ادارة الخدمات البيطرية ·
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تنشيط المناعه الخلوية في الجمـــــــــــال

باستخدام لقاح ال بي سي جي

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أجريت هذه الدراسة على تسعة جمال تابعة لقوات حرس الحدود وقسمت الـــى ثلاثـة مجموعات متساوية العدد بالاضافة الى مجموعة اخرى من ثلاثة جمال للمقارنـة·

تم حقن المجموعات الثلاثة بجرعات مختلفه من لقاح الـ بي سي جي مع اختبـار التأثير المناعي الخلوى لهذا اللقاح بعمل اختبار الجلد وتبرعم الخلايا الليمفاوية·

أكدت النتائج في هذا البحث أن مجموعة الجمال التي حقنت بجرعة ٥ر١× ١٠^٧ أعطيت أحسن النتائج الايجابيه لرد اللقاح عند مقارنتها بالمجموعات الأخـــــرى المحصنه بنفس اللقاح أو مجموعـــة المقارنـــة·

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LYMPHOCYTE PROLIFERATION AND DELAYED TYPE HYPERSESITIVITY RESPONSES IN CAMELS IMMUNIZED WITH BCG VACCINE

(With 4 Tables)

By

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SUMMARY

Nine males camels were divided into 3 groups and inoculated intradermally with different doses of BCG vaccine. Enhancement of cell-mediated immunity was detected as determined by the in vivo skin test and the in vitro lymphocyte proliferation assays.

The results obtained in this investigation indicated that camels inoculted with 1.5 X 10^7 attenuated living tubercle bacilus of Calmette and Guerin gave the greater skin reaction as well as responsivness of lyphocytes.

INTRODUCTION

In a previous study AWAD et al. (1980), it was demonesterated that camels vaccinated with BCG vaccine, were able to produce a certain degree of immunity against Trypanosoma evansi infection.

Moreover, in a field experiment among camels belonging to the Military Borders Guard, which had been vaccinated with BCG anualy at years 1983; 1984 and 1985, the data obtained recorded that the mortality rate throughout these camels was greatly reduced when compared with that recorded at the former years before the begening of the vaccination programe.

Therefore, our purpose in this investigation was to detect the immune responses following the vaccination of camels with BCG vaccine by the in vivo delayed type hypersensitivity (DTH) and the in vitro lymphocyte proliferation assays. In addition, determining the proper dose of the vaccine that should be inoculated in camels to induce the optimum stimulation for cell-mediated immunity (CMI) in camels.

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MATERIAL and METHOD

Camels :

Twelve male camels belonging to the Military borders guard ranging from 6-8 years old were used in this study. The camels were clinicaly healthy and free from infectious diseases. Their wheight was in the average of 350 - 400 killogrammes.

The camels were divided into 4 groups each of 3, they were placed in open desert stable under the same environmental conditions.

Animal inoculation :

The first group, each camel was inoculated intradermally with 100 human doses of BCG vaccine. The three camels of group 2 each was injected with 20 human doses, camels of group 3 each with 10 human doses. While those of the 4th group were acted as control. The vaccine used was a product of Institute Marieux Lyon - France in the form of lyophilized matter on reconstitution with its diluent had a concenteration of 1.5×10^7 attenuated BCG mycobacterium per 1.0 ml.

Skin test :

Four weeks following the vaccination of camels skin reaction was measured by estimating the difference in double skin fold thickness before and 72 hours after intradermal inoculation of 0.1 ml tuberculine (PPDb). Increase of skin thickness of 4mm and more was considered positive and 3mm and less called negative.

Lymphocte proliferation assay :

Heparinized blood samples were collected from the vaccinated and control camels 4 weeks after BCG inoculation.

The cells were separated by histopaque 1077 density graduated centrifugation by adding 5 ml of blood to 2 ml histopaque, allowing the blood to run very slowly down the sides of the tube so that 2 layers are formed then centrfiguge at 2000 rpm for 20 minutes.

Mononuclear cells were arrested at interface. They were resuspended in tissue culture RPMI 1640 at pH 7.3 with hepes buffer (2 ml to be added to each bottle of tissue culture 250 ml, pencillin 200 i.u/ ml and Streptomycin 200 ug/ml). The tissue culture media were supplemented with L-glutamine.

These cells were washed 3 times by centrifugation at 1500 rpm for 15 minutes followed by resuspension in the standard tissue culture fluid.

