ادراة الخدمات البيطرية للقوات المسلحة .
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تنشيط المناعة المخلوية في العجول حديثة الولادة من أبقار محصنة بلقاح الـ بي سي جي

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- أجريت الدراسة على ثمانية بقرات عشار في الشهر السادس وذلك بحقنهـا في الحلد بلقاح الـ بي سي جي . أعطت هذه الابقار رد فعل ايجابـي لهذا التحصين عند ما أختبرت بواسطة اختبار الجلد واختبار تبرعم الخلايا الليمفاويـــة .

بقياس مدى تأثير الجهاز المناعي للعجول المودة من هذه الابقار بعمـــل اختبار تبرعم الخلايا الليمفاوية بها وبمقارنتها بعجول حديثة الولادة مـن أبقار غير محصنة بلقاح الـ بي سي جي وكذا عجول حديثة الولادة من أبقار غير محصنة وحصنت هذه العجول فور ولادتها بلقاح الـ بي سي جي ، وجـد أن التأثر المناعي بدرجة كبيرة في العجول الناتجة من الابقار المحصنـــة عن باقي مجموعات العجول كما كانت الحالة الصحية أصح ولا يوجد نفـــوق في العجول المولودة من الابقار المحصنـــة .

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IN VITRO LYMPHOCYTE PROLIFERATION RESPONSES OF BOVINE NEONATES DELIVERED FROM DAMS VACCINATED WITH BCG AND OF CALVES DELIVERED FROM VACCINATED DAMS AFTER INOCULATION WITH BCG AT BIRTH

(With 3 Tables)

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SUMMARY

Eight pregnant cows at 6 months gestation period were inoculated intradermally with BCG vaccine. The animals gave detectable cell-mediated immune response as determined by the in vivo skin test and the in vitro lymphocyte proliferation assays.

Proliferative responses of cells from calves delivered from BCG vaccinated dams and calves from non vaccinated dams and vaccinated with BCG postpartum, were eliezed by PPD stimulation, while mitogen responsiveness to Concanavaline A was demonstrated in the calves from BCG vaccinated dams only.

INTRODUCTION

Humoral immunity of the bovine neonates have the subject of numerous investigations (BROWN et al., 1978; LSON & WAXIER 1978, SCHULTS, 1973; STORTS et al; WAMUKEYA and CORNNER 1978 and YAMINA & SLEGHT 1980). Cell-mediated immunity (CM) has been more recently studied (ROSSI et al. 1981, and WOODARD et al. 1979).

Actually, the newborn calf is delivered wih poor protection. This is attributed to the physiologic and anatomic charactaristics of bovine placenta (OSBURN et al., 1974). In addition, the bovine foetus produces detectable quantities of glucocorticoids which remain demonestrated in the plasma up to 12 days and are known to be immunosuppressive with the most pronouced effect upon T. lymphocytes (EERHART and PATT, 1971 and ZEMAN et al. 1972). The newborn calve recieves antibodies in colostrum from its mother establishe within hours a state of humoral immunity. There is some evidence suggested that maternal T.cells may be transferred to neonat through colostum,but the contribution of this to cellular immunity is unclear (SOLOMAN, 1971).

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From these observations, it was our purpose to determine the cell-mediated immunity in bovine neonates delivered from dams vaccinated with BCG and in calves from non vaccinated dams which were inoculated with BCG immediatly after birth employing the in vivo by lymphocyte proliferation assay.

MATERIALS and METHODS

Animal Vaccination :

Twenty six pregnant cows belonging to Shubra Shehab Military complex farm were used. The cows were dried and at approximatly the 6th month of gestation. These cows were divided into groups of 8, 10 and 8 animals.

The 8 cows of group 1 were inoculated each with 1.0 ml BCG vaccine, while the other groups were not vaccinated. The vaccine used was a product of institute Merieux Lyon - France in the form of lyophilized matter on reconstitution with its diluent had a concentration of 0.15×10^6 attenuated living BCG mycobacteria per 1.0 ml. The calves delivered from group 2 (10 dams) were inoculated intradermally with 0.5 ml BCG vaccine at birth, while the calves of the third group were not inoculated.

Separation of the animals :

The three groups of pregnant cows were placed in a pen at the dry stable, fed on green fodders and concentrates before parturition, each cow was transferred to the calving barn a few days perior to the expecting date of calving.

After birth, the calves were collected and separated into 2 experiments each of three groups as following :

Group 1 , normal calves from non vaccinated cows to use as control.
Group 2 , calves delivered from BCG dams.
Group 3 , calves delivered from non vaccinated dams but inoculated with BCG vaccine at parturition.

