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Hematological effects for rabbits immunized by *Corynebacterium pseudotuberculosis* sonicated antigen

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Abstract

Background: Analyzing Hematological parameters is usually in use to monitor various circumstances such as infection, inflammation and anemia. For that we studied blood parameters which are in touch with immunized laboratory animals (rabbits) after immunizing these animals with *Corynebacterium pseudotuberculosis* then used an adjuvant of *Pseudomonas aeruginosa* to stimulate the immune response cells after 2 to 3 weeks of immunization. Different concentrations were used to examine the effect on the animals' blood parameters changing.

Methods: *Corynebacterium pseudotuberculosis* bacterium was used at current study for evaluating the effect of immunizing laboratory rabbits with two different immune stimulators working together as adjuvant on the blood picture with the aid of 12 breed rabbits from different genders, dividing them at the base of inoculation with pre-killed and sonicated cells of bacteria (antigens) onto 4 categories as following: the group-1 members of rabbits were injected with the antigen, group-2 and group-3 included rabbits injected with the two inoculations at various concentrations; final group, group-4 was the control group.

Results: Thus, white blood cells known to be our main line of defenses act firstly in our body towards different infecting microorganisms, their count always was increased through the immunization correlated with other measured parameters. From the results we can see that the lymphocytes percentage had no alteration between the infected and the immunized compared to control group, while the percentage of MID that refers to the monocytes was at the same range in both infected and the immunized control rabbit's groups. Granulocyte cells percentage, which is including heterophilic, basophilic and eosinophilic cells, was significantly decreased ($P>0.05$) compared with control at all groups.

Conclusion: We conclude that *C. pseudotuberculosis* might function as a potent immunogen to notice the complete blood picture variations.



Introduction

Corynebacterium pseudotuberculosis is a gram-negative bacterium which has a vigorous exotoxin that is called phospholipase D (PLD) acts as an essential virulence factor in developing caseous lymphadenitis (CLA) in animals [1]. PLD was described for the first time in 1940 from this bacterium, then it had been detected PLD in every *C. pseudotuberculosis* studied isolate [2], either in its type I or Type II from all mammals studied species [3]. Research done assured that the CLA at its initial stage might facilitate multiplication of *C. pseudotuberculosis* within the macrophages themselves [4,5]. However, Phospholipase D used to play a major role in infecting through aiding in escaping for the organism at the hydrolysis process inside macrophages, effecting by that at the inner phospholipid layer for the macrophage cell membrane [6].

Infectious diseases cause blood alteration leading to pathological conditions associated with these alterations [7]. The immune system of the host varies at its responses that different components reveal different inflammatory reactions, pathogenesis, and pathogens; increased neutrophilic activity resulted from acute bacterial infections, monocytosis and lymphocytosis are resulted from chronic bacterial infections [8, 9]. CLA incubation period ranges between three and twenty weeks with the incubation periods are shorter [10] thus, few animals might express signs clinically as the showing changeable heart or respiratory conditions, fever and minimization nutritional consumptions. The CLA, however, noticed changes but minor ones at the hematological rates in some kinds of animals that were challenged with *C. pseudotuberculosis* [11,12]. This bacterial genus cell walls have a complex structure act as a virulence factor [13]; That it lipids are containing 2-branched 3-hydroxy of fatty acid under the name mycolic acid (MA) [14] as a result, *C. pseudotuberculosis* buildings units giving it virulence, and helping this bacteria at its survival acting as facultative intracellular parasite [15]. Besides, Mycolic acid contained in this bacterium expresses cytotoxic effects that might stimulate immune response [16]. It was proved (in-vitro) that phagocytic activity of white blood cells significantly degenerated during

C. pseudotuberculosis infection [17] and it could resist cellular digestion by phagocytes [18].

Methods

Experimental Animals

Rabbits were used as a model for the demonstration of the doses that twelve rabbits were divided into 4 groups, each healthy animal was of nearly 1Kg weight,

and was placed in a proper plastic cage at a good, air-conditioned room, they were left for adaptation for 2 weeks at the animal housing unit. Chaw pellets, herbs and tap water were the feeding materials used for nutrition.