The lymphocytes were counted and the cell density in the suspension adjusted to 2 X 10 cells/100 uL medium in each well, 50 uL of PHA solution was added and 50 uL of plain culture medium was added to the control wells, (the concentration of the PHA was 10 uL/ml RPMI 1640). There after, 25 uL of the autologous plasma was added and the plates were covered with adhesive tape and incubated at 37°C and 5% CO for 72 hours, BROWN (1977).

The results were recorded. The mean of the means $\pm$, the standard errors of the mean (S.E.M) and immunostimulation index derived from the ratio of the stimulated culture over the media control culture were calculated.

RESULTS

A comparison of skin test results of BCG immunized and control camels are shown in Table (I). These results indicate the positive skin responses in the three groups of camels inoculated with the different doses of the vaccine.

In vitro, lymphocyte proliferative activity of the control and BCG vaccinated camels demonesterate that the lympohocyte activity of the groups of camels inoculated with BCG was deectable as illusterated in Table (2).

Table (3) shows the immunostimulation index of control and different immunized group of camels. It is observed that the proliferatve index of the vaccinated camels were greater than those of the controls after stimulation with PHA mitogen.

DISCUSSION

It is known in human and veterinary medicine that BCG has an immunopotentiated activity, it is mainly a T. Lymphocyte and macrophage stimulant; (CLARK et al. 1976 and FUDENBURG et al., 1976).

It was demonesterated that the application of the nonspecific immunostimulant agent BCG is capable to raise the resistance of cattle and sheep (BARAKAT, 1978; BARAKAT et al, 1981; AWAD et al., 1982; SALEH et al., 1984 and SALEH et al., 1986).

Thus, it was interesting to investigate the ability of the immune system of camel immunized with BCG vaccine by detecting the immune responses with the in vivo and the in vitro by measuring the delayed type hypersensitivity and lymphocyte proliferation reactivities respectively. These assays have been done to determine the stimulation of cell-mediated immunity in animals vaccinated with Mycobacterium compound as reported by HORLAND et al. (1978), ROSSI (1979), ROSSI et al. (1981) and WOODARD et al. (1978).

In this study, a comparison of skin tested camels vaccinated with BCG and controls are shown in Table (1). It is observed that all camels inoculated intradermally with 10 human doses, 20 doses and 100 doses gave clear positive skin reactions. The average of skin thickness was 13.2 mm $\pm$ 3.01, 12.5 mm $\pm$ 0.84 and 7.3 mm $\pm$ 0.67 in group 1, 2 and 3 respectively. But skin reaction was greater in camels of group 1 than those of other groups while the control camels gave very low skin response, it was 0.07 mm $\pm$ 0.01 in average.

The lymphocyte proliferation reactivities of camels immunized with BCG and controls are illusterated in Table (2). It is observed that the cell proliferative responses of vaccinated camels in the different groups were highly stimulated following the culture in PHA when compared with the control camels. These results were evaluated by measuring the immunostimulant indexes of each group. It is indicated as shown in Table (3), that lymphocytes from camels in group 1 gave the greatest immunostimulant index; it was 4.77. While the indees of group 2 and 3 were 4.57 and 2.20 respectively. The immunostimulation index of control camels was 1.70.

This study indicates that there is a relation shipbetween the skin and lymphocyte proliffration reactivities in camels immunized with BCG vaccine and the results obtained showed that the dose of 10 human doses (1.5×10^7) gave the greater skin and lymphocyte proliferation responses. So, it is cosidered to be the dose of choise.

Moreover, the health condition of camels was highly improved after the vaccination with BCG vaccine as it is shown in Table 4. The mortality rates among the immunized camels at year (1983) were 11.2% and 6.4% and 4.3% at years 1984 & 1985 respectively. While the mortality rates among camels not vaccinated with BCG at years (1980, 1981 & 1982) were 21.2%, 22.9% and 17.8% respectively. These data indicate that the mortalities amon BCG immunized camels were greatly reduced. The explanation of this is that the capacity of camel's immune system was highly stimulated nonspecificaly and had the ability to minimize the infection against most of the infectious agents which cause deaths among camels.

Thus, the authers advice the application of this procedure of vaccination for camels in the military camel camps as will as camels in quarantines and markets to reduce the mortality and morbidity rates among this animal.

In addition, this vaccination has an economic value by discounting the costs for the treatment of camel blood parasites as will as the prices of insenticides used for irradicating the flies.

