The Effect of Two Antigen Preparations From Group A Streptococcus on Phagocytosis and Cellular Counts of Neutrophils and Monocytes in Albino Mice

A. H.AD'hiah*, S. S. Al-kashaly and T. A. A. Abbas
***Tropical Disease Research Unit, Al- Kindy College of Medicine, University of Baghdad**
Department of Biology, College of Education, Ibin Al-Haitham, University of Baghdad

Abstract

The phagocytic activity of peritoneal and blood cells counts of neutrophils and monocytes were evaluated in albino mice treated with two antigen preparations (A and B) from group A streptococci (GAS). Antigen A included water bathed bacteria at 70 °C for 60 minutes, while in Antigen B the bacteria was autoclaved at 121° C for 15 minutes. The animals were treated with 12 intraperitoneal doses of the antigens with intervals of three days (36 days). The 12^{th} dose was a challenge dose (live bacteria). The first three doses of Antigen A increased the phagocytic index (PI) to a range of 76.46-78.69%, then a gradual decreased percentage was observed, especially at the challenge dose (PI=6.42%). The count of neutrophils and monocytes followed a similar behavior. Treating the animals with Antigen B also elevated the PI, especially at the first three doses (range: 73.86-77.17%). The rest of doses showed a gradual decreasing even at the challenge dose, but the PI values were still higher than the corresponding one in the control (PI=18.66%). Similarly, Antigen B shared the effect of Antigen A in effecting the count of neutrophils and monocytes with some dose fluctuation.

Introduction

Phagocytosis is an important defence mechanism in the innate immune system. Mainly three professional cells carry it out. They are neutrophils, monocytes and tissue macrophages (1). Quantitative and/ or qualitative defects in this process can result in an increased

incidence of infections, and the phagocytes fail to engulf and kill microorganisms (2). The pathogens, in particular some bacteria, have developed their own strategies to avoid or to hamper phagocytosis. In this regard, the *Streptococcus pyogenes* group A streptococci (GAS), which by far are the most common pathogen in pharyngnotonsillitis, have several virulent factors (M protein, hyaluronic acid capsule, C5a pepetidase and leucocidin) by which the bacteria can escape phagocytosis and to establish the state of infection (3).

In the present investigation, it is aimed to evaluate the phagocytic activity of mouse peritoneal phagocytes, and to correlate such activity with the blood counts of neutrophils and monocytes in albino mice treated with two types of antigen preparations of GAS.

Materials and Methods

The Bacteria:*Streptococcus pyogenes* group A streptococcus was isolated from patients with tonsillis, attending the (Ear, Nose and Tonsilites ; E.N.T.) unit at the Medical City Hospital in Baghdad. The culture conditions, isolation, identification, purification and propagation were previously described (4,5).

Antigen Preparations:Two types of antigens were prepared. The first one (Antigen A) included purified GAS water bathed at 37° C for 60 minutes (6). In the second preparation (Antigen B), the bacteria were autoclaved at 121° C for 15 minutes (7). For each preparation, the cellular count of bacteria 9 x 10^9 cell/ml phosphate buffer saline (PBS), which was adjusted accrding to methods presented by Verssyr and El- Habib (8).

Laboratory Animals: Balb/C albino mice (*Mus musculus*)were the subjects of the study. The Institute of Sera and Vaccines in Baghdad supplied them. Their age range was 8-10 weeks at the time of experiments.

Experimental Design: The animals were divided into 12 groups, each with eight mice (4 males and 4 females). Each animal was injected intraperitoneally with 0.1 ml of the antigen preparation (A or B). The injection included 12 doses with intervals of three days (36 days). After the third day of each injtion, eight animals were scarified to evaluate the phagocytosis, and to count neutrophils monocytes. The 12^{th} dose was a challenge dose (live bacteria). Four control groups, injected with PBS (32 animals), were included to cover the period of experiments (36 days). The interval was nine days.

Laborator Methods: peripheral blood was obtained from the tail, for cellular counting of neutrophils and monocytes, and in which the total leucocyte count was made. Additionally, a blood film was made and stained with Leishman stain to assess the percentages of neutrophils and monocytes. Then, the cellular count (cells/cu.mm.blood) of the the two types was obtained from the total count of leucocytes (9). The phagocytosis was evalualuated in the peritoneal phagocytes. The cells were obtained injecting the animal with 3 ml s of PBS in the peritone, and after three minutes of a gentle massage of the region, the phagocytes were collected and washed three times. The harvested cells were counted (adjusted to 3 x 10^6 cells/ml), and the viability was assessed by a dye exclusion method using trypan blue. Then, 0.2 ml of cell suspension was mixed with 0.2 ml of human AB serum and 0.2 ml of killed yeast; *Saccharomyces cerevisiae* (10^8 cells/ml). The mixture was incubated in a water bath (37° C) for 60 minutes. After that, it was transferred to an ice bath to terminate phagocytosis. The percentage of yeast-phagocytic-cells was evaluated microscopically and expressed as a phagocytic index (PI) (10).

