Upon its release due to cell lysis, bacterial DNA has the ability to stimulate the immune system causing serious damage in tissue. Intraperitoneally injection of P. aeruginosa DNA in rats caused several histopathological changes in the splenic tissue included; a marked germinal center hyperplasia in the white pulp which results in the increase of follicles number within germinal center. Furthermore, apoptotic debris engulfed by macrophages in the marginal zone was noticed. Hemosiderin and degenerated cells were observed as well. In a conclusion, even though the bacterial cells are dead, their remains, especially DNA can cause damage to tissue.
INTRODUCTION

The two major functional zones of the spleen are the hematogenous red pulp and lymphoid white pulp. The white pulp located around a central arteriole, is composed of the periarteriolar lymphoid sheath (PALS, T-cell area) and marginal zone (B-cell area). The marginal zone contains specific population of macrophages (Devico et al., 2008). Typical cellular changes that can be observed after exposure to an immunomodulatory agent are alterations in the size and density of the PALS and / or margin zone and changes in the number of follicles with germinal centers (Germolec et al., 2004).

Pseudomonas aeruginosa is an opportunistic pathogen. Virulence of this bacterium is very low since it rarely causes diseases as a primary pathogen and the resultant infection progress is very slow. It is commonly causing septicemia, chronic infections (in cystic fibrosis patients) and urinary tract infections in catheterized patients (Kolter, 2010).

Bacterial DNA fragments have the ability to bind to Toll-like receptors and stimulate immune cells. They induce natural killer cell activity and proinflammatory cytokines release from mononuclear cells (Bossola et al., 2009) and effector molecules such as nitric oxide (Francés et al., 2004). Furthermore, bacterial DNA leads to septic shock and death in sensitive mice (Amoureux et al., 2005). DNA of periodontal pathogens, Porphyromonas gingivalis and Tannerella forsythia, stimulate cytokine production in human monocytic cells through Toll like receptor 9 (TLR-9) and nuclear factor kappa B signaling (Sahingur et al., 2011).

Anders et al. (2003) hypothesized that CpG-DNA would aggravate a preexisting immune complex glomerulonephritis and the glomerular damage characterized by obvious macrophage infiltration. Such finding also noted by others in various disease models; lung (Schwartz et al., 1997), central nervous system (Deng et al., 2001), genital tract (Galichan et al., 2001), heart (Knuefmann et al., 2008) and bowel (Gutiérrez et al., 2009).

Some studies suggest that the spleen produces inflammatory mediators and other splenic substances that could collaborate in the bacterial persistence in the host and therefore they could act in the disease pathogenesis (Harrus et al., 1998). Moreover, Rabinstein et al. (2000) pointed out that splenectomized human beings develop a more severe type of ehrlichiosis than non-splenectomized spouses do. Such severity could be attributed to a reduction in the antigen’s clearance, a decrease in responses against new antigens, and a decrease in phagocytosis and in other protective responses (Davidson and Wall, 2001).

The aim of this study was to investigate the effect of a single dose of P. aeruginosa DNA challenge on spleen of rats.

MATERIALS AND METHODS

Isolation and identification

P. aeruginosa was isolated from sputum of 3 years old child suffering from cystic fibrosis, 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 2nd edition of Bergey's Manual (Bernere and Farmer, 2005). Biochemical tests were carried out according to Forbes et al. (2007). API-20E system was employed to confirm the identification.

Bacterial DNA extraction and purification

Genomic DNA was extracted and purified from the P. aeruginosa isolate using Wizard genomic DNA purification kit (Promega, USA) and according to the protocol stated by the kit manufacturer. The concentration of the purified DNA was determined by measuring the absorbance on OD260 using a UV-Visible spectrophotometer (Cary, Australia). Whereas the DNA purity was determined using the OD260/280 ratio; which ranged from 1.81 to 1.93.

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 were used in this study. Animals were housed in plastic cages and fed ad libitum 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 intraperitoneally. 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.

Histological preparations

After 2 days of injection they were sacrificed, spleen was aseptically removed, fixed with 10% formalin and dehydrated through graded alcohol series (5 – 100%) for 24 hours at room temperature, cleared in xylene and then embedded in paraffin. Sections of 5 µm thickness were made and stained with haematoxylin and eosin (Bancroft and Gamble, 2002)

RESULTS
Injecting rats with phosphate buffered saline (control group) did not affect their splenic tissue as they appear in normal texture (figure 1).