The calves of each experiment were placed in one pen. The pens were bedded with fresh rice straw and cleaned daily. These calves each recieved colostrum and milk from its mother until 2 weeks of age. Afterwards, they were provided with milk replacer and creep ration until weaned to dry feed and hay.

Skin test :

Four weeks after inoculation, skin reaction of vaccinated and control cows were tested by measuring the diffrence in double skin fold thickness before and 48 hours after intradermal inoculation of 0.1 ml tuberculin (PPBb). Increase of skin thickness of 4 mm and more was considered positive and 3 mm and less called negative.

Lymphocyte proliferation and culture :

Heparinized peripheral blood samples were obtained from the vaccinated and control cows 4 weeks after BCG inoculation and from the calves 4 weeks following parturition. The blood was washed once in RPMI (1640) medium and the cells were subsequently layed over FICOLI - Hypaque (d : 1.09 g/cc) and centrifuged at 1500 X g for 30 ninutes at 37°C. The

interface cells were washed once by centrifugation (1000 rpm, 8 minutes). The residual red cells were removed by treatment of the pallet with ACK lysing buffer. The cells were washed twice, counted and subsended in RPMI- 1640 medium supplimented with 2%. 1- glutamene 1% nonessenteal amino acids. 1% vitamins. 10 nm HEPES, 5 X 10^{-5}M. 2% mercaptoethanol, 50 ug/ml gentamycin and 5% fetal bovine serum, BROWN (1977).

One tenth of the cells were cultured with mitogen in flat bottom plates at 37°C and 5% CO_2 atmoshpere for 5 days. After this time the cells were harvasted on Whatman glass filter strip and the radioactivity was measured by liqued scantilation spectroscopy.

The results were recorded as the mean of the means ± the standdard errors of the mean (S.E.M) and immuno-stimulation index derived from the ratio of the stimulated cultures over the media control cutures.

Mitogen and antigen :

The mitogen and antigen used were Concanavalin A and Purified protein drivative (PPD). Each was titrared and used at the concentration of 10 ug/ml and 25 ug/ml, respectively.

RESULTS

Stimulation of cows and calves with BCG :

A comparison of skin test results with the proliferative reaponeses of immunized cows and controls are shown in Table (1). These results indicate a relationship between positive tubrculine tests and enhanced proliferative responses of lymphocytes after stimulation with PPD and with the T.cell mitogen, concandvallin- A. In general, the data suggestes that BCG vaccinated cows developed Sensitized T.cells.

In vitro lymphocyte proliferative activity of the control calves, calves from BCG vaccinated dams and calves from non vaccinated dams and received BCG immediatly after birth to Con A and PPD were determined and the results of the two separeted experiments are shown in Table (2).

These data were simplified by callculating an immunostimulation index for each of the experiments by dividing scantalation counts per minute for Con A stimulated or PPD stimulatd cells by the counts from cells not exposed to Con A or PPD.

Table 3, shows the immunostimulation index of dams and different groups of calves. It was observed that the proliferative index of the pregnant cows vaccinated with BCG were greater than that of the controls after stimulation with Con A and PPD. Indexes of neonates delivered from BCG inoculated dams are in general higher than of other groups.

DISCUSSION

In Egypt, many studies have demonestrated that the application of nonspecific immunostimulant agent such as BCG vaccine, is capable of raising resistance of cattle and sheep to soma viral, bacterial and parasitic diseases (BARAKAT 1978, BARAKAT, et al. 1981 and AWAD, et al. 1982). In addition, we observed in a previous study (SALEH et al. 1984) that the bovine neonates delivered from BCG vaccinated dams had morbidity and mortality rates lower than those of calves from non vaccinated calves.

Therefore, it was interesting to investigate the ability of the immune system of dams vaccinated with BCG at gestation period and their neonates as well as calves inoculated with BCG immediatly after birth and dilivered from non vaccinated dams. The detection of the immune responses was determined with the in vivo skin tuberculin test and in vitro lymphocyte proliferation assays. These assays have been applied by many investigators to demonestrate CMI responses in bovines with Mycobacterial compounds (HORLAND et al. 1978, ROSSI et al. 1978, 1979 and 1981, WOODARD et al. 1978 and 1979).

