosa* DNA showing swelling of the central vein which contained neutrophils , dilation of sinusoids, proliferation of apoptotic cells and necrotic cells.

Discussion

Inflammation is the multi-step process by which the body attempts to remove or contain foreign material or organism from the body or repair any injury sustained by tissue. This process may be acute or chronic, reflecting the time frame over which this takes place(15). The inflammatory effects of DNA may result from the production of cytokines and chemokines(16). Recognition of microbial DNA by TLR9 in mammalian phagocytic cells triggers an immunostimulatory cascade that culminates in the maturation, differentiation, and/or proliferation of multiple cell types. Together, these cells secrete cytokines and chemokines that create proinflammatory immune stimuli and other mediators of inflammation in addition to reactive oxygen species generation(17,18,19). Thus, this cell population and its soluble products are crucial to the observed inflammatory response to DNA(20), and might participate, directly or indirectly, in the pathogenesis of liver infection.

Since, the small fragments of bacreial DNA are ubiquitous contaminants, they are capable of passing through dialyser membranes causing stimulation of immune system cells(20), such cells are kupffur cells.Results of this current work showed the proliferation of kupffur cells which are resident macrophages of the liver and play an important role in its normal physiology and homeostasis as well as participating in the acute and chronic responses of the liver to toxic compounds. Activation of Kupffer cells directly or indirectly by toxic agents results in the release of an array of inflammatory mediators, growth factors, and reactive oxygen species. This activation appears to modulate acute hepatocyte injury as well as chronic liver responses including hepatic cancer. Understanding the role Kupffer cells play in these diverse responses is key to understanding mechanisms of liver injury(21). Swelling of central vein indicate the increasing blood flow(vasodilation) with structural changes that permit plasma proteins to leave circulation (increased vascular permeability) and move into extracellular tissue. These changes are reflected microscopically by sinusoidal dilation packed with erythrocytes, presense of apoptotic and necrotic cells. According to these changes and loss of architecture of liver in the experimental animals, these findings suggest that bacterial DNA may play an important pathogenic role in liver infection,and this may be accompatible to a study applied by Ae-Kyung *et al.* which demonstrated that CpG DNA induces a fulminant liver failure with subsequent shock-mediated death by promoting massive apoptotic death of hepatocytes in D-galactosamine(D-GalN) sensitized mice(22). A recent study has shown that human primary hepatocytes and hepatocyte cell line(HepG2) express TLRs, including TLR4 and TLR9 on kupffur cells and initiate the pathogenic process(23).On the other hand, Luyer *et al.*(24) denoted that the exposure to bacterial DNA strongly aggravates the inflammatory response,disrupts the intestinal barrier,and up-regulates TLR4 expression in the liver following hemorrhagic shock. Investigators have previously shown that some patients with cirrhosis

have bacterial DNA in their serum and ascitic fluid,and that the DNA is always present simultaneously in both body fluids(25). Recently, it has shown that in experimental cirrhosis,systemic activation of the immune system occurs before ascites development and is driven by recirculation of cells activated in hepatic lymph nodes(HLNs), in addition, in compensated cirrhosis, bacterial DNA fragments reach the mesenteric lymph nodes(MLNs), where they elicit a local inflammatory response(26). In issue of urinary tract infection, Al-Mathkhury and Abdul Gaffar had reported that bacterial DNA has the ability to cause damage in renal tissue in a dose dependent mannar, and that bacterial DNA cause more intense damage than candida DNA(27). Anders *et* al. found that *E. coli* DNA increased serum DNA autoantibodies in association with progression of glomerulonephritis(19). Another study showed that *E. coli* DNA caused shrinkage of glomerulus and increased capsular space, edema and inflammatory cells infiltration in kidney tissue while the urinary bladder suffered from infiltration of inflammatory cells(17). Heart may also be affected by bacterial DNA. Pascal *et al.* revealed that bacterial DNA induces myocardial inflammation and reduces cardiomyocyte contractility(28)In case of arthritis ,it had been reported that intra-articular bacterial DNA induces arthritis, since the histological signs of the arthritis were evident within two hours and lasted for at least three weeks,and it was characterized by an influx of monocytic, Mac-1+cells and a scarcity of T-lymphocytes(9). Finally, bacterial DNA may be important in development of inflammatory complications in surgical patients with bacterial infection(24).