Rats received intraperitoneally injection with *P. aeruginosa* DNA showed several alterations in the splenic tissue included; a marked germinal center hyperplasia (periarteriolar lymphoid sheath; PALS and T- lymphocytes area) in the white pulp which results in the increase of follicles number within germinal center (figures 2 and 3). Therefore, white pulps are starting to fuse together (figure 4). Furthermore, figure 2 depicts apoptotic debris engulfed by macrophages in the marginal zone (B-cells area). Furthermore, hemosiderin and degenerated cells were observed as well as it shown in figure 5.

**Figure 1:** Normal murine spleen structure which consists of white pulp (W) and red pulp (R). 100X. H&E.

**Figure 2:** A cross section in spleen of rat treated with *P. aeruginosa* DNA showing marginal zone (white arrow), germinal center hyperplasia (G), macrophages with cytoplasmic engulfed apoptotic debris (dotted arrows) and apoptotic cells (black arrows). H&E. X400.
Figure 3: A cross section in spleen of rat treated with *P. aeruginosa* DNA showing red pulp (R) and germinal center hyperplasia (white arrow). H&E. X400.

Figure 4: A cross section in spleen of rat treated with *P. aeruginosa* DNA germinal center hyperplasia (white arrow) and white pulps fused together (black arrow). H&E. X400.
DISCUSSION

Spleen is the largest secondary lymphoid organ (Cesta, 2006). Owing to the presence of B and T lymphocytes, the inflammatory effects of microbial antigens or their extracellular products on these cell populations may be reflected in the spleen. Consequently, histopathological examination of spleen is highly recommended to evaluate the immune system (Elmore, 2006). An acute immune response to antigens may result in an increased cellularity in the B-cells areas and an increase in secondary follicles with prominent germinal centers (Suttie, 2006).

Recently we have shown that bacterial DNA can cause tissue damage in skin (Al-Mathkhury and Al-Shaybani, 2011) and renal system of mice (Al-Mathkhury and Al-Zubeidy, 2009; Al-Mathkhury and Abdul-Ghafar, 2011). Furthermore, we have demonstrated that high GC content DNA of E. coli and Low GC content DNA of S. aureus succeeded in stimulating the immune system of rats to produce anti DNA antibodies, IL-6 and IL-12. However, response to E. coli DNA was markedly higher (P<0.05) than that to S. aureus DNA. What's more, time had a significant effect (P<0.05) on the levels of anti DNA antibodies, IL-6 and IL-12 (Al-Mathkhury et al. (2012).

Knuefermann et al. (2007) suggested that bacterial CpG-ODN causes spleen inflammation via TLR9. Itagaki et al. (2011) stated that bacterial DNA increases neutrophils adherence capacity to endothelial cell (EC) alongside with upregulated adhesion molecules in both cell types. These results strongly support the conclusion that bacterial DNA can initiate spleen injury by stimulating neutrophils-EC adhesive interactions predisposing to endothelial permeability. Bacterial DNA stimulation of TLR-9 appears to promote enhanced gene expression of adhesion molecules in both cell types. This leads to PMN-EC cross-talk, which is required for injury to occur. On the other hand, Mizgerd et al (2004) demonstrated that bacterial growth and metabolism were not responsible for the inflammatory cells recruitment.

Evidence from Olishovsky (2005) study indicates that hyperplasia of spleen in bacterial CpG DNA-injected mice is due to strong proliferative response.

Taking together, bacterial DNA is capable of damaging tissue as much as living bacterial cells. These data approve that bacterial DNA is a strong bacterial antigen with highly immunotoxic activity.