Experimental bacteria and their source

Sheep infected lymph nodes were the *Corynebacterium pseudotuberculosis* source, while *Pseudomonas aeruginosa* was isolated from Human lymph node (swelling one).

Experimental Procedure

Different gender rabbits at body weight of 1000±30gm were used in the current experiment divided into four divisions, they were inoculated with the pre-killed and sonicated cells of bacteria (antigens) that group-1 included 4 rabbits, they were injected with 1 mg/ml antigen, group-2, included 4 rabbits and were injected with both (1 gm/ml of *C. pseudotuberculosis* and 1 gm/ml *Pseudomonas aeruginosa*) antigens; group-3 included 4 rabbits injected with antigen of both (1 gm/ml of *C. pseudotuberculosis* and 0.5 gm/ml *Pseudomonas aeruginosa*). Final group, group-4 included 4 rabbits as control group injected with Phosphate buffer saline (PBS). After 14 days of the immunization, all of the experimental first 3 groups were injected with booster doses of same sonicated bacterial antigen from *Cor. Pseudotuberculosis* to enhance immune response. Finally, animals were sacrificed, and the hematological changes were examined. At the laboratories of the department of Microbiology/ College of Veterinary Medicine/ University of Baghdad, both types of the bacteria (*C. pseudotuberculosis* and *P. aeruginosa*) were isolated, biochemically identified and molecularly ensured. Preparing the antigens submitted to the method of Motiva and his co-workers [19].

Results

Hematological parameters are used to provide information on the body's health conditions (such as infection, inflammation and anemia). Thus, our investigation was to research the effect of enhanced immunization on various hematological parameters in rabbits as shown in table 1 below.

Discussion

During infection of tissue, many inflammatory mediators were released producing a local inflammatory response resulting in accumulation of serum proteins and phagocytic cells at the site such as phagocytic cells which include eosinophils and neutrophils, they have the ability to engulf and terminate the bacteria [20].

Parameter	Positive Control	Immunized with 1 vaccine	Immunized 2 vaccines	Negative Control (Non-immunized)	Reference range numbers
WBC*10 ⁹ /L	8.9	11.2	13.6	5.4	4 -12*10 ⁹ /L
Lymph (%)	81.5	82	83.2	83.1	20-40 (%)
MID (%)	3.7	5.5	5.2	4.5	3-15 (%)
Gran (%)	14.8	12.5	11.6	12.4	50-70 (%)
HGB g/dL	11.3	11.7	11.1	10.3	11.0- 16.0 g/dL
RBC *10 ¹² /L	5.46	6.14	5.87	4.85	3.5- 5.5*10 ¹² /L
HCT(PCV) (%)	41.3	46.6	40.7	38.8	37- 50 (%)
MCV fL	75.8	76	69.4	80	80-100 fL
MCH pg	20.6	19	18.9	21.2	27 - 34 pg
MCHC g/dL	27.3	25.1	27.2	26.5	32 -36 g/dL
RDW-CV (%)	19.3	21.4	25.9	19.9	11- 16 (%)
RDW-SD fL	47.4	57.9	56.9	51.1	35 - 56 fL
PLT*10 ⁹ /L	545	62	33	153	150- 400*10 ⁹ /L
MPV fL	6.1	6.5	6.7	6.9	6.5 -12.0 fL
PDW	15.2	15.2	14.6	16.2	9 - 17
PCT (%)	0.351	0.040	0.022	0.105	0.108- 0.282(%)

WBCs: White Blood Cells (leucocytes), Lymph: Lymphocytes, Mid: Monocytes, Gran: Granulocytes, HGB: Hemoglobin, RBCs: Red Blood Cells or erythrocytes, HCT or PCV: Hematocrit or Packed Cell Volume, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW-CV: Red Cell Distribution Width - Coefficient of Variance, RDW-SD: Red Cell Distribution Width - Standard Deviation, PLT: Platelets or thrombocytes, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, PCT: Procalcitonin Test.

