Pronounced Production of Polyclonal Immunoglobulin G1 in the Synovial Fluid of Goats with Caprine Arthritis-Encephalitis Virus Infection

GAYLE C. JOHNSON,†‡ D. SCOTT ADAMS, AND TRAVIS C. MCGUIRE

Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164

Received 6 December 1982/Accepted 25 April 1983

Infection of goats with caprine arthritis-encephalitis virus, a lentivirus, resulted in arthritis characterized by the production of intrasynovial immunoglobulin G1 concentrations that were 2 to 5.3 times the serum concentrations in the inoculated carpi at 6 months postinoculation. The intrasynovial immunoglobulin was polyclonal, and its presence was accompanied by increased albumin leakage into the joints. Synovial fluid immunoglobulin levels fluctuated temporally but remained elevated compared with medium-inoculated controls for 38 months after infection. Elevated immunoglobulin G1 concentrations correlated with focal sublumenal plasmacytic infiltrates in the synovia of inoculated carpi at 5 months postinoculation. Inflammation in the uninoculated joints of infected goats was also accompanied by increased intrasynovial immunoglobulin G1 levels. Antibody to systemically administered antigens was a greater proportion of the immunoglobulin population in sera than in synovial fluids of infected goats, suggesting that antibody production to local antigens was responsible for increased intrasynovial immunoglobulin G1 levels.

Caprine arthritis-encephalitis virus (CAEV), a retrovirus that was originally isolated from the arthritic carpi of adult goats (7), produces a nonsuppurative synovitis after intravenous and intracarpal injection into previously unexposed goats (2, 6). The resultant morphological lesions are similar to the synovitis of rheumatoid arthritis in humans (26, 32).

Viral inoculation results in the development of a sustained inflammatory response that is compatible with continued immune stimulation in the infected joint. This is despite an apparent paucity of viral expression in inflamed joints, as demonstrated by the minimal amounts of cell-free virus present and the difficulty in demonstrating viral antigens by immunofluorescence (2). However, consistent recovery of CAEV by synoviocyte explantation (2), an increase in serum antiviral antibody titers (as measured by an enzyme-linked immunosorbent assay) with the development of experimentally induced synovitis, and long-term positive agar gel immunodiffusion tests against CAEV (1) argue for continued stimulation of the immune system by viral antigens. Data on the nature of the immune response in the inflamed synovia is lacking, and the role of CAEV in perpetuating these events is not understood.

One path to understanding the genesis of CAEV synovitis is to examine the amount and characteristics of intrasynovial immunoglobulin produced by experimentally infected goats. In this paper we describe the presence of substantial increases in polyclonal immunoglobulin G1 (IgG1) concentrations in the synovial fluids of virus-infected goats 6 months after infection, with concentrations 2.0 to 5.3 times those in serum. The immunoglobulin concentrations correlated well with the presence of plasma cells in the inflamed synovia. The relative abundance of antibody to systemically administered antigens in the sera of infected goats demonstrated that the synovial fluid immunoglobulin population did not mirror that of serum, suggesting that the synovial immune response was directed toward locally occurring antigens.

MATERIALS AND METHODS

Design. Experimental goats were derived by cesarean section, fed virus-free gamma globulin as a colostral substitute, and housed isolated from other goats, as described previously (2). A total of 15 goats that were 10 to 14 days old were inoculated intravenously with 0.5-ml portions of virus in Dulbecco modified Earle medium supplemented with 2% fetal calf serum; an additional 0.5 ml of the virus preparation was
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inoculated into one radiocarpal joint of each goat. The 75-63 prototype isolate of CAEV was cloned by triplicate limiting dilutions on primary goat synovial cells eight passages after isolation from synovioyte explants from a spontaneously arthritic goat (2). The pool contained $10^8$ to $10^9$ cells per ml and was clarified by low-speed centrifugation before injection. Seven age-matched controls received equal amounts of virus-free medium from uninfected synovioyte cultures. Sera and synovial fluids from the inoculated carpi were obtained from 10 infected and 5 control goats at monthly intervals for 1 year and at 15 months postinoculation (p.i.). Another five infected and two control animals were sampled monthly from both carpi for 5 months and were euthanized for use in morphological studies.