Conclusion

Bacterial DNA has the ability to play an important pathogenic role in liver,cause hepatic inflammation and may lead to liver failure.

References

1- Dalpke A, Frank J, Peter M, Heeg K.Activation of Toll-like receptor 9 by DNA from different bacterial species.2006; 74:940-946.

2-Ishii K, Koyama S,Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond:making sence of microbial infection.Cell Host. Microbe.2008; 3:352-363.

3- Amoureux M, Rajapakse N, Stipkovits L, Szathmary S. Peptidoglycanand bacterial DNA Induce Inflammation and Coagulation Markers inSynergy.Mediators Inflam.2005; 2:118-120.

4- Tokunaga T, Yamamoto H, Shimada S, Abe H, Fukada T, Fujisawa Y,Furutani Y, Yano O, Kataoka T, Sudo T, Makiguchi N, Sugamura T.Antitumor activity of deoxyribonucleic acid fraction from Mycobacterium bovis BCG.1 .Isolation,physicochemical characterizationand antitumor activity.J.Natl.Cancer Inst.1984; 72:955-962.

5- Chuang T, Lee J, Kline L, Mathison J, Ulevitch R. Toll-likereceptor 9 mediates CpG-DNA signaling. J. Leukoc. Biol. 2002; 71:538-544.

6- Wagner H.Tool Meets Bacterial CpG DNA.Immunity. 2001; 14:499-502.

7- Krieg A, Yi A, Matson S, Waldschmidt T, Bishop G, Teasdale R,Koretzky G, Klinman D. CpG motifs in bacterial DNA trigger direct B-cells activation.Nature. 1995; 374:546-549.

8- Wagner H.Bacterial CpG DNA activates immune cells to signal infectious danger.Adv. Immunol.1999; 73:329-368.

9- Deng GM, Tarkowski A.Synovial cytokine mRNA expression duringarthritis triggered by CpG motifs of bacterial DNA.Arthritis Res.2001;3:48-53.

10- Wagner H.Interaction between bacterial CpG-DNA and TLR9 bridgeinnate and adaptive immunity.Curr Opin.Microbiol.2002; 5:62-69.

11- Schwart D, Quinn T, Thorne P, Sayeed S, Yi A, Krieg A.CpGmotifs in bacterial DNA cause inflammation in the lower Respiratory tract. J. Clinic. Invest.1997; 100:68-73.

12- Beutler B.Inferences,questions and possibilities in Tool-like receptor signaling.Nature.2004; 257-263.

13- Holt J, Sneath P, Mair N.Bergey's manual of systemic bacteriology.Willins&Wilkins.Baltimore.London.1986; 2-1013-1035.

14- Harley Jp, Prescott ZM.Laboratory exercises in microbiology.5th ed.The McGraw-Hill Companies,New York.2002; 335:173-175,201-203.

15- Johnson A,Ziegler R, Lukasewycz O, Hawley L.Broard review series Microbiology and immunology.4th ed.Lippincott Williams&Wilkins.2002.

16- Cazzavillan S, Ratanarat R, Segala C, Corradi V, Cal M, Cruz D. Inflammation and Subclinical Infection in Chronic Kidney Disease:A Molecular Approach. Blood Purif 2007; 25: 69-76.

17- Al-Mathkhury HJF,Al-Zubeidy SQ.Bacterial DNA induces inflammations in murine renal system. Proceeding of 3rd scientific ofthe college of science, university of Baghdad, 24-26 March. Baghdad- Iraq; 2009.

18-Takeshita A, Imai K, Hanazawa S. CpG Motifs in Porphyromonas gingivalis DNA Stimulate Interleukin-6 Expression in Human Gingival Fibroblasts. Infect Immun 1999; 67: 4340-4345.

