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CHARACTERIZATION OF LYMPHOID CELLS IN GOATS WITH CASEOUS LYMPHADENITIS

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CHARACTERIZATION OF LYMPHOID CELLS IN GOATS WITH CASEOUS LYMPHADENITIS

by

Jane Ann Hedden

A Thesis Submitted to the Faculty of the
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In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
WITH A MAJOR IN MICROBIOLOGY

In the Graduate College
THE UNIVERSITY OF ARIZONA

1985
STATEMENT BY AUTHOR

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ABSTRACT

The purpose of this investigation was to study the role of cell mediated immunity (CHI) in caprine caseous lymphadenitis. FITC conjugated lectins and anti-goat IgG were used in direct immunofluorescence assays upon lymphoid cells from tissues of young, adult, and diseased goats. Studies reveal 1) in young goats, thymus and spleen have the greatest number of cells binding peanut agglutinin and anti-IgG respectively, 2) young goats have fewer peripheral blood lymphocytes (PBL) binding suboptimal concentrations of concanavalin A and wheat germ agglutinin, and both concentrations of peanut agglutinin, than adults, 3) diseased goats have fewer PBL binding optimal concentrations of concanavalin A, Lens culinaris agglutinin and peanut agglutinin than healthy adults, and 4) diseased and healthy adults can be distinguished from healthy adults based upon the lectin binding ability of their PBL indicating a possible compromised CHI in the goats with caseous lymphadenitis.
CHAPTER 1

INTRODUCTION

Caseous lymphadenitis is a debilitating chronic bacterial disease caused by *Corynebacterium pseudotuberculosis*. The bacterium was formerly called *C. ovis* as its main reservoir is sheep, (1,2) but the organism is also pathogenic for other animals such as goats, horses, deer, cattle and with rising frequency, man (3-5). Once in the host, *C. pseudotuberculosis* is carried to regional lymph nodes where it multiplies leading to enlarged caseous nodes which may be visible externally. The organism may also spread throughout the host causing systemic infections, the appearance of purulent lesions in internal organs, and eventually pronounced lethargy in the host (2,6).

*C. pseudotuberculosis*—the etiological agent

*C. pseudotuberculosis* is a gram-positive, non-motile, non-acid fast, non-sporeforming coccobacillus yielding small, opaque white colonies with minimal beta-hemolysis on 5% sheep blood agar. Further biochemical characterizations indicate that the organism is catalase and urease positive and Voges-Proskauer and nitrate reduction negative for caprine strains. The use of cystine trypticase agar sugar slants and peptone water sugars indicate that *C.*
*C. pseudotuberculosis* produces acid from glucose and maltose, but not from sucrose or lactose. Gelatin inoculated with *C. pseudotuberculosis* is liquified after 14 to 21 days at 30°C. *C. pseudotuberculosis* may be differentiated from a similar organism, *Corynebacterium ulcerans*, by its inability to ferment starch and trehalose (3,7,8).

*C. pseudotuberculosis* resides in soil, manure, on skin and in the tissues of infected animals. While infection may occur through skin or mucous membranes via ingestion of contaminated foodstuffs or soil inhalation (9), it usually originates from direct contact with an infected animal. In the latter case, purulent material containing the bacteria enters the healthy host through a break in the skin barrier (1). In the former case, *C. pseudotuberculosis* has been shown to survive for up to 55 days on barnyard fomites, up to 60 hours in river water, and up to 8 months in soil (10-12). Consequently, the healthy animal with or without skin abrasions need only contact an area previously contaminated by an infected animal shedding bacteria from a ruptured abscess to become infected (13).

**Pathogenesis**

*C. pseudotuberculosis* manufactures an exotoxin possessing phospholipase D activity which cleaves membrane bound sphingomyelin on erythrocytes and reticuloendothelial cells leading to increased vascular permeability and
cellular infiltration. The resulting influx of plasma from blood vessels at the infection site moves \textit{C. pseudotuberculosis} to the regional lymph nodes via the afferent lymphatic ducts (4,14). In the node, exotoxin induced polymorphonuclear leukocytes and macrophages phagocytose \textit{C. pseudotuberculosis} which is surrounded by a waxy lipid-like layer. This waxy layer allows the organism to survive within the phagolysosome (4,5,15). In murine macrophages, the lipid functions much like an endotoxin causing a decrease in glycolysis within the host cell as well as damage to nuclear and lysosomal membranes (15). The degenerative effect on membranes is not evident in caprine macrophages (6). The lipid coat also promotes adhesion of bacteria to one another thus concentrating the exotoxin and helping to establish caseous lymphadenitis (5,16).