Six additional goats, which were 4 to 28 days old, were inoculated as described previously (2) with uncloned CAEV after 11 passages in goat synovioyte cultures; this inoculum contained $10^2$ to $10^3$ cell ml per ml. These goats and four goats receiving virus-free medium from uninfected synovioyte cultures were sampled at 38 months p.i. At the time of sampling, joint swelling and dysfunction were present in contralateral as well as inoculated joints, whereas control goats had no carpal abnormalities.

**SRID.** The immunoglobulin subclasses used as standards and as antigens in preparing antisera for single radial immunodiffusion (SRID) were isolated from anti-dinitrophenol (DNP) antibodies removed from hyperimmune goat serum on a DNP-containing agarose affinity column (21). IgG1 and IgG2 were separated by DEAE-cellulose ion-exchange chromatography, using a 0 to 0.3 M sodium chloride gradient in 0.005 M sodium phosphate buffer (pH 8) (20). Caprine IgM was purified from anti-DNP antibodies by gel filtration chromatography, followed by a second cycle of antigen affinity and gel filtration chromatography (21). All purified antigens reacted as expected during immunoelectrophoresis against rabbit anti-goat whole serum or rabbit anti-goat immunoglobulin serum (22).

Anti-goat IgG2 antibodies for SRID were prepared by immunization of a sheep with fractions that were eluted from the ion-exchange column by buffer without NaCl. The resultant antiserum produced a single line during immunoelectrophoresis and reacted only with goat IgG2 at dilutions suitable for use. Anti-goat IgG1 antibodies were made by immunizing a horse with the first protein-rich salt gradient fractions that were eluted from the ion-exchange column. This antiserum was absorbed with a goat IgG2-agarose affinity column before a monospecific reaction with goat IgG1 was obtained. Rabbit anti-goat IgM serum did not require absorption. Standard SRID curves were constructed by using serial dilutions of purified goat immunoglobulins in concentrations from 0.05 to 1.25 mg/ml. A goat serum pool was calibrated with purified immunoglobulins, and dilutions of the pool were used in subsequent tests as standards. SRID was done with 1.2% Noble agar in phosphate-buffered saline or Vero- nal buffer (10). Precipitin cylinders were measured after 18 to 24 h by using a TG calibrator (Transidyne General Corp., Ann Arbor, Mich.). Samples were examined at two different dilutions on two plates.

**Cellulose acetate electrophoresis.** Samples (2 μl) of undiluted serum or of diluted bovine testicular hyaluronidase-treated synovial fluid (12) were applied to cellulose polyacetate strips and electrophoresed in Gelman high-resolution buffer (pH 8.8) (Gelman Instruments, Inc., Madison, Wis.) at 10 mA for 45 to 60 min. Electrophoretograms were stained with Ponceau S dye and examined by densitometry (Gelman Digi-screen).

**Protein concentration.** Coomassie brilliant blue dye binding (Bio-Rad Laboratories, Richmond, Calif.) was used to measure the protein contents of serum and synovial fluid samples that were not treated with hyaluronidase compared with a standard mixture containing equal amounts bovine serum albumin and bovine gamma globulin (3). The albumin concentration was calculated by multiplying the total protein content by the percentage of albumin in the sample, as determined from the densitometric tracing.

**Isoelectric focusing.** Synovial fluid samples were diluted with distilled water to a protein concentration of 1 to 2 mg/ml and then were dialyzed against 0.01 M sodium phosphate buffer (pH 8). Samples were applied to 5% polyacrylamide gels containing 2.4% ampholines (pH 3.5 to 10; LKB, Broma, Sweden) and were electrofocused at 4°C to a maximum of 1,500 V at 35 mA; the current became constant after 3 h, and electrophoresis was discontinued. The bands were stained with Coomassie brilliant blue and were compared with proteins having known isoelectric points.

**Western blots.** Three weeks before death, seven goats were each immunized intramuscularly with 1 mg of keyhole limpet hemocyanin (Pacific Biomarine Supply, Venice, Calif.) and 1 mg of ovalbumin in Freund complete adjuvant. The same antigens were given in Freund incomplete adjuvant 7 to 8 days before necropsy.