19- Anders H, Banas B, Schlondorff D. Signaling Danger: Toll-Like Receptors and their Potential Roles in Kidney Disease. J Am Soc Nephrol 2004; 15: 854-867.

20- Mölne L, Collins LV, Tarkowski A. Inflammatogenic properties of bacterial DNA following cutaneous exposure.J Invest Dermatol. 2003;121:294-299.

21- Ruth AB, Patricia EG, Cynthia JU,Lisa MK, Ivan R,James EK. Role of kupffur cell in Mediating Hepatic Toxicity and Carcinogenesis. Toxicol. Sci.2007; 96(1):2-15.

22- Ae-Kyung Y, Hyunsook Y, Jeoung EP, Beam SK, Hae JK, Antonio MH.CpG DNA –mediated induction of acute liver injury in D-Galactosamine-sensitized mice.The American Society forBiochemistry and Molecular Biology, Inc.2006.

23- Miura K, Kodama Y, Inokuchi S et al..Toll-like receptor 9 promotessteatohepatitis in mice. Gastroenterology.2010; 139:323-334.

24- Luyer MD, Buurman WA, Hadfoune M, Wolf T, Van't Veer C, Jacobs JA, Dejong CH, Greve JW. Exposure to bacterial DNA before hemorrhagic shock strongly aggravates systemic inflammation and gut barrier loss via an IFN-gamma-dependent route.Ann Surg.2007;245(5):795-802.

25- Such J, Francés R, Muñoz C, Zapater P, Casellas J, Cifuentes A, Rodriguez-Valera F, Pascual S, Sola-Vera J, Carnicer F, Uceda F, Palazón M, Pérez-Mateo M.Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative,nonneutrocytic ascites.Hepatol.2002;36:135-141.

26- Ubeda M, Muñoz L, Borrero MJ, Diaz D, Francés R, Monserrat j, Lario M, Lledó L, Such J, Álvarez-Mon M, Albillos A. Critical role of the liver in the induction of sysremic inflammation in rats with

preascitic cirrhosis.Hepatology.2010;52:2086-2095.

27- Al-Mathkhury HF, Abdul-Ghaffar SN. Urinary tract infections causedby staphylococcus aureus DNA in comparison to the candida albicans DNA. North Am J Med Sci 2011;3:565-9.

28- Pascal J, Markus S, Markus V, Peter K, Heidi E, Myriam R, Olafboehml Klaus F, Ulrike D, Christian G, Andereas H, Georg B, Alexander K, Kai Z, Rainer M. Bacterial DNA induces myocardial inflammation and reduces cardiomyocyte contractility. Cardiovasc. 2008;78 (1):26-35.

دراسة تأثير الحامض النووي الرايبوزي منقوص الأوكسجين لبكتريا *Pseudomonas aeruginosa* على نسيج الكبد.

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الخلاصة

يحتوي الحامض النووي منقوص الاوكسجين البكتيري على تتابعات سايتوسين–كوانيدين غير المثيلية وقد اثبت مؤخرا ان لهذه التتابعات تأثيرات تحفيزية في الخلايا المناعية، لذا من المتوقع أن يكون للدنا البكتيري دور إلتهابي في الكبد، وللتحري عن هذا الدور تم حقن الحامض النووي منقوص الاوكسجين البكتيري المعزول من بكتريا الزوائف الزنجارية داخل غشاء البريتون لإناث الأرانب ثم اجريت دراسة إمراضية نسيجية للكبد بعد 48 ساعة من الحقن.أظهرت النتائج إرتشاح الخلايا الإلتهابية في الكبد وخصوصا خلايا كبفر والتي تلعب دورا مهما في الإستجابة الإلتهابية، مع حدوث توسع في الجيوب والوريد المركزي وإرتشاح خلايا الدم العدلة داخله، بالإضافة إلى وجود الخلايا المنتحرة والخلايا المتنخرة، وأخيرا فقدان شكل الكبد. تشير هذه النتائج ان للحامض النووي منقوص الاوكسجين البكتيري دورا إمراضيا مهما ومحفزا للعملية الإلتهابية في الكبد.