Thus in this study, a comparison of the skin tested results with the proliferative responses of the BCG vaccinated dams and control are shown in Table (1). This results indicated that the principale tested animals gave clear positive skin reactions, the average of skin thickness was in the average of 7 mm $\pm$ 1 mm, while no detectable reactions were shown in control groups. The cell proliferative activities of BCG vaccinated dams was greater than those of control following stimulation with Con A or PPD. From Table (3) it is shown that the stimulation index of vaccinated dams was doubled to PPD antigen (2.4) when compared to control dams (1.2), while the index of Con A was increased from (13.4) of normal cows to almost its 3 fold value (39.9) of immunized dams. These results indicated that BCG vaccinated pregnant cows have increased to respond in vivo and in vitro to antigen or to Con A. The effect of pregnancy and its associated immune suppresion on these responses were not investigated.

The lymphocyte proliferative reactivity of neonates delivered from BCG vaccinated dams, neonates from non-vaccinated dams and immediatly inoculated with BCG after birth as well as control calves delivered from non-vaccinated cows and not inoclated with the vaccine after birth were studied and the immune responses to Con A and PPD are shown in Table (2). The immunostimulation index of the same groups of calves were calculated and summarized in Table (3).

It appears that the proliferative responses of calves from BCG immunized cows were greater than that of the other groups. The stimulation index for calves from vaccinated dams were 16.9 and 28.1 for lymphocytes challenged with Con. A, and 6.4 and 37.2 after PPD stimultion, as compared to indexes of 7.1, 7.1 and 2.1, and 2.8 after Con A and PPD stimulation of cells from control calves.

These data suggested that the neonates delivered from BCG immunized dams had received CMI from their mothers and developed lymphocyte proliferation responses at rates comparable to adult cattle.

The question is, how CMI was transmitted from BCG was vaccinated dams to their neonates ?. Three general possibilities should be considered.

1- Sensitized T. cells may be transmitted through colostrum and milk from the vaccinated dams to their neonates. TIZARD, 1982).

2- The attenuated Tubercle bacilli may pass via the colostrum and milk to the newborn calves.

3- Transfere factors or lymphokines from the cells of the BCG stimulated dams may have passed the placenta and reach the foetal circulation.

Furthur studies should provide additonal information on the role of cell - mediated immunity transferred from bovine dams to their neonates in the enhanced nonspecific resistance stimulated by BCG vaccine.

REFERENCES

Awad. F.I., Y.Z. El-Abdin, A.A. Fayed and S.M. Saleh, (1982): Vaccination of camels and mice against Trypanosoma 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 bacillus of Calmette and Guerin (BCG). Bull. Off. Int. Epis. 1979, 91: (9 - 10), 679 - 692.

Barakat, A.A., M.S. Saber, Nafei Emad, S. Abdel Ghaffar, M.M. H. El-Nimr, K. Sobhy, M. Fathia, A.T.A. Mohsen, and S. El-Mahashly (1981): Primininary studies on the use of BCG as an immunopotantiating 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 vito lymphocyte transformation to specific antigens. J. Immunol. Meth. 18: 17 - 32.

Brown, T.T., Shults, E.D. and Duncan, J.R. (1979): Serological responses of the bovine foetus to bovine viral diarrhea virus. Infec. Immunol. 25: 93 - 97.

Eberhart, R.J. and John, A.P. (1971): Plasma cortisol concentrations in neoborn calves. Am. Jour. Vet. Res. 32: 1921 - 1927.

Horland, W. Renshaw, William, P. Exbland, Dale, O. Everson, Patricia, D, Tessinari and Dabolie Anos. (1971): Otogeny of immunocompotance in cattle. Evaluation of phytomitogen induced in vitro on bovine foetal lymphocyte Bastogenesis, using a whole blood culture technique. Am. Jour. Vet. Res. 33: 1141 - 1150.

Osburn, P.I., Stabenfeild, C.H. and Ardans, A.A. (1974): Prenatal immunity in calves. Am. Jour. Vet. Res. 164: 298 - 300.

Olson, D.P. and Waxler, G.L. 61979): Immune response of the bovine foetus and neonate to Ecsherichia coli. quantitative and qualitative of the humoral immune response. Am. Jour. Vet. Res. 37: 693 - 697.

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

Rossi, C.R., Kiesel, G.K. and Hudson, R.S. (1979): Kinities of detection of Blastogenetia responses of nenoatal calves inoculated in utero with tetanus toxoid killed Mycobacterium bovi and killed Brucella bovis and killed Brucella abortus. Am. Jour. Vet. Res. 40: 578 - 679.

Rossi, C.R., Kisie, 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 Hypersnsitivity responses. Am. Jour. Vet. Res. 42: 1369 - 1370.