For western blotting, 160 μg of each antigen in 0.1 M phosphate-buffered saline (10 mM NaH₂PO₄, 150 mM NaCl, pH 7.4) was electrophoresed at 30 mA on a slab (10 × 10 cm; 0.75% sodium dodecyl sulfate-containing 7.5 to 17.5% polyacrylamide gradient slab gel under reducing conditions (31). The antigens and 14C-methylated standards of known molecular weights were then transferred to nitrocellulose paper (pore size, 0.2 μm; Schleicher & Schuell Co., Keene, N.H.) at 4°C over a period of 23 h at 90 mA. The blots were washed as described previously (4). After transfer, the charged groups on the paper were blocked by incubation with 3% bovine serum albumin-containing Tris-saline buffer (10 mM Tris, 150 mM NaCl, pH 7.4). Antigen-containing strips 4 mm wide were incubated with serum and synovial fluid samples containing varying amounts of immunoglobulin in 10 ml of this buffer for 1 h with constant rotation. After the strips were washed six times in phosphate-buffered saline, two times in Tris-saline buffer supplemented with 0.1% Nonidet P-40, and once in Tris-saline buffer, the location of the goat antibody bound to the strips was determined as follows. The strips were incubated for a second 1 h with rabbit anti-goat immunoglobulin serum (diluted 1:50) and, after a similar washing procedure, were incubated with 1.5 × 10⁶ cpm of 125I-labeled staphyloccocal protein A (Sigma Chemical Co., St. Louis, Mo.) radiolabeled to a specific activity of 6 × 10⁶ cpm/μg by using a chloramine T reaction (14). The final washing cycle consisted of three washes with Tris-saline buffer containing 0.1% Nonidet P-40. The strips were wrapped in plastic and exposed for 24
from 0

VOL. 41, total

antibody titer to

infected goats control. The controls. infected, globulin in IgG2 infected, could be compared with synovial fluid ly, ug. Titers exsanguinated. Samples intravenous overdose infected and were single penverlie, Ill.) (Lab-Tek Products, Div. Miles Laboratories Inc., Naperville, Ill.) and frozen in liquid nitrogen. To detect immunoglobulin-containing cells in the synovia, rabbit anti-goat immunoglobulin conjugate was used on frozen tissue sections fixed in 95% cold ethanol. Serum from rabbits injected with ammonium sulfate-precipitated goat immunoglobulins was conjugated with fluorescein isothiocyanate as described previously (2); the conjugate had a ratio of F to P of 1:3 (15) and contained 0.1 mg of protein per ml at the dilution used. Positive staining was evaluated relative to the inability of the tissues to bind a normal rabbit serum-fluorescein isothiocyanate conjugate with a similar ratio of F to P and to the blocking of specific conjugate binding by goat serum.

RESULTS

Immunoglobulin concentrations in serum and synovial fluid. To examine immunoglobulin production after CAEV infection, concentrations of IgG1 and IgG2 were determined by SRID in serum and synovial fluid samples from 15 infected and 7 control goats for 5 months p.i. and from 10 infected goats and 5 controls for the remaining 10 months. The mean intrasynovial IgG1 concentrations increased dramatically in the virus-infected group at 2 months p.i., peaked at 6 months p.i., and remained statistically elevated (P < 0.05, Student's t test) above control values for the months thereafter (Fig. 1a). At 6 months

FIG. 1. Mean IgG1 and IgG2 concentrations as determined by SRID in synovial fluids and sera of virus-infected and mock-infected goats. Synovial fluids from inoculated carpi (a) or sera (b) were obtained at intervals from 0 to 15 months p.i. The values for months 0 to 5 are the mean values determined from 15 virus-infected and 7 control goats for serum and from 15 infected and 3 control goats for synovial fluid. During months 7 to 15 10 infected and 5 control goats were sampled. The 6-month control synovial fluid value represents a pool of five controls. The vertical bars indicate the standard error of the mean. Symbols: ●, CAEV infected, IgG1; ■, CAEV infected, IgG2; ○, mock infected, IgG1; □, mock infected, IgG2.

