قسم: طب الحيوان
كلية: الطب البيطري - جامعة أسوان
رئيس القسم: أ.د. إبراهيم سيد أحمد

صورة الخلايا الدموية البيضاء في الدورة الورمية

بعد التحصين ب المادة ال BCG والعدوى الصناعية بالبانية في الحمير

شريف نافع، أحمد عامر

تم تقييم تأثير الحقن بعيدة ال BCG على تنظيم المناعة الخلوية ضد العدوى الصناعية بالبانية في مجموعة من الحمير الذكور قبل وبعد العدوى، وكذلك بعد إزالة الطحال في مجموعة منهم.

وأوضح النتائج أن حقن مادة ال BCG قد وفر الحماية ضد البانية في جميع الحمير الغير مزال طحالها بينما نفقت الحمير المزال طحالها. وأوضحت النتائج بعض التغييرات المعنوية في نسب الخلايا البيضاء الكلية والثلاجية قبل وبعد العدوى وكذلك بعد إزالة الطحال.
CIRCULATING LEUKOCYTES AFTER B.C.G. AND EXPERIMENTAL BABESIA EQUI INFECTION IN DONKEYS
(With Two Tables and 4 Figures & 3 Plates)

By

TH.S. NAFIE and A.A. AMER
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SUMMARY

A group of 6 donkeys were used to evaluate the effect of B.C.G. injection on the activation of non-specific cell mediated immunity against the experimental infection of Babesia equi.

The experimental donkeys were previously treated against internal parasites until proved its freedom from infestation. The animals were proved to be free from tuberculosis using tuberculin test.

The experimental animals were injected by B.C.G. intradermally. After one month they were challenged experimentally by B. equi infected blood (98% parasitaemia) intravenously.

Out of the experimental group three donkeys were splenectomized to induce relapses.

All animals were clinically observed, blood samples and bone marrow smears were routinely examined for estimation of leukocytic picture.

The study revealed that the injection of B.C.G. had protected donkeys from acute babesiosis, however it failed to protect splenectomized animals from acute relapses and severe parasitaemia was observed, and all splenectomized animals were died.

The observations of leukograms revealed a highly significant leukocytosis to reach maximum (19.2 ± 3.4 X 10^3 /μl) two weeks post B.C.G. injection, and one week post experimental babesial infection.

The relative count revealed a highly significant neutrophilia and eosinophilia post B.C.G. up to one month post infection with the increase of the relative number of non segmented cells, however, monocytes achieved non significant increase in the peripheral blood.

Leukograms of splenectonized animals revealed a highly leucocytosis (47×10^3 /μl) in two of them, however, the 3rd one showed severe leukopenia (2.7×10^3 /μl). The relative values of segmented and non segmented neutrophils achieved a highly significant increase (neutrophilia), while the relative number of lymphocytes was decreased, however, the relative values of eosinophils and monocytes were significantly increased.

It was observed that the phagocytic processes were highly activated post experimental infection. Neutrophils were able to engulf more than one parasitic cell in the peripheral blood, however monocytes were able to phagocyte a lot of parasitic cells and others could
NAFIE and AMER

engulf more than one of the infected erythrocytes (erythrophagocytosis) especially in bone marrow smears.

INTRODUCTION

Equine piroplasmosis [Babesiasis]; a world-wide disease was diagnosed in Assiut province, A.R.E. (NAFIE, 1980). Since that date, several studies were conducted on the pathological effects of Babesia equi in donkeys.

The effect of induced equine piroplasmosis on the haematological picture and some biochemical constituents in splenectomised and non-splenectomised donkeys was conducted by NAFIE et al. (1982). It was observed that the increase in total leukocytes was more prominent in splenectomised animals than in non-splenectomised ones. Russian investigators (ERCHOV, 1963) found a shift to the left in the neutrophils and a mild lymphocytosis as well as monocytosis in 50% of the diseased animals. ROBERTS et al. (1962) observed leukopenia, neutropenia and lymphopenia just prior to the appearance of parasites in the blood. The same observations were recorded by TAYLOR et al. (1969) in the United States. Transient leukopenia and lymphopenia which progressed to lymphocytosis in about 10 days was observed by the authors. However, RUDOLPH et al. (1975) observed wide variation in the leukograms however, monocytosis and eosinopenia being the most common variations.