In the infected node, the influx of leukocytes and the accumulation of cellular debris cause the node to take on a caseous appearance. In sheep, the node appears laminated much like the layers of an onion. In goats, however, the node is filled with an amorphous, greenish-white purulent material enclosed in a fibrous capsule (6,9,14,15). From this point, the infection may spread to internal organs via hematogenous and lymphogenous routes.
Treatment and Prevention

Although *C. pseudotuberculosis* is susceptible to antibiotics such as erythromycin and tetracycline, infected individuals usually require excision of the infected node. Even then, the recovery process is lengthy (3). Consequently, a vaccine seems to be a practical solution to preventing the spread of caseous lymphadenitis.

Current vaccines are unfortunately proving to be unsuccessful at preventing infection. In some cases the vaccine only decreases the number of caseous lesions in *C. pseudotuberculosis* challenged animals while in other cases, the vaccine permits the formation of sterile abscesses (17-19). To compound the problem, a vaccine useful in one species may not protect a different species (20).

The development of a successful vaccine for use in goats depends in part on determining the relative roles of CMI and humoral immunity in resolution of chronic *C. pseudotuberculosis* infection (5,20). Much controversy exists as to the relative importance of these branches of host defense in caseous lymphadenitis. Part of the controversy may be associated with lack of a clear understanding of CMI and a lack of appropriate methods for measuring the various aspects of CMI. Moreover, CMI and humoral immunity must be examined both separately and in conjunction with one another. It may be that in chronic
caseous lymphadenitis, a compromised CHI leading to less than optimally functioning humoral immunity has led to disagreement over which plays the dominant role.

**Humoral Immunity**

Several studies support the role of antibody as being of primary importance in the resolution of chronic caseous lymphadenitis. Cameron and Fuls (21) measured agglutination titers in sheep which had been immunized with formalin inactivated *C.* pseudotuberculosis plus an aluminum phosphate adjuvant. They found that the vaccine caused a moderate elevation in anti-*C.* pseudotuberculosis titers and appeared to provide the sheep with greater resistance to subacute infection.

Using the hemolysin inhibition test, Lund et al. (22) studied anti-*C.* pseudotuberculosis antibodies in the colostrum and serum of infected female goats and in the serum from their kids to see if anti-hemolysin could passively confer protection upon the kids. They reported a positive correlation between anti-hemolysin levels in the serum and colostrum of each dam and a moderately positive correlation between the quantities of anti-hemolysin in the colostra of the dams and the sera of their kids. The anti-hemolysin levels in the kids began to decrease at 3 days postpartum to undetectable levels at two and one-half months of age. As caseous lymphadenitis is rarely diagnosed in
kids under three months of age, it appears that anti-
hemolysin in the dams is transferred to the kids via
colostrum and may be protective against infection.

Desiderio, Turillo and Campbell (23) examined serum
proteins photometrically and electrophoretically in normal
goats three months to one year of age, normal goats one year
of age and older and C. pseudotuberculosis infected goats
one year of age and older. The mean concentration of total
serum proteins was 59 mg/ml in the young goats, 69 mg/ml in
normal goats and 74 mg/ml in goats with caseous
lymphadenitis. Analysis of electrophoresis patterns showed
that the higher protein concentration in infected goats was
due to an increased gamma globulin fraction and appeared to
be antibody directed towards circulating C. pseudotuberculosis antigen in the host.

Burrell (24), using a hemolysis inhibition test to
detect antibody to C. pseudotuberculosis, showed that sheep
infected with non-toxigenic strains of C. pseudotuberculosis
possessed no antibody three weeks post infection while sheep
infected with toxigenic strains had antibody titers that
increased from 2 to 137 in the same time period (25).

Using C. pseudotuberculosis cell wall antigen in an
enzyme linked immunosorbent assay, Shen, Jen and Gorham (26)
demonstrated an increase in anti-C. pseudotuberculosis
antibody in the serum of experimentally infected goats.
This response was first seen 2 weeks post infection, it peaked 5 weeks post infection and although it declined slightly, remained elevated throughout the infection.

**Cell Mediated Immunity**

The branch of host defense responsible for phenomena such as transplant rejection, destruction of virus infected cells and delayed type hypersensitivities is known as cell mediated immunity (CMI). Effectors of CMI include macrophages and T-lymphocytes; antibody appears to be insignificant. CMI may also be of primary importance in certain intracellular bacterial diseases such as caseous lymphadenitis (26).

Elberg (27) and Hackaness (28) first demonstrated that immunity to facultative intracellular parasites was associated with an enhanced killing ability of reticuloendothelial cells subsequent to host infection. North (29) showed the primary role of CMI in controlling chronic infection caused by the facultative intracellular bacteria *Listeria monocytogenes* and *Mycobacterium tuberculosis*.

Gameel and Tartour (30) infected sheep subcutaneously with *C. pseudotuberculosis*. The peripheral blood of the animals showed increased leukocyte and polymorphonuclear leukocyte counts 2 days post infection. The counts decreased on day 4, but rose again during the
second week of the infection. While monocyte and eosinophil counts remained fairly stable, lymphocyte numbers decreased. Concurrently, total protein, albumin and gamma globulin levels dropped and remained low.