Statistiical Methods: The student t-test was employed to assess the significance of differences between treated animals and controls.

Results

It is worth mentioning that there were no differences between males and females in the investigated parameters. Therefore, the results of both sexes were considered together.

The percentage of PI (mean±S.D.) and the counts of neutrophils and monocytes (x10^3 cells/cu.mm.blood) for the Antigens A and B are presented in table (1,2) respectively.

The activity of phagocytosis scored a PI of 18.66% in the control grouq. Treating the animals with Antigen A disturbed the PI, positively and negatively, and this was a dose-dependent. The first three doses increased the PI to the range 76.46-78.69%. Starting from the fourth dose, the PI decreased gradually, and a value of 25.33% was recorded at the 10^{th} dose. These values were still higher than the control ones, and the differences reached a highly significant level ($P<0.001$). In dose 11, the decrease continued (PI=9.28%), and much more reduction was observed in the challenge dose (PI=6.42%). Both values were less than the control counterpart, and a significant difference was reached ($P<0.001$).

The neutrophil count in the control group was 2.76, and treating the animals with Antigen A disturbed such value. The first three doses elevated the count to the range 3.57-5.94. Such differences were significant ($P<0.001$) for the 2nd and 3rd doses. From dose 4 and up to dose 8, the count declined, but is was still higher than the control value and maintained at a level of about 3.00, but the differences were not significant. However, the control count was almost restored at the last four doses (9^{th}, 10^{th}, 11^{th}, and 12^{th}). The monocyte count shared the manner of neutrophils in response to Antigen A. However, the challenge dose may contradict this, and the monocyte count was significantly ($P<0.001$) higher than the control ane (0.84 *vs.*0.22).

Treatihg the animals with Antigen B aloso elevated the PI, especially at the first three doses with a range of 73.86-77.17%. the rest of doses showed a gradual decreased level of phagocytosis. In spite of this decrease, the PI values remained higher than in the control, even at the challenge dose (35.07 vs. 18.66%), and the differences maintained a significant level ($P<0.001$).

The neutrophils, and in their response to Antigen B, showed some fluctuation. At the first five doses, an increased count was observed (range: 3.29-5.87), while the next three doses (6^{th}, 7^{th} and 8^{th}) approximated the the control count (2.76). At the doses 9,10 and 11, a significant ($P<0.05$) reduction in neutrophil counts was observed. However, the challenge dose restored the level of the first five doses with a count mean of 4.18, which was highly significant ($P<0.001$) compared to controls. When the monocytes werer considered, a significant increase was observed at the 2^{th}, 3^{th}, 4^{th} and 5^{th} doses, although the levels of significance were different. The 10^{th} and 11^{th} doses restored the control count of monocytes, while the challenge dose showed around 79% increase, which was highly significant ($P<0.001$).

Discussion

The present study demonstrated that GAS can modulate, positively or negatively, the phagocytosis and the cellular counts of neutrophils and monocytes. This is dependent on the method employed in antigen preparation, as well as, the dose, and whether the bacteria were killed or alife. The present procedure of bacterial processing yields two kinds of GAS antigens. In the first one, the whole bacteria may be recovered, and their outercellular components may be preserved (6).