Morphological studies. At 5 months p.i., five virus-infected and two control goats were each given an intravenous overdose of sodium pentobarbital and exsanguinatad. Samples of synovial were fixed in McDowell fixative (19); other tissues were immersed in 4% neutral buffered Formalin. Paraffin-embedded tissues were processed by standard methods. The synovial membranes used in immunofluorescence studies were encased by O.C.T. embedding medium (Lab-Tek Products, Div. Miles Laboratories Inc., Naperville, Ill.) and frozen in liquid nitrogen.
synovial fluid IgG1 concentrations were more than 20 times those of controls. Wide fluctuations in intrasynovial IgG1 concentrations in individual infected goats occurred with respect to time. Two- or threefold changes in concentration were not uncommon over a 1- to 2-month interval, and peaks above serum values were relieved by intermittent returns to concentrations resembling serum IgG1 concentrations or below. Three or more such fluctuations were noted within 15 months after infection, with diminishing amplitude after 6 months p.i. In contrast, the concentrations of intrasynovial IgG2 were consistently low in both groups of goats, with no significant differences between them.

In addition to elevation of synovial fluid IgG1 levels, virus-infected goats also had increased serum IgG1 levels compared with controls (Fig. 1b), although the differences between the two groups were not always as great as the differences in synovial fluid concentrations. The values were significantly higher ($P < 0.05$, Student’s $t$ test) than the values of controls during the first 4 months after exposure and plateaued at a significantly higher level from 6 to 9 months p.i. Thereafter, the IgG1 levels were similar in the two groups. Serum IgG2 concentrations were slow to increase with age in both groups, approaching adult values only when the animals were 15.5 months old. The serum IgM concentrations remained below 1.5 mg/ml throughout the period of study; mean values for virus-infected and uninfected goats were virtually identical (data not shown).

A comparison of the serum and synovial fluid IgG1 values for individual virus-infected goats demonstrates how much more IgG1 was present in synovial fluid than serum after infection (Fig. 2). By 3 months postinfection, the synovial IgG1 concentrations increased from levels below serum values to levels that were equal to or somewhat above serum values. By 6 months, considerably more IgG1 was present in the inoculated carpi of all infected goats than in their sera, and ratios of synovial fluid IgG1 levels to serum IgG1 levels ranged from 2.0:1 to 5.3:1. This suggests considerable local IgG1 synthesis within the joints. The ratios were still greater than 1.0:1 in some individuals 7 to 10 months p.i. (as demonstrated by the 9-month samples), but by 12 months p.i. sera and synovial fluids again had similar IgG1 concentrations.

Early increases in intrasynovial immunoglobulin levels were not limited to the carpi directly inoculated with virus. Table 1 shows the relative IgM and IgG1 concentrations in the inoculated and contralateral carpi of five virus-infected and two control goats at 2 and 4 months p.i. Because the serum IgG1 concentrations were significant-

![FIG. 2. Comparison of serum (●) and synovial fluid (○) IgG1 concentrations in CAEV-infected goats. Serum IgG1 concentrations are compared with intrasynovial IgG1 concentrations for individual virus-infected goats at months 0, 3, 6, 9, and 12 p.i. The mean control synovial fluid IgG1 concentrations are indicated by bars for comparison. The zero time data represent the preinoculation IgG1 values of the 22 goats used in this study.](http://iai.asm.org/)

ly different for infected and control goats, the data were expressed as percentages of serum immunoglobulin levels, so that direct comparisons could be made between the two groups. By 2 months p.i., the virus-inoculated carpi had increased IgG1 levels, whereas the contralateral carpi had IgG1 levels similar to those of controls. However, by 4 months p.i., the IgG1 concentrations in the contralateral joints approached those in sera. The intrasynovial IgG1 concentration exceeded the serum level in the contralateral joint in one of five infected goats by 5 months p.i. (data not shown).

Prominent increases in the relative amounts of intrasynovial IgM were also measured in the inoculated carpi of infected goats early in infection (Table 1). At 2 months p.i., the inoculated carpi averaged 10 times the relative amounts of IgM present in the contralateral or control
TABLE 1. Mean synovial fluid immunoglobulin concentrations expressed as percentages of serum immunoglobulin concentrations for inoculated and contralateral joints of CAEV-infected and control goats