Irwin and Knight (31) studied the effects of levamisole on *C. pseudotuberculosis* infection in mice. Levamisole, which potentiates CHI, was administered subcutaneously to mice on days 0 and 30 of the experiment. The mice were challenged with $6 \times 10^5$ virulent *C. pseudotuberculosis* organisms on day 41, and deaths were recorded. In spite of lowered serum immunoglobulin levels, these mice demonstrated a prolonged, non-specific enhancement of resistance to infection. Since studies showed that levamisole was not bactericidal and did not appear to have a prolonged effect upon phagocytosis the authors suggested that levamisole may exercise a mitogenic effect upon T-cells and an augmented CHI.

Husband and Watson (32) injected *C. pseudotuberculosis* into afferent lymphatic vessels of popliteal lymph nodes of healthy sheep and examined the cells in the efferent lymph. When compared with uninfected controls, the efferent lymph of the infected nodes possessed significantly greater numbers of lymphoblasts, lymphocytes and increased total cell output from day two to day sixteen post infection. The increase of cells in the efferent lymph
appears to associated with a concomitant depletion of lymphocytes in the infected node. *C. pseudotuberculosis* induced inflammation and congestion may impair the entry of peripheral blood lymphocytes into the node via the postcapillary venules. By obstructing the recirculation of peripheral blood T-cells through the node, macrophage-to-T-cell interaction in the node is prevented leading to a compromised host CI1I and infection.

As further evidence supporting the importance of CI1I in caseous lymphadenitis are the acquired cellular resistance (ACR) studies of Hard (33). Hard transferred peritoneal cells from *C. pseudotuberculosis* immune mice to non-immune mice and used suppression of abscess formation as a measure of immunity to infection. While 80% of controls developed abscesses subsequent to challenge, only 20% of recipient mice developed abscesses. Hard then separated the peritoneal cells from the immune mice into macrophage-like and lymphocyte-like cell populations and repeated the experiment. He reported macrophages and lymphoid cells to be equal in their ability to confer protection against *C. pseudotuberculosis* infection.

Host responses against *C. pseudotuberculosis* meet the prerequisites required of an ACR as suggested by Mackaness and Blanden (26) namely, that the organism should survive in host macrophages and antibody should not provide
an appreciable degree of protection against infection. It appears that resolution of chronic caseous lymphadenitis may be dependent upon an effective CMI, and as CMI and humoral immunity interact extensively, a compromised CMI may lead to a less than optimally functioning humoral immunity.

Characterization of Lymphoid Cells

To further examine the role of CMI in resolution of chronic caseous lymphadenitis in goats, one needs to examine the different cell populations involved in the CMI of both infected and uninfected animals. Current reagents for detecting populations and subpopulations of human and murine lymphoid cells include tagged monospecific antibodies against specific cell receptors and immunoglobulin heavy chains. Monospecific and monoclonal antibodies against lymphocyte determinants in sheep are currently being developed (34), but such reagents are not available for use in goats. Until such reagents become available, intermediate solutions must be sought. Caprine B-cells may be detected using indirect rosetting techniques to detect cell receptors or immunoglobulin receptors. Caprine B-cells may also be detected using anti-goat IgG in a direct immunofluorescence assay. Caprine T-cells have been separated from other lymphoid cells by passing them through a nylon wool column; however, this yields a pure population of T-cells and is not applicable to work requiring an
accurate enumeration of cell types (35). Lectins, oligomeric-proteins or glycoproteins from bacteria, plants and animals, offer a temporary solution. Different lectins bind non-covalently to specific oligosaccharides on cell membrane surfaces in much the same way antibody binds to antigen. Lectins, however, can be easily removed from the sugar moieties on the cells by competing sugars. This property and the fact that lectins are sugar specific for glycoproteins on cell membranes makes them useful in cell separation and identification (36). Schnebli and Dukor (37) first showed that lectins may specifically bind to murine lymphocyte populations. Reisner et al. (28) separated murine thymocytes into cortical and medullary cells on the basis of their ability to bind *Arachis hypogaea* agglutinin (PNA). Hellstrom et al. (39) demonstrated the usefulness of *Helix pomatia* agglutinin in separating sialidase treated human B and T lymphocytes.

Lectins may also be used to differentiate caprine T and B lymphocytes in a lymphoid cell preparation. Using fluorescent histochemical techniques, Banks and Greenlee (35) demonstrated that caprine T-cells are differentiated from B-cells on the basis of their ability to bind PNA.