Table 1: showing blood parameters compared to the reference ranges of healthy rabbits

Many studies admitted that immunization with sonicated antigens might stimulate efficient immune response at the host cells [21]. Bone marrow is responsible of producing the granulocytes which subsequently are released into peripheral blood then migrate to the tissues in order to complete their action for the body as responding to specific stimulation which requires production and mobilization. Animals' plasma which suffers leukopheresis have Leukopoietic factors, while animals which exposed to endotoxin mobilizing factors, they became responsible for releasing the granulocytes into the peripheral circulation. At the time, leukotoxin (which is a polypeptide from inflammatory exudates) was found to be responsible for increasing capillary permeability and inducing local granulocytes migration. When the antigen-antibody was reacting, eosinophils rottenly mobilized at the site of their presence leading to increment in the number of the eosinophils at that blood stream. Inflammatory conditions are always associated with the presence of neutrophils which

could be available with large numbers in tissues infected with pyogenic germs. Additionally, the inflammatory reaction might be (in low chance) initiated by the granules of the basophils which contain the heparin (responsible of inhibiting the clotting mechanism) and histamine. Lymphocytes in the animal body are constantly in a state of circulation and recirculation, this made the lymphocytic cells unknown at the body, hence, unknown factors regulating blood lymphocytes levels also occurred; Mechanisms of proliferation and differentiation for the cellular events took place after g exposure to antigen, with the contribution of lymphocytes (as presenting essential metabolites for the proliferating cells). Macrophages, which developed from monocytes, have the capability to enter inflammation sites in which fungi and protozoa are present in order to do phagocytosis in tissues [22]. The four mentioned factors could influence the levels of blood cells that circulate at the blood vessels, which affect total and differential white blood cells count as what was found by [23,24]. A report [25] assumed that the CLA at its initial stage might facilitate multiplication of *C. pseudotuberculosis* within the macrophages themselves [8], However, phospholipase-D might have a positive influence in infections through playing part in escaping of germs from hydrolysis process inside the macrophages, effecting by that at the inner layer of the phospholipid for the macrophage's cell membrane [26] for the previous reason we found our results are logically accepted for their variation between infected and immunized groups.

Our results exemplified that normal complete blood count (CBC) in the fourth control group were matching the normal readings rates while the positive first groups exhibited slight raising in the RBCs & lymphocytes which are in tune with findings of Abdulkareem and his team [27] whom mentioned that there was a severe leucocytes infiltration in the histopathological sections for the internal organs of rabbits that injected with different antigens.

The increase noticed in RBC count and Hb might be from good health and no presence of hepatotoxicity and dysfunction, that led to the thought that haem and globin molecules synthesized and sufficient liver iron storage, this agrees with the results of previous report [28], thus, it goes with studies mentioned that the increase in WBC indicate an inflammation, beside a slight descending in RBC [29].

One of the cases is that HGB value is high the chronic diseases and it is obvious from our results that the animals did not suffer from chronic diseases or anemia either. However, the minor rise in the values might be because of dehydration. Platelet count and its indices (MPV & PDW) considered parameters for maintenance

CBC checking, they considered major growth factors sources such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor- β (TGF- β) giving the platelets the importance in achieving various pathways such as repair, inflammation, regeneration of tissues and angiogenesis [30]; this agrees with our results which were showing that the PTC was normal at the negative control group and there was a raise in the levels of the PLT, MPV, PDW and PCT values, this could be attributed to the inflammations, tissues regenerating processes or repairing at the body tissues. Moreover, it was assumed that the size and the volume of the platelets altered, that in minor inflammation disorders, values of MPV increases; oppositely, at major inflammation disorders there would be decrease in MPV levels resulting from consumption for the big platelets in the inflammatory site [31], thus, from our results it is clear that there are no major inflammations occurred at the rabbits tissues, with slight calcification at the tissues of the infected group, might be due to the harmful effects resulted from *C. pseudotuberculosis* PLD or exotoxin especially in the cells of endothelium for vascular system leading to disrupt of hemopoietic pathways or damage caused at the bone marrow [32, 33].

Analyzing changes in blood due to the hematology evaluation after response to *C. pseudotuberculosis* in rabbits revealed an experimental challenge that immune stimulators could elevate a pattern in which sonicated bacterial antigens inoculation caused significant difference indicating cellular immune response, changing the infection from acute to chronic.

Author Contributions

Authors were equally contributed to the current study.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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