<table>
<thead>
<tr>
<th>Synovial fluid from:</th>
<th>No. tested</th>
<th>Concn at 2 months p.i.</th>
<th>Concn at 4 months p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG1</td>
<td>IgM</td>
</tr>
<tr>
<td>Infected carpi</td>
<td>5</td>
<td>186 ± 6 (111–275)³</td>
<td>49 ± 40 (17–113)³</td>
</tr>
<tr>
<td>Contralateral carpi</td>
<td>5</td>
<td>18 ± 3 (15–23)²</td>
<td>5 ± 3 (2–11)</td>
</tr>
<tr>
<td>Control carpi</td>
<td>4</td>
<td>25 ± 8 (16–34)</td>
<td>4 ± 1 (3–5)</td>
</tr>
</tbody>
</table>

³ Values were calculated from the following expression: (synovial fluid IgG1 concentration/serum IgG1 concentration) × 100. The results are expressed as mean ± standard deviation. The values in parentheses are ranges.

² Significantly greater than control values (P < 0.01), as determined by Student’s t test.

joints; at 4 months after infection, both carpi of infected goats showed less striking increases in IgM levels. Although relatively high IgM levels occurred in these joints, IgM concentrations greater than those of serum were not evident.

After 38 months of viral infection, the IgG1 predominance in synovial fluid samples had disappeared. Goats showed a tendency toward elevated IgG1 and IgG2 concentrations in the synovial fluids of both inoculated and uninoculated carpi compared with similarly aged, medium-inoculated controls (Table 2). Although the IgG1 and IgG2 levels were elevated in one or both carpi of some goats, only the uninoculated carpi differed significantly from controls. The concentrations of intrasynovial immunoglobulin were always less than the serum values. The ratios of IgG1 to IgG2 were similar in serum and synovial fluid, in contrast to the preponderance of intrasynovial IgG1 in goats infected for 15 months or less.

Cellulose acetate electrophoresis. Because synovial fluid is an ultrafiltrate of plasma (18), one might expect increased leakage of serum proteins into the synovial fluids of infected goats. To analyze this possibility, the protein and albumin contents of infected and control sera and synovial fluids were determined (Table 3). Cellulose acetate electrophoresis of synovial fluids from mock-infected goats demonstrated the presence of some albumin (the smallest and most diffusible of the serum proteins) and small amounts of gamma globulin, which correlated with the low immunoglobulin concentrations found by SRID. Albumin and the small amount of immunoglobulin accounted for nearly all of the protein present in control synovial fluid (Table 3). The control ratio of albumin to globulin was high (6.6) compared with that of serum (4.1), and there was much less albumin in synovial fluid than in serum. In contrast, infected goats had considerable synovial gamma globulin (as expected), which was distributed in a wide peak during electrophoresis. Large amounts of albumin and minor amounts of other serum proteins were also present. More than 83% of the synovial fluid protein in infected goats was immunoglobulin and albumin, but the ratio (0.7) of these constituents was quite different from the ratio in controls. The albumin concentration in CAEV-infected joints was also higher than that in control joints, approaching serum concentrations (mean, 34 mg/ml in synovial fluid versus 42 mg/ml in serum), indicating increased diffusion of serum constituents into the infected carpi. Serum values were similar in the two groups.

Isoelectric focusing. To determine the number of clones involved in intrasynovial IgG production, infected and control synovial fluids were electrophoresed over a pH range (pH 3.5 to 10), producing the protein-staining patterns shown in Fig. 3. For comparison, antibodies specific to DNP and IgG1 antibodies to DNP were also focused. Like the control synovial fluid (Fig. 3, lane F), the infected synovial fluids (lanes D and E) contained a prominent albumin band with an

TABLE 2. Mean immunoglobulin concentrations in sera and synovial fluids of goats infected with CAEV 38 months previously

<table>
<thead>
<tr>
<th>Goats</th>
<th>No. tested</th>
<th>Serum concn</th>
<th>Concn in synovial fluids from inoculated carpi</th>
<th>Concn in synovial fluids from contralateral carpi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG1</td>
<td>IgG2</td>
<td>IgG1</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>10.9 ± 5.4</td>
<td>6.8 ± 3.5</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Experimentally</td>
<td>6</td>
<td>16.1 ± 8.9</td>
<td>12.0 ± 5.6</td>
<td>7.0 ± 5.9</td>
</tr>
<tr>
<td>infected</td>
<td></td>
<td>6.8 ± 3.6b</td>
<td>3.8 ± 0.7c</td>
<td></td>
</tr>
</tbody>
</table>

³ Determined by SRID.