The purpose of this study is to explore the CHI status of goats chronically infected with *C. pseudotuberculosis* using choice lectins. PNA will be used
to differentiate T-cells and rabbit anti-goat IgG will be used to detect B-cells in lymphoid tissues from healthy and diseased animals. In addition, the ability of goat lymphocytes to bind lectins of other specificities will be evaluated and compared to ascertain the value of these lectins for the differentiation of normal and diseased altered lymphocytes.
CHAPTER 2

MATERIALS AND METHODS

Animals

Goats were obtained from several sources in southeastern Arizona, and consisted of various breeds and crossbreeds of either sex. The animals were divided into three groups of fifteen animals each which were housed separately. Group one consisted of kids aged two to six months from non-affected herds having no apparent signs of caseous lymphadenitis and group two was comprised of goats over two years of age from non-affected herds exhibiting no clinical signs of caseous lymphadenitis. Group three consisted of animals over two years of age having abcessation of superficial lymph nodes. Isolation of \textit{C. pseudotuberculosis} from the abcessed nodes confirmed the disease as being caseous lymphadenitis. The study consisted of three experiments which utilized the groups of animals as described later in Experiments I, II, and III.

Reagents

Fluorescein isothiocyanate (FITC) conjugated Peanut agglutinin (PNA) was purchased from Vector Laboratories, Burlingame, Ca. and FITC conjugated concanavalin A (ConA), Wheat germ agglutinin (WGA), and \textit{Lens culinaris} agglutinin.
(LCA) were purchased from Calbiochem Co., San Diego, Ca.
All lectins were resuspended to a concentration of 1 mg/ml in sterile distilled water. FITC conjugated rabbit anti-
goat IgG (H & L) was purchased from Cappel Laboratories,
Westchester, Pa. Caseous lymphadenitis vaccine was supplied by Dr. Brad LeaMaster from the USDA Sheep Station,
DuBois, Id.

**Cell Sources and Initial Preparation**

10 ml of peripheral blood was drawn from the jugular vein into heparinized syringes and mixed gently with 10 ml of sterile phosphate buffered saline (PBS) and 10 ml of sterile cells' balanced salt solution (CBSS). This suspension was divided equally between two 50 ml siliconized glass tubes.

Cells from goat thymus and spleen were obtained as follows. Goats were exsanguinated and thymuses and spleens were removed. Sections of cortical thymus, medullary thymus and spleen were teased to single cell suspensions in 10 ml of sterile CBSS. The cell suspensions were placed into 50 ml siliconized glass tubes.

Tubes containing peripheral blood and tissue suspensions were underlayed with 15 ml and 10 ml respectively of Ficoll-Hypaque solution (Pharmacia, Uppsala, Sweden), $d = 1.077 \pm .003$, and centrifuged at 700 X g for 25
minutes (40). Cells at the interface were removed and placed in 5 ml siliconized glass tubes.

**Preparation of Lymphoid Cells for Immunofluorescence Staining**

Cells were washed three times by adding sterile PBS to the 5 ml tubes to yield a total volume of 3 ml and centrifuging the tubes at 470 x g for 3 minutes. The supernatant was decanted and the cells again resuspended to 3 ml with PBS.

Following the second wash, cells were counted and viability determined using the trypan blue exclusion test (41). Cell suspensions were adjusted to 5 x 10^5 cells/0.1 ml using sterile PBS.

**Determination of Optimal Reaction Conditions**

FITC conjugated lectins (1 mg/ml) and rabbit anti-goat IgG (5 mg/ml) were diluted ten-fold in sterile PBS. Volumes of each lectin were added to 100 ul portions of cell suspensions to equal 1 ug, 5 ug, 15 ug and 30 ug of each lectin. Volumes of rabbit anti-goat IgG equal 10 ug, 20 ug, 40 ug and 80 ug of antibody were added to 100 ul portions of lymphoid cell suspensions.

All reaction mixtures were mixed and incubated in the dark at 4°C for 1, 7, 20 and 45 minutes. At these times the reactions were stopped and the cells fixed by the
addition of 1.0 ml of 15% paraformaldehyde. Fixed cells were allowed to sit in the dark at 4°C overnight and were then centrifuged at 470 x g for 3 minutes and the supernate decanted.

**Microscope Slide Preparation**

The cell pellet was resuspended in 100 ul of sterile PBS. Glass microscope slides were coated with poly-L-lysine (50 μg/ml in PBS) for 45 minutes, rinsed in PBS and dried (42). Using templates and slide carriers (International Equipment Co., Needham Hts., Mass.), 10 ul of each cell preparation was pelleted in duplicate onto the treated slides by centrifugation at 470 x g for 5 minutes.

Slides were air dried, coverslipped with Aquamount (Lerner Laboratories, New Haven, Conn.) and permitted to sit in the dark at 4°C for 2 hours. The percentage of fluorescence positive cells per 200 cells was determined using a Leitz phase-contrast microscope equipped with an incident-light fluorescent illuminator (E. Leitz Inc., San Francisco, Ca.).

