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The Differences in Inflammation Changes between Lymphoid and Non Lymphoid Tissues of Mice after i.p. *E.Coli* Injection and its Effect on Renal Function

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Abstract

Splenic microenvironments are suitable for the definition of immunologic processes, which may different than other non lymphoid organs. Splenic changes have been identified after Leishmania and viral infections, but after bacterial need more study. In kidney, as a non lymphoid organ, the bacterial infection associated with acute kidney injury. Acute kidney injury can lead to chronic kidney disease which can lead to kidney failure. The present work amid to study the differences between bacterial infection stages in lymphoid organ, spleen, and nonlymphoid organ, kidney; and the effect of bacterial infection stage on the renal function. 20 males Balb/c mice were used in this study, aged about 2 months and weight about 24g. 15 mice were injected intraperitoneal with 200µl *E.coli*, isolated from children have urinary infection, in final concentration (10^8cell /ml), and 5 mice were injected with only normal saline as control group. The mice were killed in different time after *E.coli* injection: 24, 48, and 72h. Blood was collected and used in serum creatinine and blood urea determinations. Spleen and kidney were collected, fixed in 10% formalin solution, and processed by standard procedures. The body weights were recorded before sacrifice. The results showed that: significantly higher body weight in infected groups within 24, 48, and 72h compared to zero time and control group, significantly negative correlations between body weight and infection duration, white pulp widening, back off red pulp

size, and activation of germinal center with the time after bacterial injection, infiltration of inflammatory cells and appearing of the focal necrosis of renal epithelial tubules within 48h after bacterial infection and increased after 72h, significantly higher serum creatinine (S.Cr) and blood urea (B.Urea) in the infected groups within 48 and 72h after bacterial injection compared to control group, and significantly positive correlations between S.Cr and B.Urea levels in infected groups and all groups together but not in the control group.

Keywords: Inflammation Changes; Lymphoid and Non Lymphoid Tissues' Organs of Mice, i.p. E.Coli Injection

Introduction

The spleen is the largest lymphatic organ just like a large lymph node. It is subdivided into lobules. The spaces within these lobules are filled with blood instead of lymph. Within the lobules, two types of tissues were found, white and red pulp. White pulp consists of lymphatic tissue, mostly lymphocytes. It appears as circular whitish gray areas. It composed of splenic nodules, and containing the branch of splenic artery. It surrounded by red pulp, which consist of venous sinuses and contains many red blood cells along with numerous lymphocytes and macrophages. The spleen has both morphologic and immunologic filtering function [1].

Splenic cellular diversity and compartmentalized microenvironments are suitable for the definition of immunologic processes, which may influence the outcome of infections more than other non lymphoid organs [2, 3]. It has been identified the changes in the microenvironment of the spleen after experimental infection of mice by a variety of pathogens, including Leishmania [4] and viral [5] infections.
Secondary lymphoid organs tissues, such as spleen, facilitate the induction of adaptive immune response. These devices pick up germs to limit the spread throughout the body, and bring the antigen-presenting cells into a productive contact with specific lymphocytes, and provide outlets for the differentiation of immune effectors cells. Therefore, the microscopic anatomy of the spleen determines the ability of the organism to respond to pathogens. Its microarchitecture is, at the same time, adaptable to very environmental changes [6].

Kidney, one of nonlymphoid organs, consists of an outer cortex and an inner medulla. It contains of many functional units called nephrons which divide to: **Renal corpuscle**, the site of blood filtration located in the cortex; **Proximal tubule** , located in the cortex and part enters the medulla; **Loop of Henle**, in the medulla; **Distal tubule** , consisting of a thick straight part ascending from the loop of Henle back into the cortex; **and Connecting tubule**, linking the nephron to collecting ducts [7].

Kidney has a pivotal role in a number of physiological functions including blood pressure, salt and water balance, acid-base and calcium balance, and blood cell production. Therefore, it is not surprising that kidney failure can result from, or cause, and a variety of diseases [8].
Bacterial infection associated with acute kidney injury. Events that promote kidney disease can be quite different. However, acute kidney injury can lead to chronic kidney disease. Both, if unchecked, can lead to kidney disease at the end of the stage. More importantly, inflammation and stimulate the immune system represents a common core feature of both acute kidney injury and chronic kidney disease [9].