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

Shultz, R. (1973): Developmental aspects of the foetal bovine immune responses. A review, Cornell, Vet. 63: 503 - 535.

Soliman, I.M. (1971): Frontiers of biological series. Foetal and neonatal immunity. Vol. 20 North Holand American Elaevier. Amesterdam and New York.

Stortz, G., Young, S. Coroll, E.G. (1976): Parvo virus infection of the bovine foetus. Distribution of infection, antibody response and age - related suseeptibility. Am. Jour. Vet Res. 3: 1099 - 1102.

Tizard, I. (1982): An introduction to vetrinary immunology. W.B. Saunbara Company Bhiladelphia, California. Wamukoye, G.P.

Wamukoye, G.P. and Cornner, G.A. (1978): Local immune response in the bovine foetus vaccinated with Eschereshia coli antigens in utero. Am. Jour. Vet. Res. 37: 159 - 163.

Woodard, L.F., Renashw, H.W. and Burger, D. (1978): Cell-mediated immunity in neonatal calves: Delayed Hypersensitivity and Blastogenesis following immunization with tuberculo protein of Mycobacterium bovis. Am. Jour. Vat. Res. 39: 579 - 584.

Woodard, L.F., Ranshaw, H.W. and Burger, D. (1979): Onset kenitics and duration of in vivo and in vitro Delayed Hypersensitivity responses of neonatal calves sensitized with Mycobacterium compounds. Vet. Micriol. 2: 169 - 176.

Yamini, B. Slieght, S.D. (1980): Immunoglobuline responses of the bovine foetus and neonate to Escherishia coli. Am. Jour. Vet. Res. 37: 639 - 647.

Zeman, B.C., Coben, G. Budrys, M. Williams, G. and Gabor, H.H. (1972): The effect of plasma cortisol biols on the lymphocyte transformation test. Jour. Allergy. Clin. Immunol. 49: 10 - 15.

Table (1)

Comparison of in vivo skin test reactivity and in vitro proliferative responses of control and BCG vaccinated pregnant cows

Group	(N)	Skin test (mm)	Counts per min ± S.E.M (a)		
			Media	Con A	PPD PPD
1. Cont	8	0	8661± 1748	62934 ± 12033	6500 ± 1962
2. BCG	8	7 ± 1	13414 ± 4328	85311 ± 14639	29610 ± 9154

(a) Lymphocytes from individual calves were cultured as described in the materials and methods section. Results represented the mean of the means from triplicate cultures ± S.E.M.

(N) Number of animal tested.

Table (2)

Proliferative responses of control calves, calves from BCG vaccinated dams and calves of unvaccinated dams inoculated with BCG after birth

Group	(N)	Counts per minutes ± S. E. M.		
		Media	Con A	PPD
Experiment I	5	7643 ± 3354	54069 ± 15133	11147 ± 13786
I. Control (a)	5	1818 ± 811	30708 ± 5574	11652 ± 4390
II. BCG dams (b)	6	5567 ± 1974	19809 ± 3388	28845 ± 2086
III. BCG postpart.(c)	6	5567 ± 1974	19809 ± 3388	28845 ± 2086
Experiment II:				
I. Control (a)	3	5168 ± 2702	21949 ± 3842	11287 ± 3419
II. BCG dams(b)	3	320 ± 66	7230 ± 3000	11119 ± 4454
III. BCG postpat.(c)	4	3620 ± 2183	15193 ± 2055	23581 ± 7540

(N) Number of animals tested.
(a) Normal calves from non vaccinated dams.
(b) Calves from BCG vaccinted dams.
(c) Calves from normal dams and vaccinated with BCG postpartum.

Table (3)

Mean Immuno-stimulation Index of dams vaccinated with BCG and control group, and calves delivered from immunized cows, calves vaccinated with BCG postpartum and control group

Animals	Groups	(N)	Con A	PPD
Preg. Cows	I	8	33.4	1.2
	II	8	37.9	2.4
Calves	Expt.(1)			
	I	5	7.1	2.1
	II	5	16.9	6.1
	III	6	2.7	4.6
	Expt. (2)			
	I	3	7.1	2.8
	II	3	28.1	37.2
	III	4	8.7	12.7

- (N) Number of animals. - Preg. Cows: Group I Dams not vaccinated with BCG (control). Group II Dams vaccinatec with BCG.
- Calves Group I (control). Group II Calves from BCG vaccinated dams. Group III Calves from non vaccinated dams and inoculated with BCG postpartum.