**Detection of Lymphoid Cell Surface Receptors**

The numbers of receptor positive cells were ascertained using the previously determined optimal reaction conditions and times as well as a suboptimal reagent concentration. These include: 1) 1 μg and 15 μg of each
lectin, 2) 10 ug and 80 ug of rabbit anti-goat IgG and 3) reaction times of 7 minutes for ConA, 20 minutes for rabbit anti-goat IgG, 30 minutes for WGA and LCA, and 45 minutes for PNA. Cells were then fixed by the addition of 1.0 ml of 1% paraformaldehyde. The percentage of fluorescent cells was then determined as described previously.

**Immunization of Goats**

Goats were immunized at time 0 and at week 4 of the experiment with 2.0 ml of caseous lymphadenitis vaccine using multiple intramuscular injections.

**Statistical Analysis of Data**

Percentages were converted to arcsin transform values and subjected to an analysis of variance using statistical programs available on the Control Data Computer Cyber 175 and Dec 10 computers at the University of Arizona Computer Center. Results were expressed as the mean arcsin transform values ± the 95% confidence intervals or least significant intervals. The results of Experiment II were also subjected to a linear discriminant analysis to determine whether the animals could be differentiated on the basis of the lectin binding ability of their peripheral blood lymphoid cells.
Experiment I

This experiment was done to characterize lymphoid cells in various goat tissues as to their ability to bind ConA, PNA and rabbit anti-goat IgG, and to confirm PNA as a marker of caprine T-cells and rabbit anti-goat IgG as a marker of caprine B-cells.

Lymphoid cells from the cortical and medullary thymus, spleen and peripheral blood of each kid were analyzed for the presence of ConA, PNA and rabbit anti-goat IgG receptors. The data from each animal were analyzed to determine the percentages of cells with each type of receptor in each of the tissues tested.

Experiment II

Peripheral blood lymphoid cells were obtained from goats in each of the three groups. Each lymphoid cell sample was assayed for the presence of cells having surface receptors for ConA, PNA, WGA, LCA and rabbit anti-goat IgG.

In each group of animals, the mean number of cells possessing each type of receptor was determined at both suboptimal and optimal concentrations of reagent. The values obtained from each group were compared to determine if differences existed in the lectin binding ability, the number of T-cells and the number of B-cells in the peripheral blood lymphoid cells of the goats.
In addition, data from each subject were analyzed using a linear discriminant analysis to ascertain if healthy kids, healthy adults and diseased adults could be separated on the basis of the lectin binding ability of their peripheral blood lymphoid cells.

**Experiment III**

This experiment was performed to examine the changes in the numbers of B-cells, T-cells and ConA receptor positive cells that may occur when healthy adult goats are immunized with caseous lymphadenitis vaccine.

Peripheral blood lymphoid cells were obtained from each goat at time 0 prior to immunization and once each week for the subsequent 8 weeks. At week 4, the goats were immunized a second time.

Each sample was examined for the percentages of cells with ConA, PNA and rabbit anti-goat IgG receptors. The percentage of lectin and rabbit anti-goat IgG receptor positive peripheral blood lymphoid cells were subjected to an analysis of variance. The mean percentage of ConA, PNA, and rabbit anti-IgG receptor positive cells at each concentration ± the least significant interval was plotted as a function of time to ascertain changes in percentages of cells in response to immunization.
CHAPTER 3

RESULTS

Determination of Reaction Conditions

The appropriate amounts of FITC conjugated ConA, PNA, WGA, LCA and rabbit anti-goat IgG required for the optimal detection of lymphoid cell surface receptors was determined by incubating various amounts of lectin and antibody at 4°C with $5 \times 10^5$ peripheral blood lymphoid cells. The cells were obtained from adult goats without visible signs of caseous lymphadenitis. The cell preparations were incubated at time intervals ranging from 7 minutes to 24 hours to determine the appropriate time period required for maximum binding of reagent to receptor. The optimum amount of FITC conjugated ConA, PNA, WGA and LCA for receptor detection was 15 µg/150 µl per $5 \times 10^5$ cells, and for FITC-rabbit anti-goat IgG, the optimum amount was 80 µg/160µl per $5 \times 10^5$ cells. The appropriate reaction times for maximum receptor detection was 7 minutes for FITC-ConA, 20 minutes for FITC-rabbit anti-goat IgG, 30 minutes for FITC-WGA and FITC-LCA, and 45 minutes for FITC-PNA. These conditions were employed as optimal in all subsequent research. In addition, suboptimal concentrations of 1 µg of each lectin and 10 µg of antibody were used to detect
possible differences in receptor affinity or saturation of available receptors as the result of age or disease.

**Experiment I**

Experiment I characterized the lymphoid cells in cortical thymus, medullary thymus, spleen and peripheral blood of apparently healthy kids to substantiate PHA as a caprine T-cell marker and rabbit anti-goat IgG (H & L) as a goat B-cell marker, and to quantitate the lymphoid cells from these tissues having surface receptors for ConA, PNA, and rabbit anti-goat IgG.