REFERENCES

Al-Mathkhury, H. Al-Zubeidy S. 2009. Bacterial DNA induces inflammations in murine renal system. In the Proceeding of third scientific conference of the college of science, University of Baghdad: 24-26 March 2009; Baghdad-Iraq, pp. 976-980.
Al-Mathkhury, H. and Abdul-Ghaffar, S. 2011. Urinary tract infections caused by Staphylococcus aureus DNA in comparison to the Candida albicans DNA. North Am. J. Med. Dis., 3: 562-566.
Al-Mathkhury, H. and Al-Shaybani, M. Murine skin damage caused by Staphylococcus aureus DNA and Propionibacterium acnes DNA. Int. J. Sci. Sci. in press
Al-Mathkhury, H., Utba, N. and Al-Alusi, N. 2012. Low GC and High GC bacterial DNA impact on anti DNA antibodies, IL-6 and IL-12 level in rats. Int. J. Sci. Technol., 7: 90-94.
Amourex, M., Rajapakse, N., Stipkovits, L., Szathmary, S. 2005. Peptidoglycan and Bacterial DNA Induce Inflammation and Coagulation Markers in Synergy. Med. Inflam., 2: 118–120.
Anders, H., Banas, H., Linde, Y., Wellr, L., Cohen, C., Kretzler, S., Vielhauer, V., Schindorff, D., Grone, H. 2003. Bacterial CpG-DNA Aggravates Immune Complex Glomerulonephritis: Role of TLR9-
Mediated Expression of Chemokines and Chemokine Receptors. J. Am. Soc. Nephrol., 14: 317–326.

Bancroft, J. and Gamble, M. 2002. Theory and practice of histological technique. 5th ed. Churchill, Livingston, New York.

Berenre, D. and Farmer, J. 2005. Family Enterobacteriaceae. In: Garrity et al (eds) Bergey’s manual of systematic bacteriology, 2nd ed. Vol. Two: The Proteobacteria Part B: The Gammaproteobacteria, Springer, USA, pp. 587–607.

Bossola, M., Sanguinetti, M., Scribano, D., Zuppi, C., Bancroft, J. and Gamble, M. 2002. Theory and practice of histological technique. 5th ed. Churchill, Livingston, New York.

Harrus, S., Waner, T., Keysary, A., Aroch, I., Voet, H. and Bark, H. 1998. Investigation of splenic functions in canine monocytic ehrlichiosis. Vet. Immunol. Immunopathol., 62: 15-27.

Itagaki, K., Adinına, Y., Sun, S., Zhao, C., Sursal, T., Chen, Y., Junger, W. and Hauser, C. 2011. Bacterial DNA induces pulmonary damage via TLR-9 through cross-talk with neutrophils. Shock, 36: 548-552.

Knuefermann, P., Baumgarten, G., Koch, A., Schwederski, M., Velten, M., Ehrentraut, H., Mersmann, J., Meyer, R., Hoeft, A., Zacharowski, K. and Grohé, C. 2007. CpG oligonucleotide activates Toll-like receptor 9 and causes spleen inflammation in vivo. Respir. Res., 8: 72-80.

Knuefermann, P., Schwederski, M., Velten, M., Krings, P., Ehrentraut, H., Rudiger, M., Boehm, O., Fink, K., Dreiner, U., Grohe, C., Hoeft, A., Baumgarten, G., Koch, A., Zacharowski, K., Meyer, R. 2008. Bacterial DNA induces myocardial inflammation and reduces cardiomyocyte contractility: role of Toll-like receptor 9. Cardiovasc. Res., 78: 26–35.

Kolter, R. 2010. Biofilms in lab and nature: a molecular geneticist’s voyage to microbial ecology. Int. Microbiol., 13:1-7.

Mizgerd, J., Lupa, M. and Speik, M. 2004. NF-κB p50 facilitates neutrophil accumulation during LPS-induced pulmonary inflammation. BMC Immunol. 5: 10-20.

Olishevsky, S., Shlyakhovenko, V., Kozak, V. and Yanish Y. 2005. Response of different of different organs of immune system of mice upon administration bacterial CpG DNA. Exp. Oncol., 27: 290-297.

Rabinstein, A., Tikhomirov, V., Kaluta, A., Gelfman, N., Iannini, P. and Edwards, L. 2000. Recurrent and prolonged fever in asplenic patients with human granulocytic ehrlichiosis. Q. J. Med., 93: 198-201.

Sahingur, S., Xia, X., Schifferle, R. 2011. Oral Bacterial DNA Differ in Their Ability to Induce Inflammatory Responses in Human Monocytic Cell Lines. J. Periodontol., 13: doi:10.1902/jop.2011.110522.

Schwartz, D., Quinn, T., Thorne, P., Sayeed, S., Yi, A. and Krieg, A. 1997. CpG Motifs in Bacterial DNA Cause Inflammation in the Lower Respiratory Tract. J. Clin. Inv., 100: 68–73.

Suttee, A. 2006. Histopathology of spleen. Toxicol. Pathol. 34: 466-503.