The use of Bacillus Calmette-Guerin (B.C.G.) in the activation of non-specific cell mediated immunity against piroplasma was attempted by CLARK et al. (1975) in mice and NAFIE (1983) in donkeys and NAFIE et al. (1985). All authors reported that B.C.G. gave protection in non-splenectomised animals against acute babesial infection.

The present work was carried out to study the variations in circulating leukocytes after the immunization by B.C.G. and post challenge by Babesia equi in splenectomized and non-splenectomized donkeys.

MATERIAL and METHODS

A group of 6 donkeys aged between 3.5-4 years were used in the experimental work. All animals were treated by Ivomec* against internal parasites until it proved negative egg count by faecal examination. Scheme of the experimental work is shown in Diagrams.

Whole anticoagulated blood samples were drained from jugular vein using disodium-Ethyline Diamine Tetra Acetate (Na-E.D.T.A.). One blood sample was taken before B.C.G. injection and the other two-after 2 and 4 weeks post injection. Animals were then infected by Babesia equi infected blood (100 ml of 98% infected blood i.w.).

Total leukocytic count and blood smears were examined daily, however, three samples every two weeks post infection were representative. In splenectomized animals blood samples were examined daily until the day of death.

Total and differential leukocytic cell count were carried out according to SCHALM (1979).

Statistical analysis was conducted using t-test according to SNEDECOR and COCHRAN (1967).

*: Developed by the research Dept. of MSD Co.

LEUKOCYTES, B.C.G, BABESIA EQUI

RESULTS

It was observed that the injection of B.C.G. deparrd the appearance of the clinical signs of piroplasmosis in B.C.G. injected donkeys. However, in B.C.G. splenectomized animals the typical acute clinical signs of babesia were evident and all splenectomized animals were dead within few days. Results of the total leukocytic count and differential cell count were recorded in table (1) leukogram (1) and table (2) leukogram (2,3 & 4) for B.C.G. injected and splenectomized groups respectively.

The activity of leukocytes against babesia equi parasites were recorded in Pictures (1 & 2).

DISCUSSION

It is of important to observe that B.C.G. injection before babesial infection protected the infected animals against acute illness in non-splenectomized animals. However, in splenectomized animals, B.C.G. failed to protect them from acute relapses and the clinical signs of acute babesia were appeared. High temperature, severe anaemia, haemoglobinurea, yellowish mucous membranes and emaciation were the most prominent clinical signs. It was observed that all splenectomized donkeys were dead. These results could be interpreted depending on the fact that B.C.G. stimulates cell mediated immunity which depends mostly on the presence of large number of sensitized leukocytes and the presence of spleen as a lymphoid tissue which act as a precursor for leukocytes (CLARK, et al, 1975; SELBITZ et al, 1980 and NAIE, 1983).

Regarding leukocytic count, it was observed that leukograms achieved a highly significant leukocytosis to reach the maximum (19.2 ± 3.4 X 10^9 / ul) two weeks post B.C.G. injection and one week post experimental babesial infection. The absolute number of segmented, non-sigmented neutrophils, eosinophils and lymphocytes were, however, significantly increased post B.C.G. injection (Table 1 & Diag. 1) and declined slowly but not reached the before injection figures. It returned to increase again post babesial infection to reach the maximum elevation at the 6th week post babesial infection with appearance shift to the left. These results coincided with those obtained by ERCHOVE (1982) and NAIE (1980 & 1983). However, it contradict with those obtained by ROBERT et al, (1962) and TAYLOR et al, (1969), while, RUDOLPH et al, (1975) recorded a wide variation in the leukograms.