The results are presented in Table 1. At suboptimal concentrations of ConA, medullary thymus had significantly greater numbers of lymphoid cells with ConA receptors (mean arcsin percentage = 71%) than the other tissues. This difference was not apparent when the higher concentration of ConA was employed. At low concentrations of PNA both areas of the thymus contained higher numbers of cells with PNA receptors (mean arcsin percentage was approximately 53%) compared to the spleen and peripheral blood. Comparable results were observed when optimum concentrations were employed.

Suboptimal and optimal concentrations yielded similar results for numbers of cells with receptors for rabbit anti-goat IgG, however, as expected, results varied greatly among the four tissues. The spleen possessed twice
Table 1. Mean Arcsin Transforms of the Percentage of Receptor Positive Lymphoid Cells in Young Goat Tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>1 μg ConA</th>
<th>1 μg PMA</th>
<th>10 μg Rb-Go IgG</th>
<th>Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORTICAL THYMUS</td>
<td>57</td>
<td>52</td>
<td>11</td>
<td>±7</td>
</tr>
<tr>
<td>MEDULLARY THYMUS</td>
<td>71</td>
<td>55</td>
<td>3</td>
<td>±7</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>48</td>
<td>35</td>
<td>41</td>
<td>±8</td>
</tr>
<tr>
<td>PERIPHERAL BLOOD</td>
<td>57</td>
<td>39</td>
<td>21</td>
<td>±7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissues</th>
<th>15 μg ConA</th>
<th>15 μg PMA</th>
<th>80 μg Rb-Go IgG</th>
<th>Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORTICAL THYMUS</td>
<td>70</td>
<td>52</td>
<td>14</td>
<td>±7</td>
</tr>
<tr>
<td>MEDULLARY THYMUS</td>
<td>69</td>
<td>53</td>
<td>14</td>
<td>±7</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>65</td>
<td>37</td>
<td>49</td>
<td>±8</td>
</tr>
<tr>
<td>PERIPHERAL BLOOD</td>
<td>73</td>
<td>44</td>
<td>28</td>
<td>±7</td>
</tr>
</tbody>
</table>

a. Mean value of nine animals as detected by direct immunofluorescence assay using FITC conjugated lectins and antibody.

b. Confidence limits are expressed at the 95% level (CI_{95%}) computed from the mean square error.
the number of lymphoid cells binding rabbit anti-goat IgG as the peripheral blood. Numbers of rabbit anti-goat IgG receptor positive cells in both areas of the thymus were extremely low or non-existent (mean arcsin transform of $8 = .02\%$).

**Experiment II**

The results of Experiment II are seen in Tables 2 and 3. Table 2 presents data on the lectin and antibody binding ability of peripheral blood lymphocytes from young and adult goats free of caseous lymphadenitis.

Significantly lower numbers of receptor positive peripheral blood lymphocytes were detected with low concentrations of all lectins in young and older animals. These differences were not evident for rabbit anti-goat IgG. Using optimal concentrations of each lectin and antibody, the young animals differed from the adults only in numbers of PNA receptor positive cells, values being 34% for the kids and 54% for the adults.

Table 3 shows the results from Experiment II comparing the lectin and antibody binding ability of peripheral blood lymphoid cells from healthy adult goats and goats with caseous lymphadenitis. There were no differences in numbers of receptor positive cells between diseased and healthy goats for any of the suboptimal concentrations of lectins or antibody. However, at the optimal concentration
Table 2. Lectin and Antibody Binding Ability of Peripheral Blood Lymphoid Cells from Young and Adult Goats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Group</th>
<th>1 ug Con A</th>
<th>1 ug PNA</th>
<th>1 ug WGA</th>
<th>1 ug LGA</th>
<th>10 ug Rb-agO Tag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YOUNG GOATS</td>
<td>17±5&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15±3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13±3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25±3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17±2</td>
</tr>
<tr>
<td></td>
<td>ADULTS GOATS</td>
<td>29±5</td>
<td>25±3</td>
<td>20±2</td>
<td>20±3</td>
<td>21±2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>15 ug Con A</th>
<th>15 ug PNA</th>
<th>15 ug WGA</th>
<th>15 ug LGA</th>
<th>30 ug Rb-agO Tag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YOUNG GOATS</td>
<td>60±4</td>
<td>34±4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35±5</td>
<td>47±4</td>
</tr>
<tr>
<td></td>
<td>ADULTS GOATS</td>
<td>63±4</td>
<td>54±4</td>
<td>35±5</td>
<td>42±4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value of fifteen animals as detected by direct immunofluorescence using FITC conjugated lectins and antibody ± the least significant interval.

<sup>b</sup> A total of 200 cells were examined for immunofluorescence for each animal in each group.

