VITAMIN A DEFICIENCY: SERUM CORTISOL AND HUMORAL IMMUNITY IN LAMBS

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ABSTRACT

Serum cortisol and antigen-specific and polyclonal immunoglobulin G (IgG) concentrations were measured to investigate the relationship between vitamin A status and immune function in lambs. Twenty-four 3-mo-old crossbred ewe lambs weighing approximately 10 kg were each fed 900 g/d of a carotene-deficient diet. The 12 control lambs also received a 100,000 IU oral dose of vitamin A palmitate every 2 wk. All lambs were given primary and secondary antigenic challenges. Lambs were slaughtered at the end of the secondary challenge period. Liver vitamin A concentrations were greater (P < .0001) in the control animals (69.5 vs 1.3 μg/g wet tissue). Both groups of lambs exhibited a similar growth response until d 105, after which daily gain of the control lambs exceeded (P < .03) that of the A-deficient lambs. Polyclonal serum IgG concentrations were greater (P < .05) in the A-deficient lambs on d 49 to 124 and on d 151 (P < .10). Ovalbumin-specific serum IgG concentrations tended to be greater in the control lambs throughout the primary and secondary challenge periods. Control lambs had greater titers on d 164 (P < .07) and d 190 (P < .03). Vitamin A status appeared to have no consistent effects on serum cortisol concentrations. Spleen weights were greater (P < .002) in the A-deficient lambs. Lungs from 11 of 12 A-deficient lambs contained abscesses, as opposed to 1 of 12 for the control lambs. Both polyclonal and antigen-specific IgG concentrations were affected by vitamin A status. Serum cortisol concentrations did not appear to mediate this effect.

(Key Words: Vitamin A Deficiency, Hydrocortisone, Immunoglobulins, Immunity, Sheep.)


Introduction

Both the frequency and severity of bacterial, viral and protozoal infections are increased in vitamin A-deficient (A-def) animals (Bang and Foard, 1972; Cohen and Elin, 1974; Darip et al., 1979). Vitamin A-deficient animals have decreased splenic and thymic mass (Bang et al., 1973; Krishnan et al., 1976), decreased splenic lymphocyte response to mitogens (Nauss et al., 1979), decreased serum and secretory immunoglobulin (Ig) levels (Ludovici and Axelrod, 1951; Sirisinha et al., 1980) and decreased number and activity of macrophages (Krishnan et al., 1976) but normal serum complement levels (Madjid et al., 1978). The physiological bases behind these observations, however, are not clearly understood.

Vitamin A has been proposed to affect glucocorticoid production. Glucocorticoid concentrations are elevated in vitamin A-deficient rats (Gruber et al., 1976), sheep (Webb et al., 1969) and chicks (Perek and Kendler, 1969). In turn, glucocorticoids decrease serum Ig levels (Butler and Rossen, 1973), decrease proliferation of T lymphocytes, decrease production of gamma interferon (Munck et al., 1984) and decrease monocyte chemotaxis as well as phagocytosis (Rinehart et al., 1974). Any circumstance that may elevate glucocorticoids could have a detri-
mental effect on the ability of an animal to respond to an antigen.

The objective of the present study was to investigate the relationships between vitamin A deficiency, serum cortisol concentrations and humoral immune function in lambs.

**Experimental Procedure**

Twenty-four crossbred ewe lambs weighing approximately 10 kg were blocked according to weight and assigned randomly within block to either a control or A-def treatment. The lambs were treated for internal parasites and vaccinated against clostridia. The animals were housed under constant lighting conditions on a raised, expanded metal floor in a pen 2.5 m x 10.0 m equipped with automatic nipple waterers and were fed twice daily. The lambs were fed as a group at the rate of 900 g.hd\(^{-1}\).d\(^{-1}\) of a whole oats diet (Table 1) and injected monthly with vitamins D\(^6\) (50,000 IU/mo) and E\(^7\) (600 IU/mo) and with Se\(^8\) (0.05 mg.kg\(^{-1}\).mo\(^{-1}\)). The control lambs also received 100,000 IU oral doses of vitamin A palmitate\(^9\) in capsule form every 2 wk. Eight weeks into the feeding period, a bleeding regimen was initiated in which the animals were bled via jugular puncture and weighed every 2 wk for 4 mo. Throughout the study, blood samples were taken at 0900. Serum was harvested and frozen (-20°C) for later analysis of vitamin A, IgG and cortisol concentrations.

Upon completion of the 4-mo bleeding period, jugular catheters were placed into each ewe lamb on d 108 and the animals were placed into metabolism stalls to facilitate accessibility to the catheters. Blood samples were obtained via catheter during the morning feeding because lambs seemed most interested in eating then and were disturbed minimally by this sampling process. Blood samples were taken on d 112, 113, 115, 117, 124 and 151 with serum samples again being frozen for later analysis. The lambs were challenged on d 151 with 1 mg ovalbumin\(^10\) in 1 ml of Freund’s complete adjuvant. Half of this solution was injected i.m. into the hind leg with the remainder of the solution injected s.c. in five different sites along the lateral thorax. Blood was obtained on d 157, 164, 171 and 185. Upon completion of the first challenge period, the lambs were rechallenged on d 185 as before with blood taken daily for 6 d. At the end of the second challenge period, the lambs were weighed and slaughtered and spleen weights were obtained. Liver samples were taken at this time and frozen (-20°C) for analysis of liver vitamin A concentrations. Gross postmortem observations also were made at this time.