In splenectomized animals the total count reached a highly significant increase (47 X 10^9/ul) in two of them, however, the 3rd one showed gradual decrease to reach (2.7 X 10^9/ul). The absolute values of segmented and non-sigmented neutrophils recorded a highly significant increase (Neutrophilia) while the absolute number of lymphocytes was decreased, however, the relative values of eosinophils and monocytes were significantly increased. With respect to the 3rd splenectomized donkey, it was observed that the total leukocytic count was decreased just post splenectomy. The typic count revealed a sharp decrease in the segmented neutrophils, however the non-sigmented one showed a significant increase up to the 4th day when it began to decrease again. These picture indicated a degenerative shift to the left (Table 2 & Diags 2,3 & 4).

These pictures were similar to those obtained by NAIE (1980); NAIE et al. (1982) and SALEM et al. (1986).

The careful examination of stained blood and bone marrow smears (Fig. 1 & 2) revealed that the phagocytic characters of neutrophils and monocytes were highly activated post B.C.G. injection. Neutrophils thus could engulf and lyse a great number of babesial agents, while monocytes were able to phagocyte more than one parasitic cell. Furthermore, monocytes

were able to engulf infected erythrocytes (Target cells). More than one monocyctic cell coalesced with each other to increase the phagocytic affinity. Similar observations were recorded by Allen et al. (1975) and Nafie (1980 and 1983).

The overall observations revealed that the use of B.C.G. activated leukocytes through the mechanism of cell mediated immunity and increased the total number of circulating leukocytes specially segmented, non-segmented and lymphocytes (The cells which are encountered in the cell mediated immunity). The functions of these cells were supported by increasing the number of monocytes and to a little extent by eosinophilic cells.

REFERENCES


### Table 1. Total and Absolute Differential Leukocyte Count of BCG Infection Experiment

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<tr>
<th>Time of Experiment</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Basophils</th>
<th>Neutrophils</th>
<th>Absolute Total Count</th>
<th>Differential Leukocyte Count</th>
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<td>Before BCG</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.0 x 10^4</td>
<td>2.0 x 10^4</td>
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<tr>
<td>6 weeks post BCG</td>
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<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.0 x 10^4</td>
<td>2.0 x 10^4</td>
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<tr>
<td>1st Injection</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.0 x 10^4</td>
<td>2.0 x 10^4</td>
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<tr>
<td>1st ST.</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.0 x 10^4</td>
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<td>2nd Injection</td>
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<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
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<tr>
<td>2nd ST.</td>
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<td>3rd Injection</td>
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<td>3rd ST.</td>
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<tr>
<td>4th Injection</td>
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<td>4th ST.</td>
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<td>2.0 x 10^4</td>
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Note: The table shows the percentage of each cell type in the total leukocyte count. The total count remains consistent throughout the experiment, indicating no significant changes in the absolute count.
<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Before</th>
<th>Days post 1</th>
<th>Days post 2</th>
<th>Days post 3</th>
<th>Days post 4</th>
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Table (2): Total and absolute differential leukocyte count of BCC splenectomized group.
6 DONKEYS treated against intestinal parasites. First tuberculin test. Post 2 weeks inject BCG I.D. Post 2 weeks 2nd tuberculin test. Post 2 weeks infected by 98% B. equi infected blood.

3 DONKEYS non-splenectomized
Survived

3 DONKEYS splenectomized
Died

Outlines of the experimental work.

Fig. (1) - Leukogram of BCG immunized group.
- TLC - SN - NSN - EOS - L - MON.

Fig. (2) - Leukogram of splenectomized donkey NO. (1).
- TLC - SN - NSN - EOS - L - MON.
Fig. (3) - Leukogram of splenectomized donkey No. 2.

--- TLC --- SN --- NSN --- EOS --- L --- MON.

Fig. (4) - Leukogram of splenectomized donkey No. 3.

--- TLC --- SN --- NSN --- EOS --- L --- MON.
Pict.(1): Babesia equi within
the Red Blood cells and
the neutrophil cell.

Pict.(2): Infected Erythrocytes
within large monocyte.

Pict.(3): Neutrophil and
monocyte performing phago-
cytic and lysing processes
on Babesia equi.