<sup>c</sup> Young goats have significantly (p < .05) fewer cells binding ConA, PNA and WGA.
Table 3. Lectin and Antibody Binding Ability of Peripheral Blood Lymphoid Cells from Healthy and C. pseudotuberculosis Infected Goats

<table>
<thead>
<tr>
<th>Group</th>
<th>1 ug ConA</th>
<th>1 ug PNA</th>
<th>1 ug WGA</th>
<th>1 ug LCA</th>
<th>10 ug Sb-go IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEALTHY GOATS</td>
<td>29±5(^b,c)</td>
<td>25±4</td>
<td>20±3</td>
<td>20±3</td>
<td>21±2</td>
</tr>
<tr>
<td>DISEASED GOATS</td>
<td>22±5</td>
<td>29±4</td>
<td>18±3</td>
<td>21±3</td>
<td>24±2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>15 ug ConA</th>
<th>15 ug PNA</th>
<th>15 ug WGA</th>
<th>15 ug LCA</th>
<th>80 ug Sb-go IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEALTHY GOATS</td>
<td>63±4</td>
<td>54±3</td>
<td>35±5</td>
<td>43±6</td>
<td>24±2</td>
</tr>
<tr>
<td>ADULT GOATS</td>
<td>44±4(^c)</td>
<td>43±3(^c)</td>
<td>34±5</td>
<td>33±4(^c)</td>
<td>24±2</td>
</tr>
</tbody>
</table>

a. Mean value of fifteen animals as detected by direct immunofluorescence using FITC conjugated lectins and antibody ± the least significant interval.

b. A total of 200 cells were examined for immunofluorescence for each animal in each group.

c. Goats with caseous lymphadenitis have significantly (p < .05) fewer cells binding ConA, PNA and LCA.
of ConA, PHA and LCA, goats with caseous lymphadenitis had fewer numbers of cells binding these lectins (44%, 43% and 33% respectively) than apparently healthy goats (63%, 54% and 43% respectively). No differences in numbers of WGA or rabbit anti-goat IgG receptor positive cells were seen between the diseased and healthy animals.

Relative numbers of peripheral blood T and B lymphocytes among young, adult, and diseased goats are seen in Figure 1. When compared with healthy adults, young and diseased goats had fewer peripheral blood T-cells as detected by PNA.

To ascertain whether healthy kids and adults, and \textit{C. pseudotuberculosis} infected goats could be differentiated by the lectin binding ability of their peripheral blood lymphoid cells, a linear discriminant analysis was utilized. As seen in Figure 2, young and adult goats could not be differentiated but healthy adults and goats with caseous lymphadenitis could be distinctly separated based on the ability of their peripheral blood lymphoid cells to bind ConA, PNA, WGA and LCA.

\textbf{Experiment III}

The changes in the mean arcsin percentages of caprine peripheral blood lymphoid cells binding ConA, PNA and rabbit anti-goat IgG in response to immunization are seen in figures 3, 4 and 5.
Figure 1. Mean arcsin transforms of the percentage of T and B Lymphocytes in the peripheral blood of young, adult and diseased goats as detected by PNA and anti-goat IgG.
Figure 2. Linear discriminant analysis separating normal and diseased goats based upon lectin binding by peripheral blood lymphoid cells.
Figure 3. Mean arcsin transforms of the binding of ConA to peripheral blood lymphoid cells in response to immunization.
Figure 4. Mean arcsin transforms of the binding of PNA to peripheral blood lymphoid cells in response to immunization.
Figure 5. Mean arcsin transforms of the binding of anti-goat IgG to peripheral blood lymphoid cells in response to immunization.
The percentages of ConA, PNA and rabbit anti-goat IgG receptor positive cells in the peripheral blood of goats immunized against \textit{C. pseudotuberculosis} showed no significant change during the 8 eight weeks of the study when tested with the optimal concentration of each lectin. Differences in responses were noted in the numbers for ConA receptor positive and PNA receptor positive cells when detected by the suboptimal concentration of lectins. In each case, lesser numbers of cells were detected after the vaccine was introduced into the animals. Recovery of normal numbers of cells occurred within the following one to two week period. No significant changes were apparent in the numbers of B-cells at any time period following immunization.
Caseous lymphadenitis is a debilitating chronic bacterial disease caused by *C. pseudotuberculosis*, and studies to date concerning the relative importance of CMI and humoral immunity in the resolution of this disease are conflicting. An objective of this research was to assess the status of CMI in goats with caseous lymphadenitis by characterizing lymphoid cell populations in the peripheral blood of these animals. As CMI is mediated by T-lymphocytes, enumeration of T as well as B lymphocytes was considered valuable in establishing the relative importance of CMI and humoral immunity in caseous lymphadenitis.