Serum vitamin A, cortisol and both ovalbumin-specific and polyclonal IgG concentrations were monitored throughout the study. Vitamin A was extracted by the method of Kimble (1939) and the color was developed by the trifluoroacetic acid method of Dugan et al. (1964). Liver vitamin A levels were determined as described by Gallup and Hoefer (1946). Polyclonal serum IgG concentrations were elucidated by a modification of the radial immunodiffusion technique (Mancini et al., 1965) and serum cortisol concentrations were determined using a single antibody radioimmunoassay\(^11\).

Antigen-specific serum IgG concentrations were determined using a modification of the enzyme-linked immunosorbent assay (ELISA) as described by O’Sullivan et al. (1979). The concentration of antigen used to coat the microtiter

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<table>
<thead>
<tr>
<th>TABLE 1. COMPOSITION OF DIET FED TO LAMBS(^a)</th>
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<tbody>
<tr>
<td><strong>Item</strong></td>
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<tr>
<td>Whole oats</td>
</tr>
<tr>
<td>Molasses</td>
</tr>
<tr>
<td>Limestone</td>
</tr>
<tr>
<td>Trace mineral salt(^b)</td>
</tr>
<tr>
<td>Vit. D(^6)</td>
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<tr>
<td>Vit. E(^7)</td>
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<tr>
<td>Se(^8)</td>
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<tr>
<td>Vit. A(^9)</td>
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\(^{a}\) As-fed basis.

\(^{b}\) Composition: NaCl (98.5%), Fe (.232%), Mn (.225%), Zn (.250%), Mg (.100%), S (.040%), Cu (.023%), Co (.007%) and I (.007%).

\(^{c}\) Individual monthly injection of 50,000 USP.

\(^{d}\) Individual monthly injection of 600 IU.

\(^{e}\) Individual monthly injection of .05 mg/kg.

\(^{f}\) 100,000 IU vitamin A palmitate in capsule form every 2 wk for control lambs only.

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\(^4\) Levasole, Pitman-Moore, Washington Crossing, NJ.

\(^5\) Ultrabac-7, Beecham Laboratories, Bristol, TN.

\(^6\) Taylor Pharmacol Co., Decatur, IL.

\(^7\) Anchor Laboratories, St. Joseph, MO.

\(^8\) Burns-Biotic, Omaha, NE.

\(^9\) R. P. Scherer Corp., Clearwater, FL.

\(^10\) Miles Laboratories, Inc., Naperville, IL.

\(^11\) Amerlex Cortisol RIA Kit, Amersham Corp., Arlington Heights, IL.
plate was 5 μg/ml. Initial serum dilutions were 1:250 and the enzyme-labeled antibody used was a 1:5,000 dilution of a peroxidase conjugated rabbit anti-sheep IgG. A 1:5,000 dilution of a peroxidase conjugated rabbit anti-sheep IgG was used as the substrate for the peroxidase enzyme. The reaction was allowed to proceed for 30 min, at which time 5% sodium dodecyl sulfate was added to each well to stop the reaction. The optical density of each well was determined with a microtiter plate reader at 415 nm. The titer was equal to the dilution at which the optical density was equal to that of the negative control.

Treatment differences were analyzed by least squares ANOVA utilizing the GLM procedures of SAS (1979). For all variables, treatment comparisons were made within a sampling date with the model including treatment and block as fixed effects.

Results and Discussion

Serum vitamin A concentrations at the beginning of the bleeding regimen were 42.3 and 22.3 μg/dl for the control and A-def lambs, respectively. Vitamin A concentrations for the control lambs remained relatively constant due to vitamin A supplementation, whereas vitamin A concentrations for the A-def lambs were reduced to 3.5 μg/dl just prior to catheterization on d 108 and 3.1 μg/dl at the time of the first ovalbumin challenge on d 151 (Figure 1). Vitamin A deficiency symptoms included nervousness, muscular weakness, staggered gait, convulsions and death after completion of this experiment. The muscular weakness and staggered gait may have been the result of an increased cerebrospinal fluid pressure, which accompanies vitamin A deficiency (Eaton et al., 1961). Liver vitamin A concentrations observed upon completion of the study were 69.5 and 1.3 μg/g wet tissue for the control and A-def lambs, respectively.

Control and A-def lambs exhibited similar growth rates until d 105, at which time ADG (P < .03) of the control lambs exceeded that of the A-def lambs. Weights of the A-def lambs dropped from 47.6 kg on d 105 to 36.3 kg on d 207. Control lambs gained weight during this period, increasing BW from 49.6 kg to 51.8 kg (Figure 2). The weight loss observed in the A-def lambs from d 105 to d 207 probably is the result of a loss of appetite, which accompanies vitamin A deficiency (Anzano et al., 1979).