Biochemical markers of renal function, such as creatinine and urea, play an important role in accurate diagnosis [10]. Creatinine is a breakdown product of creatine phosphate in the muscles, usually are produced at a constant rate depending on the muscle mass [11]. Urea is the major end product of protein, amino acids demolition. The liver produces it and distributes it throughout the intracellular and extracellular fluid. Urea, in the kidneys, are filtered out the blood by glomerulli [12]. When the serum creatinine [13] and urea [14] is greater than the normal interval, renal failure is suspected.
The present work amid to study 1) the differences between bacterial infection stages in lymphoid organ, spleen, and nonlymphoid organ, kidney. 2) the effect of bacterial infection stage on the renal function.

Materials and methods

E.coli was isolated from children, which have urinary infection. It was isolated and diagnosis in the medicine city/ Baghdad.
20 males Balb/c mice were used in this study, from preventive research center, Baghdad, Iraq. The mice aged about 2 months and weight about 24g. 15 mice were injected intraperitoneal with 200µl *E.coli* in final concentration (10^8cell /ml), and 5 mice were injected with only normal saline as control group.
The mice were killed by cervical distraction in different time after *E.coli* injection: 24, 48, and 72h. Blood was collected from the eye in dry sterile test tube to use in determination of serum creatinine and blood urea by the creatinine and urea kit from Bio systems. Spleen and kidney were collected, fixed in 10% formalin solution, and processed by standard procedures. Sections of paraffin-embedded tissues were stained with heamatoxyline and eosin and examined by light microscopy [15]. All the experiments were done in Biology department, Science collage, Al-Mustansiriyiah University.

Results are expressed as mean ± standard error (M±SE. Data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's test for multiple comparisons, using Statview version 5.0. Differences were considered significant when $p<0.05$. Regression analysis was performed by analysis of covariance (ANCOVA) also using Stat view version 5.0.

Results

The body weights were recorded before sacrifice. The results of this determination are showing in Table-1. The body weight was significantly ($P<0.05$) lower in infected groups within 24, 48, and 72h compared to zero time and control group. The lowest body weight was within 72h. Significantly negative correlations ($P<0.05$) between body weight and infection duration were detected ($R^2= 0.619$) (figure-1).

Table-1: body weight in the mice (M ± SE)

Infection duration (h)	Body weight (g)
Zero	23.2 ± 0.374
24	22.2 ± 0.362
48	20.8 ± 0.376*#
72	20.6 ± 0.400*#

* Significantly difference in infected groups vs. zero. # significantly difference in infected group within 24h after bacterial injection vs. other infected groups.

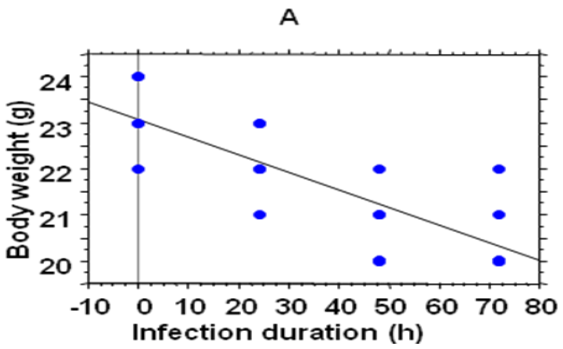

Figure-1: the correlation between Body weight and Infection duration

Figure-2A showed the normal structure of control mice spleens which consist of normal white and red pulps and sinusoid. Within 24h after bacterial injection, the white pulps begin to widen (figure-2B). This widening of white pulp was more within 48h after bacterial injection, but red pulp size started to back off (figure-2C). Within 27h after bacterial injection, the white pulp covered all the space, the red pulp was no more presence, and the germinal center was seems more active (figure-2D).

Figure-3A showed the normal structure of control mice kidney which consist of normal glomeruli, proximal and distal convoluted tubules. These structures looked like normal within 24h after bacterial injection (figure-3B), while within 48h after bacterial injection the inflammatory cells were began to infiltrate and the focal

necrosis of renal epithelial tubules were began to appear (figure-3C). These changes were increased within 72h after bacterial injection (figure-3D).

