تنشيط المناحة الخلوية في العجل حدثة الولادة
من أبقار محصنة بلقاح A بـ سي جي

صفيت صالح، ريجينا اسکاتي*، بولس بطرس*، عبد المنعم بركات**
اسماعيل رضا***، عماد نافع****، محمد زكي

أجريت الدراسة على ثمانية بقرات عشية في الشهر السادس وذلك بحفلتها
في الحلد بلقاح A بـ سي جي . أعطت هذه الأبقار رد فعل إيجابي
لهذا التحصين عند ما أختبرت بواسطة اختبار الجلد واختبار تعرُّم الخلايا
الليمفاوية .

بقياس مدى تأثير الجهاز المناعي للعجل المولد من هذه الأبقار بعمل
اختبار تعرُّم الخلايا الليمفاوية بها ومقارنتها بعجل حديثة الولادة من
أبقار غير محصنة بلقاح A بـ سي جي وكذا عجل حديثة الولادة من أبقار
غير محصنة وحصنت هذه العجل فور ولادتها بلقاح A بـ سي جي، وجد
أن التأثر المناعي بدقة كبيرة في العجل الناتجة من الأبقار المحصنة
عن باقي مجموعات العجل كما كانت الحالة الصحية أصح ولا يوجد نفوق
في العجل المولودة من الأبقار المحصنة .

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مرکز البحوث الطبية للبحوث الا مريكية.

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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)

By
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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 elicited 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; WAMUEYA and CORNER 1978 and YAMINA & SLEIGHT 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 with poor protection. This is attributed to the physiologic and anatomic characteristics of bovine placenta (OSBURN et al., 1974). In addition, the bovine foetus produces detectable quantities of glucocorticoids which remain demonstrated in the plasma up to 12 days and are known to be immunosuppressive with the most pronounced effect upon T. lymphocytes (EERHART and PATT, 1971 and ZEMAN et al. 1972). The newborn calf receives antibodies in colostrum from its mother establishes within hours a state of humoral immunity. There is some evidence suggested that maternal T-cells may be transferred to neonate through colostrum, 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 immediately 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 approximately 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 X 10⁶ attenuated living BCG mycobacteria per 1.0 ml. The calves delivered from group 2 (10 dams) were inoculated intradermically 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 prior to the expected 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 received 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 difference in double skin fold thickness before and 48 hours after intradermal inoculation of 0.1 ml tuberculin (PPDb). 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 layered over FICOLI - Hypaque (d : 1.09 g/cc) and centrifuged at 1500 X g for 30 minutes at 37°C. The
BCG VACCINATION

interface cells were washed once by centrifugation (1000 rpm, 8 minutes). The residual red cells were removed by treatment of the paillet with ACK lysing buffer. The cells were washed twice, counted and subsed in RPMI-1640 medium supplemented with 2% L-glutamine 1% nonessential amino acids, 1% vitamins, 10 nm HEPES, 5 x 10^{-5} M 2% mercaptoethanol, 50 μg/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% CO₂ atmosphere for 5 days. After this time the cells were harvested on Whatman glass filter strip and the radioactivity was measured by liquid scintillation spectroscopy.

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

Mitogen and antigen:

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

RESULTS

Stimulation of cows and calves with BCG:

A comparison of skin test results with the proliferative reponseses of immunized cows and controls are shown in Table (1). These results indicate a relationship between positive tuberculin tests and enhanced proliferative responses of lymphocytes after stimulation with PPD and with the T-cell mitogen, concandwallin-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 immediately after birth to Con A and PPD were determined and the results of the two separated experiments, are shown in Table (2).

These data were simplified by calculating an Immunostimulation index for each of the experiments by dividing scintillation counts per minute for Con A stimulated or PPD stimulated 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 demonstrated that the application of nonspecific immunostimulant agent such as BCG vaccine, is capable of raising resistance of cattle and sheep to some 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 immediately after birth and delivered 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 demonstrate 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 principal tested animals gave clear positive skin reactions, the average of skin thickness was in the average of 7 mm ± 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 suppression on these responses were not investigated.

The lymphocyte proliferative reactivity of neonates delivered from BCG vaccinated dams, neonates from non-vaccinated dams and immediately inoculated with BCG after birth as well as control calves delivered from non-vaccinated cows and not inoculated 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 stimulation, 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- Transfer factors or lymphokines from the cells of the BCG stimulated dams may have passed the placenta and reach the foetal circulation.

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

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### Table (1)

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Skin test (mm)</th>
<th>Media</th>
<th>Counts per min</th>
<th>S.E.M (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cont 8</td>
<td>0</td>
<td>8661±1748</td>
<td>62934±12033</td>
<td>6500±1962</td>
</tr>
<tr>
<td>2. BCG 8</td>
<td>7±1</td>
<td>13414±4328</td>
<td>85311±14639</td>
<td>29610±9154</td>
</tr>
</tbody>
</table>

(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.

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### BCG Vaccination

#### Table (2)

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

<table>
<thead>
<tr>
<th>Group</th>
<th>(N)</th>
<th>Media</th>
<th>Counts per minutes ± S. E. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Con A</td>
</tr>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Control (a)</td>
<td>5</td>
<td>7643 ± 3354</td>
<td>54069 ± 15133</td>
</tr>
<tr>
<td>II. BCG dams (b)</td>
<td>6</td>
<td>5567 ± 1974</td>
<td>19809 ± 3388</td>
</tr>
<tr>
<td>III. BCG postpartum (c)</td>
<td>6</td>
<td>5567 ± 1974</td>
<td>19809 ± 3388</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Control (a)</td>
<td>3</td>
<td>5168 ± 2702</td>
<td>21949 ± 3842</td>
</tr>
<tr>
<td>II. BCG dams (b)</td>
<td>3</td>
<td>320 ± 66</td>
<td>7230 ± 3000</td>
</tr>
<tr>
<td>III. BCG postpartum (c)</td>
<td>4</td>
<td>3620 ± 2183</td>
<td>15193 ± 2055</td>
</tr>
</tbody>
</table>

(N) Number of animals tested.
(a) Normal calves from non-vaccinated dams.
(b) Calves from BCG vaccinated 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

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

(N) Number of animals.
- Preg. Cows: Group I Dams not vaccinated with BCG (control).
- Group II Dams vaccinated 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.