² P < 0.05 compared with the control group, as determined by Student’s t test.

² P < 0.01 compared with the control group, as determined by Student’s t test.
TABLE 3. Protein, albumin, and IgG concentrations in sera and synovial fluids of virus-infected and control goats at 6 months p.i. 

<table>
<thead>
<tr>
<th>Goats</th>
<th>Conc in synovial fluids (mg/ml)</th>
<th>Serum concn (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>Albumin</td>
</tr>
<tr>
<td>Virus infected</td>
<td>98.1 ± 14.1</td>
<td>34.0 ± 9.4</td>
</tr>
<tr>
<td>Mock infected</td>
<td>21.4 ± 3.8</td>
<td>15.6 ± 3.0</td>
</tr>
</tbody>
</table>

a The synovial fluids were from inoculated carpi.

b The results are expressed as means ± standard deviations. The ratios of albumin to IgG in the synovial fluids were 0.7:1 and 6.7:1 for the virus-infected and mock-infected animals, respectively; in sera these ratios were 2.9:1 and 4.1:1, respectively.

c Significantly greater than the control value, as determined by Student’s t test (P < 0.01).

Acidic isoelectric point. Infected samples also had numerous protein bands with neutral to alkaline isoelectric points, which were lacking in the control. Since immunoglobulins accounted for 75% of the nonalbumin protein in these synovial fluid samples (Table 3), it was likely that most of the bands which focused between the pH 6.85 and 8.45 p.i. standards were immunoglobulins. These proteins were more numerous than the proteins present in affinity-purified immunoglobulin preparations to DNP (Fig. 3, lanes B and C). Therefore, the intrasynovial immunoglobulin in CAEV infections is polyclonal.

Western blot antibody titrations against systemically administered antigens. To determine whether synovial fluid contained the same relative amount of antibody to systemically injected antigens as serum in infected and control goats, the least amount of serum or synovial fluid immunoglobulin needed to detect 0.8 μg of keyhole limpet hemocyanin or ovalbumin was determined by western blotting (Table 4). Whereas similar amounts of serum and synovial fluid IgGs detected antigens in the control group, three of five infected goats required at least five times the amount of synovial fluid IgG to reach the titration endpoint of serum. Since control synovial fluid immunoglobulins are mostly derived by diffusion from serum (18), they reflected the serum composition. In infected goats, it was probable that some serum immunoglobulin entered the synovial fluid by leakage, but it was added to a locally produced IgG population which contained little antibody to the systemically administered antigens.

Morphological studies. Five CAEV-infected goats were killed at 5 months p.i. and examined to determine whether the morphological lesions correlated with the increased synovial fluid immunoglobulin concentrations. External swelling of the inoculated carpi was noticeable in these infected goats at 1 month p.i. The lesions in the inoculated carpal joints were similar to those previously reported (2, 6). Diffuse synovial thickening was macroscopically evident (Fig. 4) and was accompanied by villus proliferation and occasional petechiation compared with the smooth, white synovia of control goats. The synovia of less severely affected joints were tan and thickened. Two infected goats had macroscopic lesions in the contralateral carpi.
TABLE 4. Amounts of total IgG1 and IgG2 present at endpoint antibody activity on western blot titrations against intramuscularly administered protein antigens

<table>
<thead>
<tr>
<th>Goat</th>
<th>Amt of antigen (μg)</th>
<th>Keyhole limpet hemocyanin</th>
<th>Ovalbumin</th>
<th>Synovial fluid IgG concn (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Synovial fluid</td>
<td>Serum</td>
<td>Synovial fluid</td>
</tr>
<tr>
<td>Virus infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>9.0</td>
</tr>
<tr>
<td>03</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>18.3</td>
</tr>
<tr>
<td>05</td>
<td>1</td>
<td>50</td>
<td>100</td>
<td>37.6</td>
</tr>
<tr>
<td>06</td>
<td>1</td>
<td>5</td>
<td>50</td>
<td>17.2</td>
</tr>
<tr>
<td>07</td>
<td>1</td>
<td>5</td>
<td>100</td>
<td>16.3</td>
</tr>
<tr>
<td>Mock infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>08</td>
<td>0.5</td>
<td>1</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Immunization was initiated 3 weeks previously and boosted 7 to 8 days before sampling.