Cells are commonly differentiated on the basis of specific cell surface markers or receptors. The differentiation of caprine lymphoid cells is compromised by the lack of comparable reagents which are used for assessing the CMI status of other animals, but lectins offer a solution. PNA has been used as a T-cell marker in other animals such as dogs, mice, cattle and sheep (43-46). Therefore, FITC conjugated PNA and rabbit anti-goat IgG as well as other choice lectins were used to characterize lymphoid cells in goat tissues.
Quantities of PNA and anti-goat IgG receptor positive cells in healthy kid thymus, spleen and peripheral blood support the findings of Banks and Greenlee (35) as well as substantiating the value of PNA and anti-goat IgG as respective markers of caprine T and B lymphocytes. Compared with adult goats, kids had fewer peripheral blood lymphoid cells binding ConA and WGA only when suboptimal concentrations of these lectins were used, and fewer PNA receptor positive cells when both concentrations of PNA were used. Peripheral blood lymphoid cells in kids may possess fewer surface receptors for ConA and WGA or the cells may have less affinity for these same lectins, and use of the optimum concentration helped to overcome the differences. The lower number of peripheral blood lymphoid cells binding PNA observed in young goats may be a function of age. Scolay has reported the numbers of PNA receptor positive cells to increase after exposure to antigen (47). Perhaps the differences in numbers of T-cells as detected by PNA reflect the differences in prior exposure to antigen associated with young and adult goats.

When compared to apparently healthy goats, the goats with caseous lymphadenitis had significantly reduced numbers of peripheral blood lymphoid cells to bind ConA, LCA, and PNA. In other diseases, T-cell deficiencies and suppressed CHI in diseased hosts is well documented. Linch et al. (48) demonstrated an absence of T-cells in the blood of severe
combined immune deficiency affected fetuses, and other researchers have shown lowered numbers of T-helper lymphocytes in the peripheral blood of AIDS victims (49). Decreased numbers of peripheral blood T-cells were also found in multiple sclerosis victims by Reinherz et al. (50) and in individuals chronically infected with Mycobacterium leprae by Rea et al. (51). Immunity to Listeria monocytogenes (52) and Mycobacterium bovis (53) infections in mice were shown to rely upon a population of T-cells which enhance bactericidal capabilities of macrophages.

There is also evidence to suggest that T-lymphocytes are important in immunity to chronic C. pseudotuberculosis infection. Hard (33) showed that immune lymphocytes and macrophages from C. pseudotuberculosis infected mice conferred protection to non-immune mice challenged with C. pseudotuberculosis. The findings of Irwin and Knight (31) led them to speculate that the T-cell mitogen levamisole enhanced immunity to C. pseudotuberculosis infection in mice. Gameel and Tartour (30) found that C. pseudotuberculosis infected sheep had decreased numbers of lymphocytes in their peripheral blood, and this is substantiated by the findings of this study which show C. pseudotuberculosis infected goats to have lowered numbers of T-cells in their peripheral blood. This may result from obstruction of T-lymphocyte recirculation in the infected
node as suggested by Husband and Watson (32).

Data from this study show that compared to control animals goats with caseous lymphadenitis have an altered lectin binding ability by peripheral blood lymphoid cells and decreased numbers of peripheral blood T-lymphocytes. These deficiencies may indicate a compromised CHI in chronically C. pseudotuberculosis infected goats and an inability of the host to resolve this disease.
APPENDIX A

SOLUTIONS

**Phosphate Buffered Saline (1X)**

PBS was made by dissolving 8 g NaCl, 0.3 g KCl, 0.975 g Na$_2$HPO$_4$, 0.02 g KH$_2$PO$_4$ and 2 g glucose in 1000 ml double distilled water. The pH was adjusted to 7.2 and the solution was sterilized by filtration through a 0.45-μm Millipore filter. The solution was stored at 4°C.

**Cells' Balanced Salt Solution (1X)**

CBSS was made by dissolving 1.0 g dextrose, 0.06 g KH$_2$PO$_4$, 0.385 g Na$_2$HPO$_4$·7H$_2$O, 0.186 g CaCl$_2$·2H$_2$O, 0.400 g KCl, 0.200 g MgCl$_2$·6H$_2$O, 0.200 g MgSO$_4$·7H$_2$O, 8.000 g NaCl and 2 ml phenol red (0.5%) in 1000 ml double distilled water. The solution was sterilized by filtration through a 0.45-μm Millipore filter and stored at 4°C.

**Ficoll-Hypaque Gradients**

Eighteen g of Ficoll were imbibed in 230 ml double distilled water at which time 60 ml Hypaque was added. The solution was sterilized by autoclaving and stored at 4°C.
Paraformaldehyde

Paraformaldehyde was prepared by adding 2 g paraformaldehyde to 500 ml of double distilled water. The solution was heated to 70°C for 30 minutes and filtered through a Whatman #1 paper filter. To this was added 450 ml of saline (8.26 g NaCl in 450 ml of double distilled water) yielding 1% paraformaldehyde.
REFERENCES


Infected with *Corynebacterium pseudotuberculosis*. J. Comp. Path. **84**:477-484.