In the autoclaved bacteria, the cellular structucture is ruptured, and some of the intracellular constituents are released and/or inactivated by heat (7). Injecting the animals with live bacteria (challenge dose) will certainly shed some light on the immunological effects of the virulent factors, which are not preserved by the forthcoming antigen preparations. So, the three types of treatments may show the role of GAS in immunity wity much clearer picture. Comparing the PI in the animals treated with Antigens A and B revealed that both antigens increased the the phagoytosis, although the range was different. In Antigen A, the range was 9.28-78.69% for 11 doses, and the mean was 49.45% For Antigen B, the corresponding range was 54.06-77.17%, and the mean was 66.86%, which is higher than that observed for Antieg A. So, Antigen A was less effective than Antigen B in elevating the PI, although both values were significantly higher than in the controls. This observation would fit well the early presentation of antigen preparations, which suggest that Antigen A (water-bathed at 70° C) may retain more virulent factors than Antigen B (autoclaved at 121° C). The retained factors could be the cell wall, together or in part, with the peptidoglycan and lipopolysaccharides, which have an inhibitory effect to Phagocytosis (11). The challenge dosa (live bacteria), which have all the virulent factors in their native forms, may confirm such conclusion. In agreement with this scope, the challenge dose in Antigen A treatment reduced the PI to 6.42%, which is far behind the control value (18.66%). Recently, some further virulent factors have been described (C5a peptidase and M protein), and their inhibitory effect to phagocytosis has been demonstrated (12,13). In Antigen B, although the challenge dose reduced the PI, it was still higher than the control one. At the time being, it is difficult to explain such outcome, and further investigations are required to confirm the observation or to contradict it before reaching a conclusion.

Nerophils and monocytes are the most important leucocytes in combating microorganisms, and they are the first line of cellular defence in innate immunity (1). Therefore, for pathogens to establish a state of infection, they must escape this line of defence, and a fluctuation in the count of responsible cells is expected. This augmentation is apparent in the fluctuation of neutrophil and monocyte counts as a result of treatments with Antigens A and B, and the challenge dose may sapport the outcome. In agreement with this scope, it has been recently demonstrated that live GAS are able to

produce two types of streptolysin ; O and S, and both factors are able lyse leucocytes, especially, neutrophils and monocytes (14).

In conclusion, the phagocytic activitiy of peritoneal cells and the blood count of neutrophils and monocytes were affected by the current regimens of GAS treatment. However, it would be better to evaluate the phagocytosis in terms of pathways of killing. Furthermore, it would be fruitful to assess the cell surface changes of neutrophils and monocytes, as this may shed more light on their function.

References

1. Lydyard, P. and Grossi, C. (1998) *Immunology*, 5th Edition. Edited by I. Roitt, J. Brostostoff and D. Male. Mosby International Ltd., UK. 14-30.
2. Rosen, F. (1998) *Immunology*, 5th Edited by I. Roitt, J. Brostoff and D. Mosby Internatioal Ltd., U.K. 285-292.
3. Fischetti, V.A.(1989) *Clin. Microbiol. Rev.*, 2 : 285-314.
4. Kaplan, E.(1973) *Com. Med.*, 37: 45-48.
5. Baron, E.J. ; Paterson, I. R. and Finegold, S.M. (Editors) (1994) Baily scotts diagnostic microbiology, 9th Ed. Mosby Baltimore. 333-351.
6. Agren, K.; Brauner, A. and Andersson, J. (1988) *O. L. R.*, 60: 25-41.
7. Facklam, R. R. and Carey, R. B. (1985) Manual of clinical microbiology, 3rd Ed. Edited by E. H. Lennette, A. Balow, W. J. Husler and H. Shadomy. American Soociety for Microbiology, Washington D. C. 154- 173.
8. Verssyr, C. and El-Habib, R. (1989) Serodiagno. *Immunoth. Infect. Dis.*,3:193-199.
9. Sood, R. (Editor) (1985) Haematology for students and practitioners. Jayppee Brothers, India. 243-320.
10. Metcalf, J. A.; Gallin, J. I. ; Nanseef, P. W. M. and Root, R. K. (Editors) (1986) Laboratory manulal of neutrphil function. Raven Press, U. S. A. 84-90.
11. Wahl, S. M. ; Hunt, A. ; Allen, J. B. ; Wilder, R. L.; Paglia, L. and Hand, A. R. (1986) *J. Exp. Med.*, *163*: 384-402.
12. Kehoe, M. A. (1994) *Bacterial cell wall,* Elsevier Science, Holland. 21-27.

13. Perez-Caballer, D.; Alperti, S. ;Vivanco, F.; Sanchez-Corral, P. and Rodriguezde-Cordoba, S. (2002) *Eur. J. Immunol.*, 30: 1243-1253.

14. Betschele, S. D.; Borgia, S. M.; Barg, N. L.; D. E. and De Azavedo, J. C. S. *Infec. Immunol.*, 66: 1671-1679.

Table (1) Phagocytic index and cell counts of neutrophils and monocytes in mice treated with Antigen A of GAS.