Serum cortisol concentrations prior to catheterization followed a cyclic pattern of about a 56-d duration and the A-def lambs tended to have the lower mean concentration (Figure 3). Serum

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12Pel-Freez Biologicals, Rogers, AR.
13Titertek Multiscan MCC/340, Flow Laboratories, McLean, VA.
Figure 3. Mean serum cortisol concentrations in control (●) and vitamin A-deficient (●) lambs. Lambs were catheterized on d 108. To avoid clutter, SE bars were omitted on d 113 and 117; the values are 2.55 and .65, respectively.

cortisol concentrations were lower in the A-def lambs on d 91 (P < .10) and 105 (P < .02). Following catheterization (d 108), cortisol concentrations in the control lambs continued to decline, whereas cortisol concentrations in the A-def lambs rose. Just prior to the first ovalbumin challenge (d 151), serum cortisol concentrations were greater (P < .04) in the A-def lambs (Figure 4). Serum cortisol concentrations increased in both groups of lambs up to the second ovalbumin challenge (d 185) but were not different (P > .10) between treatments. Serum cortisol concentrations continued to rise in the A-def lambs to the end of the study, whereas they decreased slightly in the control lambs. Vitamin A status had no consistent effect on serum cortisol concentrations throughout the study.

Prior to catheterization (d 108), serum IgG concentrations were higher (P < .002) in the A-def lambs with means of 24.2 mg/ml vs 15.5 mg/ml for the control lambs (Figure 5). Both groups of lambs responded with a linear (P < .0001) increase in serum IgG concentrations following catheterization. Polyclonal serum IgG concentrations increased throughout the study in both treatments. Mean values for the two treatments were not different (P > .10) from d 151 to the end of the study. A breakdown in epithelial tissues lining the respiratory tract is associated with vitamin A deficiency and may explain the increased polyclonal serum IgG concentrations in the A-def lambs prior to catheterization. A breakdown in the epithelial tissues, which function as an animal’s first line of defense against
invading organisms, would result in an increased presence of antigens and a concomitant increase in polyclonal serum IgG. The increase in polyclonal serum IgG concentrations following catheterization probably was due to an increase in bacterial invasion as a result of the catheter. This could explain the increase in polyclonal serum IgG concentrations in both treatment groups.

Both the control and A-def lambs responded with an increase in ovalbumin-specific serum IgG concentrations following the primary ovalbumin challenge on d 151 (Figure 6). In the control lambs, ovalbumin-specific serum IgG concentrations reached a peak on d 164 and then declined to d 185. In the A-def lambs, ovalbumin-specific serum IgG concentrations in response to the primary challenge did not reach a maximum until d 185. Control lambs had greater ($P < .07$) ovalbumin-specific IgG concentrations on d 164. Following the secondary challenge (d 185), the control lambs again tended to have higher antigen-specific IgG titers. Titers were greater ($P < .03$) in the control lambs on d 190.

In both the primary and secondary ovalbumin challenge periods, the control lambs were able to produce a greater amount of antigen-specific serum IgG in a shorter period of time compared with the A-def lambs. The decline in antigen-specific IgG concentrations in the control lambs from d 164 to 185 may suggest a greater efficiency of antigen clearance on the part of these same animals. If the control lambs were more efficient in clearing the ovalbumin from their system, less antigen would be present to stimulate antibody production and their titers would decline. Conversely, if the A-def lambs were less efficient in removing the antigen from their systems, the animals would be exposed to the antigen for a longer period of time and, thus, antibody titers would not fall so quickly. Both a decrease in phagocytosis (Krishnan et al., 1976) and a deterioration in antigen-stimulated lymphocyte trapping (Takagi and Nakano, 1983) have been observed in vitamin A-deficient animals. These malfunctions acting alone or in concert could explain the differences in antibody production observed in the primary challenge period. The decreased antibody response in the A-def lambs during the secondary challenge period may suggest a decrease in the number and(or) activity of memory cells, which are primarily responsible for the secondary response.

Spleen weight also was observed to be greater ($P < .002$) in the A-def lambs (Table 2). This is in contrast to Nauss and Newberne (1985), who observed no differences in spleen weights in control and A-def rats and to Krishnan et al. (1976), who reported decreased splenic weights in A-def animals. The enlarged spleens in the A-def lambs may have been in response to infections, which are more prominent in A-def animals. Eleven of the 12 A-def lambs had lung abscesses, whereas this number was only 1 of 12 for the control lambs. However, all lambs survived to the end of the trial. Whether these lung abscesses were due to the effects of vitamin A on epithelial tissue, the immune system or a combination of the two is not known.

**Implications**

Results of the present study indicate that severe vitamin A deficiency in lambs is associated with alterations in immune function. These
changes do not appear to be mediated through changes in serum cortisol concentrations. The exact mechanism(s) associated with the effects of vitamin A on immune function in lambs remains unclear. Whether the nature and/or severity of these alterations would be the same in a less severe state of vitamin A deficiency is uncertain.

Literature Cited