*Goats were infected or mock-infected 5 months previously.

*The minimum amount of total immunoglobulin present in the incubation medium of a strip showing an autoradiographic line at 24 hs.

Microscopic lesions were characterized by villus proliferation, synoviocyte hyperplasia and necrosis, and non-suppurative inflammation. Plasma cells, some containing Russel bodies, were focally numerous near the synovial lumen (Fig. 5A), and follicle-like lymphocytic nodules occurred in the deeper intimal tissue of one goat. Degenerate villi with associated fibrin deposits were present in three inoculated carpi. In one of these joints extensive collagen necrobiosis and mineralization occurred in the perisynovial connective tissue. Changes in contralateral carpi were more focal and contained numerous plasma cells without degenerative changes. Immunofluorescent staining revealed that immunoglobulin-containing cells were abundant near the synovial surface in the inoculated joints (Fig. 5B). Focal collections of plasma cells with diffuse, bright cytoplasmic staining and eccentric nonstaining nuclei were present in areas of villus proliferation either within the villus core or near the luminal surface. Many more cells in the lesion contained a less brilliant rim of membraneous or intercellular fluorescence accompanied by occasional cytoplasmic granular fluorescence. This pattern of fluorescence was common in all areas where inflammation was present. Synovial lining cells often contained several large granules of fluorescent material within their cytoplasm. Immunofluorescent staining was not evident when goat serum was mixed with the conjugate or when normal rabbit serum was used instead of rabbit anti-goat immunoglobulin serum in producing the conjugated reagent.

DISCUSSION

CAEV-infected goats have elevated IgG1 concentrations but not elevated IgG2 concentrations in sera, in synovial fluids from inoculated carpi, and eventually in synovial fluids from contralateral carpi, demonstrating that immunological reactivity to the infecting antigens has a strong subclass preference. Ruminant immune responses may preferentially involve either IgG subclass, and IgG1 is produced by goats in response to a number of antigens (22). IgG1 responses also dominate the immune response in several ruminant diseases, notably in visna virus infection of sheep (11). Thus, it is not surprising that CAEV infection of goats produces a strong IgG1 response, a portion of which is directed against viral antigens, (G. C. Johnson, A. F. Barbet, P. Klevjer-Anderson, and T. C. McGuire, submitted for publication). The early differences between the IgG1 serum concentrations of virus-infected and control goats are no doubt equalized by later immunological stimulation of the immune systems of uninfected goats by other IgG1-inducing environmental antigens. However, the prolonged period of low serum IgG2 concentrations in both groups is surprising and is dissimilar to the age-related responses observed in cattle (21). When our animals were 15.5 months old, the serum IgG2 values were beginning to approach previously published normal serum values (22). The reason for this observation is not known and could reflect factors of environment, genetic background, or antigenic exposure.

The synovial fluid IgG1 concentrations in goats varied above the serum values in a transient recurrent pattern and were above levels which could be explained by leakage of serum constituents into the inflamed joints. This pattern suggests recurrent local antibody synthesis in the inoculated joints and resembles the varying antiviral lymphocyte stimulation indices in animals infected by this and other lentiviruses (1, 16, 17, 27, 28). In these diseases, less pronounced fluctuations of neutralizing antibody occur in serum, but burst production of such antibodies might be concealed by the serum halflives of the immunoglobulins. In a CAEV-infected synovium, however, the diffusion of high concentrations of locally produced intrasynovial antibody into serum could occur rapidly and thus would allow local bursts of immunoglobulin production to be reflected in elevated synovial IgG1 levels.

Western blot assessment of antibody activity against intramuscularly administered antigens.
demonstrated that the intrasynovial immunoglobulin populations in three of five infected goats contained 20% or less of the serum activity per microgram of IgG, even in the face of probable intrasynovial leakage of serum IgG. These results suggest that there is no enhanced intrasynovial commitment by B lymphocytes and plasma cells to systematically administered antigens during the stage of disease when the antigens are given. The reduced ratios of synovial fluid antibody to serum antibody in virus-infected animals are consistent with either synovial IgG1 synthesis against locally available antigens or selective trapping of IgG1-synthesizing cells with a previous immunological commitment in the synovium (13).