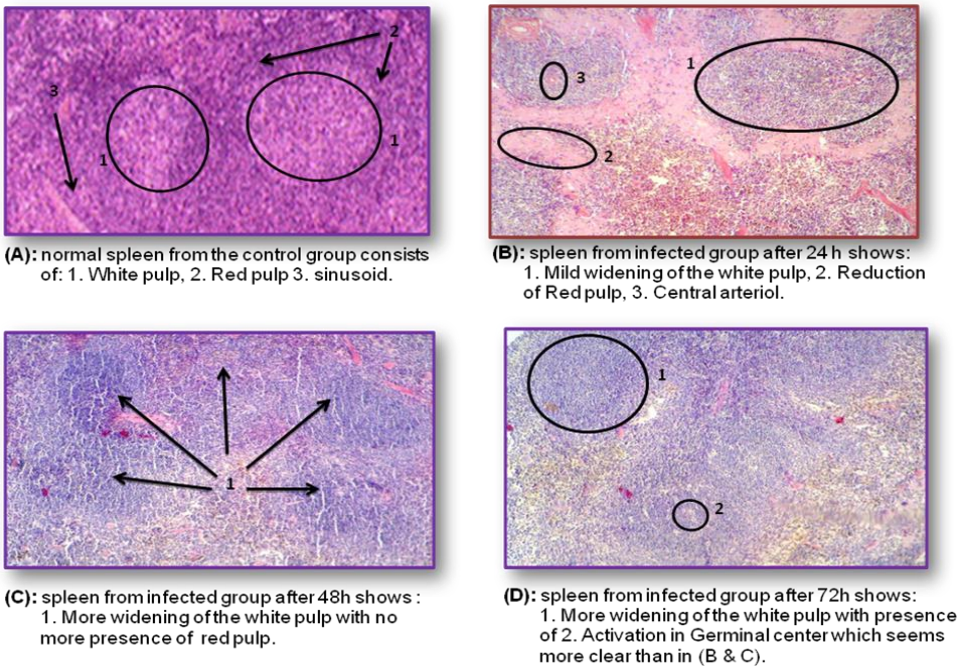

Figure-2: the changes in the spleenic structures in infected groups; after 24h **(B)**, 48h **(C)**, and 72h **(D)**; compared to normal spleen **(A).** (H&E) 400x.

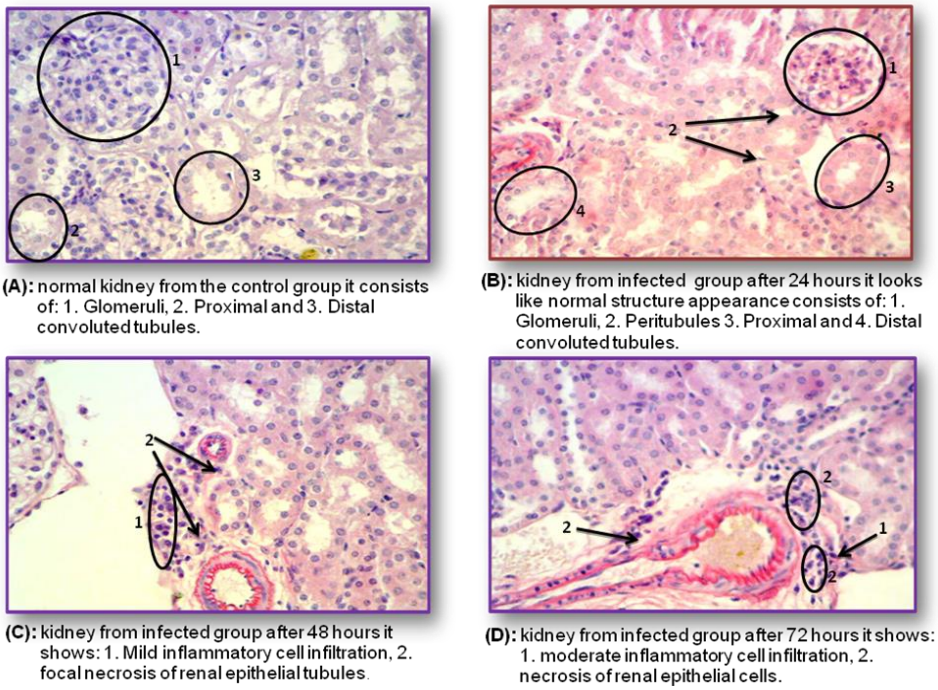

Figure-3: the changes in the kidney's structures in infected groups; after 24h **(B)**, 48h **(C)**, and 72h **(D)**; compared to normal kidney **(A).** (H&E) 400x.