Groups and Doses	Phagocytic Index (Mean±S.E.;%)	Cell Count (x10^3 cells/cu.mm.blood)	
		Neutrophils (Mean±S.E.) (Mean±S.E.)	Monocytes
Controls	18.66±2.02	2.76±0.45	0.22±0.10
Treated: Dose 1	76.46±5.64*	3.57±0.32*	0.37±011
2	76.89±6.05*	5.05±2.24*	0.95±0.33*
3	78.69±3.36*	5.94±1.08*	0.98±0.24*
4	78.56±3.22*	3.70±1.30	0.67±0.23*
5	48.39±5.77*	3.52±0.91	0.48±0.20*
6	39.26±5.75*	3.44±0.91	0.26±0.20
7	39.03±4.16*	3.30±0.77	0.26±0.17
8	37.43±2.97*	3.00±0.77	0.20±0.06
9	34.64±7.07*	2.79±0.05	0.19±0.15
10	25.33±6.83*	2.73±1.07	0.18±0.11
11	9.28±2.59*	2.63±1.06	0.16±0.11
Challenge Dose (12)	6.42±3.55*	2.88±1.39	0.48±0.25*

*Significat compared to controls.

Table (2) Phagocytic index and cell counts of neutrophils and monocytes in mice treated with Antigen B of GAS.

Groups and Doses	Phagocytic Index (Mean±S.E.;%)	Cell Count (x10^{3}cells/cu.mm.blood)	
		Neutrophils (Mean±S.E.)	Monocytes (Mean±S.E.)
Controls	18.66±2.02	2.76±0.44	0.22±0.10
Treated: Dose 1	73.86±7.12*	3.54±1.20	0.31±0.09
2	75.52±7.30*	4.62±1.09*	0.45±0.07*
3	77.17±9.54*	3.29±0.77	0.51±0.14*
4	71.47±2.78*	5.87±2.82*	0.52±0.23*
5	68.29±3.16*	4.19±0.88*	0.48±0.25*
6	67.45±3.32*	2.64±0.91	0.39±0.11
7	64.66±3.43*	2.31±0.59	0.36±0.13
8	62.23±7.59*	2.28±0.91	0.32±0.09
9	61.44±3.95*	1.67±0.21*	0.30±0.10
10	59.31±7.67*	1.73±0.40*	0.24±0.09
11	54.06±8.55*	1.85±0.83*	0.24±0.08
Challenge Dose (12)	35.07±0.90*	4.18±1.10*	0.82±0.32*

*Significat difference compared to controls.

تأثير اثنين من التحضيرات المستضدية للعقديات المحلة للدم على الفعالية البلعمية والعد الخلوي للعدلات ووحيدة النوى في الفأر الابيض

علي حسين ادحية* ، صلاح سلمان عبد الحسين و طارش عبدة عبد الله عباس
*وحدة بحوث امراض المناطق الحارة، كلية طب الكندي، جامعة بغداد
قسم علوم الحياة ، كلية التربية -ابن الهيثم ،جامعة بغداد

الخلاصة

قيمت الفعالية البلعمية لخلايا غشاء الخلب وحسبت اعداد العدلات ووحيدة النوى في الدم المحيطي للفئران البيض المعاملة بنوعين من التحضيرات المستضدية (أ ، ب) للعقديات الحلة للدم أ. تضمن التحضير أ حضن البكتيريا في حمام مائي (70 °م) لمدة 60 دقيقة، في حين حضر المستضد ب بقتل البكتيريا في الموصدة (121 °م لمدة 15 دقيقة). حقنت الحيوانات 12 جرعة من كل مستضد في غشاء الخلب وبمعدل جرعة واحدة كل ثلاثة ايام (36 يوم)، وكانت الجرعة الثانية عشرة جرع تحدي (بكتيريا حية). عملت الجرع الثلاث الاولى على رفع قيمة معامل البلعمة الى المدى 46. 76 -78.69 %، ثم بدأ هذا المعامل بالانخفاض تدريجيا ولاسيما في جرعة التحدي (6.42%)، كما لوحظ نفس منوال الانخفاض في عدد الخلايا العدلة ووحيدة النواة. وعند معاملة الحيوانات بالمستضد ب ارتفع معامل البلعمة ايضا ولاسيما في الجرع الثلاث الاولى (المدى: 86. 73-17 77 %). في حين عملت الجرع الباقية على خفض قيمة معامل البلعمة تدريجيا، الا انها مرتفعة عما هو عليه في السيطرة (18.66%). تماثل الستضد أ في تأثيره على اعداد الخلايا العدلة ووحيدة النواة مع وجود بعض الاختلاف المعتمدة على الجرعة.