Our observation of a plasma cell-rich inflammatory reaction in the carpi of infected goats, which was occasionally accompanied by lymphoid follicle-like structures in the synovia, is similar to previous morphological observations (2, 6) and supports the data presented in this paper for local IgG1 synthesis. The necrotic villi and fibrin exudation observed in this study are characteristic features of the intrasynovial reaction, particularly in spontaneous CAEV arthritis (8). These villi resemble rice bodies present in human rheumatoid joints, which may be important in the perpetuation of inflammation and joint pain (25). Hypertrophy and hyperplasia of the synovial lining may be the result of proliferation of endogenous synoviocytes or may result

FIG. 4. Macroscopic appearance of CAEV-inoculated radiocarpal joints 5 months after infection. (A) Mock-infected control. (B) Infected goat.
FIG. 5. Microscopic appearance of CAEV-infected synovium. (A) Light microscopy of synovial villus (stained with hematoxylin and eosin). (B) Immunofluorescent staining of inflamed synovial membrane 5 months p.i. The inset shows a higher magnification of plasma cells. Bar = 25 μm.
from the immigration of macrophage precursors of bone marrow origin (9).

Immunofluorescent staining of infected synovia with rabbit anti-goat immunoglobulin conjugate confirmed the presence of the focal subluminal plasma cells observed by light microscopy. In addition, a second staining pattern involved most cells of the inflammatory infiltrate, with irregular rims of fluorescence observed on the surfaces of or between cells and occasionally cytoplasmic immunofluorescent granules. The cells could be B lymphocytes, which during certain maturational stages contain minimal cytoplasmic IgG but considerable membrane-bound immunoglobulin. Alternatively, since macrophage-like cells are part of the microscopic lesions, membrane fluorescence could result from the attachment of either goat antibody or rabbit conjugate to cells by their Fc receptors. Cytoplasmic granules could be the result of phagocytosis of IgG. The low background fluorescence in areas of uninfamed synovia and IgG-specific blocking in the inflamed synovia suggest that the staining pattern was the result of specific conjugate attachment.

The synovitis produced by CAEV early in infection has many morphological similarities to rheumatoid arthritis of humans (2, 6). In a study of rheumatoid synovial effusions, the amount of intrasyovial immunoglobulin was greater than could be explained by the amount of albumin leakage (18), but synovial fluid immunoglobulin concentrations were 40 to 90% those of serum (29), not the 200 to 500% noted in early CAEV infections. However, the values for rheumatoid arthritis patients were not dissimilar to the relative amounts of intrasyovial immunoglobulins observed in infected goats by 38 months p.i. The preference for IgG3 synthesis by synovial fragments of rheumatoid joints (30) was not as striking as the IgG1 class-specific response present in CAEV infections. By using in vitro culture techniques to radiolabel antibodies produced by rheumatoid arthritis synovial fragments, workers have demonstrated that less than 2% of the immunoglobulin synthesized is specific for a systemically injected antigen, compared with 14 to 32% of the immunoglobulin produced by peripheral blood leukocytes of the same patients (13). Although the serum antibody acquired through leakage contributes to the intrasyovial immunoglobulin pool of CAEV-infected goats, it appears that the synovial lymphocyte commitment to systemically administered antigens by goats is low and similar to that observed in rheumatoid arthritis.

In conclusion, experimental CAEV infection induced a synovitis accompanied by high synovial levels of polyclonal IgG1. Although serum immunoglobulins probably contributed to the synovial immunoglobulin population, there was evidence for intrasyovial IgG1 production. This evidence included synovial IgG1 concentrations greater than serum concentrations, lower ratios of IgG2 to IgG1 in synovia than in sera, lower ratios of albumin to IgG in synovia than in sera, lower antibody titers against systemically administered antigens in synovia than in sera, and the presence of plasma cells in the inflamed synovia. The contribution of antiviral antibody to intrasyovial IgG1 is uncertain at present, although high titers of antibody to CAEV glycoproteins are present in the joints of virus-infected goats (14a). Neutralizing antibody has not been detected in infected goat sera (15a), but emergence of antigenic variants of the parental virus, as in visna virus (11, 23, 24), or intermittent expression of integrated provirus could provide part of the antigenic stimulus for recrudescences of synovial IgG1 production.

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LITERATURE CITED