Physiologically, the serum creatinine (S.Cr) and blood urea (B.Urea) were determinate in the control and infected groups. While no significantly differences were detected between S.cr and B.Urea levels in the control and infected groups within 24h after bacterial injection, the levels of S.Cr and B.Urea were significantly higher ($P<0.05$) in the infected groups within 48 and 72h after bacterial injection compared to its levels in the control group. S.Cr and B.Urea levels were more tendencies higher in the infected groups within 48h compared to 72h after bacterial injected (figure-4).

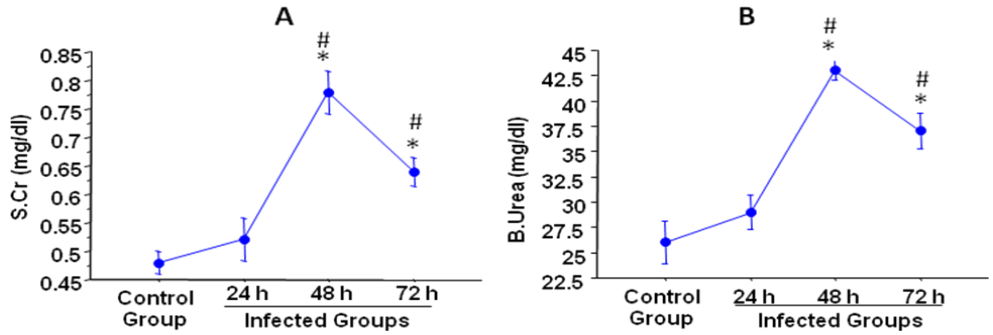

Figure-4: S.Cr and B.Urea levels in control and infected groups (within 24. 48, and 72h). (A) S.Cr and (B) B.Urea. * Significantly difference in infected groups vs. control. # significantly difference in infected group within 48h after bacterial injection vs. other infected groups.

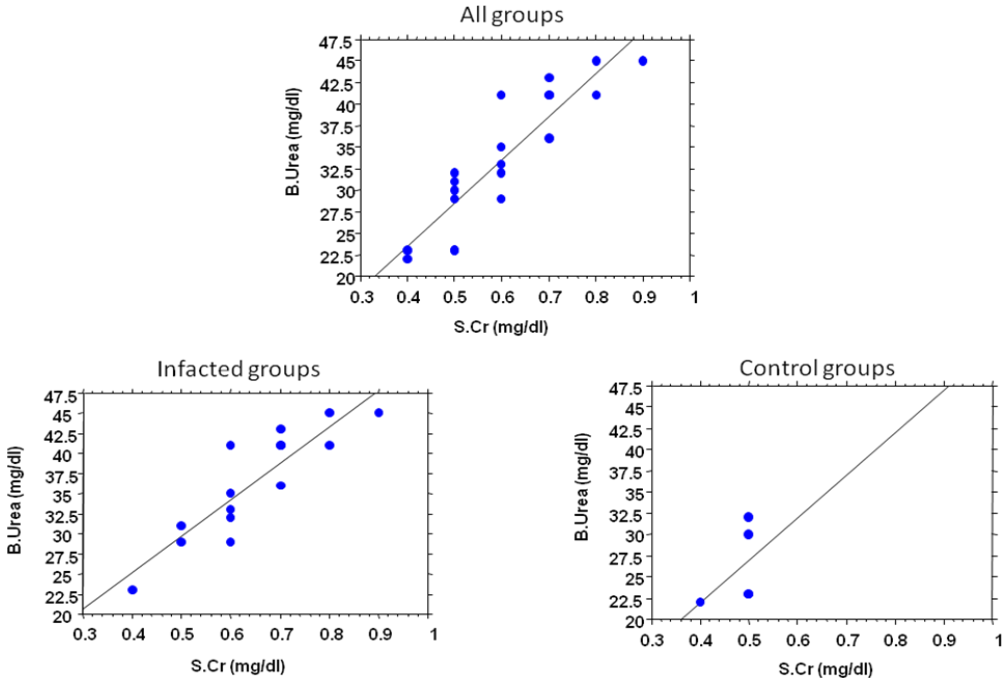

Figure-5: the correlation between S.Cr and B.Urea in the infected, control, and all groups together.

Significantly positive correlations ($P<0.05$) between S.Cr and B.Urea levels were detected in infected groups and all groups together ($R^2= 0.779$ and 0.798, respectively) but not in the control group ($R^2= 0.233$) (figure-5).

Discussion

E. coli infection causes prolonged pro-inflammatory response in adult rodents' brains [16]. The cytokines of pro-inflammatory can change many behaviors as the sickness [17], including fever, reduce the intake of food/water [17, 18], which causes body weight losing [19]. In our study, infected mice lose their body weight in positive correlation with the infection duration.