REFERENCES

Awad, F.I.; Y.Z. El-Abdin; A.A. Fayed and S.M. Saleh (1982): vaccination of camels and mice against T. evansi infection by using BCG. Egypt. J. Vet. Med. 19: 81-86.

Barakat, A.A. (1979): Immunization against Caseous Lymphadinitis of sheep using attenuated bovine tubercle of Calmette and Guerin (BCG). Bull. Off. Int. Rpis. 1979, 91: (9 - 10), 679 -692.

Barakat, A.A.; M.S. Saber; Nafei Emad; S. Abdel-Ghaffar; N.M.H. El-Nimr; K. Sobhy; M. Fathia; A.T. Mohsen and S. El-Mahashly (1981): Priminitary studies in the use of BCG as an immunopotenitating agent against Rift Valley Fever among sheep in Egypt. Bull. Off. Int. Epis. 93: (11 - 12), 1387 - 1393.

Brown, J. (1977): A micromethod for the study of in vitro lymphocyte transformation to specific antigens. Jour. Immunol. Meth. 25: 93 - 97.

Horland, W. Renohaw; Willia, P. Exbland; Dale, O. Everson, Patricia, D. Tassi-nard and Dablie. Anos. (1977): Otogemy of immuuocompotance in cattle. Evaluation of phytomitogen induced in vitro on bovine foetol lymphocyte Blastogenesis, using a whole blood culture technique. Am. Jour. Vet. Res. 35: 1141 - 1150.

Rossi, C.R.; Kiosel, C.K. and Kraer, T.T. (1978): Cell mediated and humoral immune responses in cattle to Brucella abortus, Mycobacterium bovis and tetanus toxoid : Evaluation of immunitation and assay technique. Am. Jour. Vet. Res. 39: 1738 - 1741.

Rossi, C.R.; Kiesel, G.K. and Hudson, R.S. (1981): Evidence for suppression or incomplete maturation of cell-mediated immunity in neonatal calves as determined by Delayed Type Hypersesitivity responses. Am. Jour. Vet. Res. 42: 1369 - 1370.

Saleh, M.S.; Reda, I.A. and Abdel Fatah, A.Z. (1984): Reduction of morbidity and mortality rates in bovine neonatal calves delivered from immunized dams with BCG vaccine. Vet. Med. Jour. 32: 195 - 206.

Saleh, M.S.; Regina, R. Skelly; Boulos, A.M. Botrous; A.A., Barakat; I.M. Reda; Nafei Emad and A.M. Zaki (1986): In vitro lymphocyte proliferation responses of bovine neonates delivered from dams vaccinated with BCG and of calves delivered from non vaccinated dams after inoculation with BCG at birth. Assiut Vet. Med. Jour. 15 (30): 119 - 125.

Woodard, L.F.; Banshaw, H.W. and Berger, D. (1978): Cell- mediated immunity in neonatal calves: Delayed Type Hyper Sensitivity and Blastogenesis following immunization with tuberculo protein of Mycobacterium bovis. Am. Jour. Vet. Res. 39: 579 - 584.

Table (1)

Comparison of the in vivo skin test reactivity of control and BCG vaccinated camels

Groups	N	Doses of BCG	Mean of skin thickness (mm)
Group 1	3	10 H/D	13.2 ± 3.01
Group 2	3	20 H/D	12.5 ± 0.84
Group 3	3	100 H/D	7.3 ± 0.67
Group 4	3	control	0.07±0.01

(N) Number of camels. (H/D) human doses.

Table (2)

Comparison of the in vitro lymphocyte proliferative responses of control and BCG immunized camels

Group	N	Mean counts per minutes ± S.E.M	
		Media	PHA
Group 1	3	5.133 ± 1.88	23.600 ± 4.72
Group 2	3	3.840 ± 0.58	17.900 ± 4.23
Group 3	3	6.600 ± 1.64	14.660 ± 2.36
Group 4 (cont)	3	3.870 ± 0.25	6.070 ± 0.68

(N) number of camels. (S.M.E) standard errors.

Table (3)
Mean immunostimulation index of control and immunized camels

Groups	N	PHA
Group I	3	4.77
Group 2	3	4.57
Group 3	3	2.20
Group 4	3	1.70
(cont)		

Table (4)
Mortality rate among camels at years before vaccination and years post-vaccination with BCG

Years		Mortality rate %
1980	camels were	21.2
1981	not vaccinated	22.8
1982		17.8
1983	camels were	11.2
1984	vaccinated	6.4
1985	with BCG.	4.5