After bacterial infection, spleen becomes an evident site of the interaction between the immune system and the bacteria, because all the participants in the immune response against the bacteria are present in large quantities. These include antigens (live or wreckage bacteria), cells exhibiting the antigen, and lymphocytes which able to respond to these antigens. It has been identified changes in the microenvironment of the spleen after experimental infection of mice by a variety of pathogens, including Leishmania and virus infections [4, 5].
Santana, 2008, found higher extensive structural disorganization of the white pulp areas, large follicles, and large germinal centers in the infected dogs by Leishmania [20]. These results by Leishmania infection similar the results founded in this work by bacterial infection and these changes were increase with stage of infection.
The white pulp contains mostly lymphocytes (T and B cells), while red pulp is made up mainly red blood cells. Within white pulp, the periarteriolar lymphatic sheath (PALS) is formed by lymphocytes aggregated around the central artery. The main cell type in the PLAS is T cells which surround B cells [1]. So the evident site in the spleen after bacterial infection is the white pulp, because it has the entire participant in the immune response. Bacterial infection activates the antigen-specific T and B lymphocytes which begin to divide rapidly, producing numerous nonspecific daughter cells [21] . The germinal center serves to produce memory B cells and select for high-affinity antigen-specific B cells clones [22]. These evidences may be explaining the enlargement in the white pulp against red pulp and large size, and activation of germinal center which had big cells ready to split in our results.
In our results, the immune response of bacterial infection in the kidney was later than it in the spleen. In the spleen the histological changes appeared within 24h after bacterial infection, while it appeared within 48h in the kidney. Mice in our study infected by ip bacterial injection, these bacteria were absorbed by lymph and blood, reached lymph nodes and spleen to filter the blood and lymph before reach any other organs [23]. On the other hand, in the spleen present all the participants in the immune response against the bacteria [5], but the kidney need supports from blood by inflammatory cells infiltrations to begin the adaptive immune response after initial response which need a time [24].

Neutrophils and monocytes were attracted from the bloodstream by chemokines which releases form macrophages. This causes dilation and increases blood vessels permeability to increase leukocytes migration. In the initial phases, neutrophils presents into infected tissue then followed by monocytes after short a time [24]. This can explain the different in the inflammatory cell infiltration rat in our study depend on the duration of infection.

Recent evidences showed that the variety of chemokines and cytokines regulate up by epithelial and endothelial cells activation in early initial response [25, 26]. But there is no evidence explains the role of this cellular activation in the renal perfusion. On the other hand, the dilation phase due to not only inflammatory response but also initial ischemic [27]. These events decrease blood flow and ramify in the epithelial cells which recently covered [28, 29]. Due to these events, endothelial cells damage and undergo injury and death which cause the necrosis, which is very clear in our results.

Necrosis are followed three phase; initiation due to reduce glomerular filtration rate, which caused by hypoperfusion and epithelium damage, and raise S.Cr and B.Urea levels; maintenance results to severe reduce in golmerular filtration rate and continue increase S.Cr and B.Urea; and recovery, that the tubular function is retired which causes an increase in the volume of urine and a gradual reduce in S.Cr and B.Urea [27]. During maintenance, the dysfunction of nephron is continued and the glomerular filtration increase, which lead to constriction of afferent arterioles. Then to maintain cellular, cells undergo repair and cellular function slowly improving by return the blood flow. These changes continue during recovery phase until renal function slowly returns [30]. In this study, the higher level of S.Cr and B.Urea in infected mice was within 48h compared to within 24 and 72h. While the differences between S.Cr and B.Urea in infected mice within 24 and 48h were significant, they were within 48 and 72h no significant but more tendencies.

S.Cr and B.Urea are biochemical markers of renal function, which play an important role in accurate diagnosis [10]. in the kidneys, both of them are filtered out the blood by glomerulli [12]. When the serum creatinine [13] and urea [14] is greater than the normal interval, renal disease is suspected. These evidences explain the significantly positive correlation between them in this study except in the control group, in which the positive correlation was not significant due to the different sources of them. Creatinine is a breakdown product of creatine phosphate in the muscles, usually are produced at a constant rate depending on the muscle mass [11]. Urea is the major end product of protein, amino acids demolition. The liver produces it and distributes it throughout the intracellular and extracellular fluid [12].

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