American Journal of Pathology, Vol. 151, No. 4, October 1997

Expression of Cytokine mRNA in Lentivirus-Induced Arthritis

Franziska Lechner,* Hans-Rudolf Vogt,* Heng F. Seow,† Giuseppe Bertoni,* William P. Cheevers,‡ Uta von Bodungen,* Andreas Zurbriggen,§ and Ernst Peterhans*

From the Institute of Veterinary Virology and Institute of Veterinary Neurology,§ University of Bern, Bern, Switzerland; CSIRO,† Division of Animal Health, Parkville, Victoria, Australia; and the Department of Veterinary Microbiology and Pathology,‡ Washington State University, Pullman, Washington*

Infection of goats with the lentivirus caprine arthritis encephalitis virus (CAEV) leads to persistent infection and development of chronic arthritis. We analyzed the expression of cytokines and viral RNA in the joints of goats at early time points after experimental infection with CAEV and in those of animals suffering from chronic arthritis as a result of natural infection. *In situ* hybridization experiments showed that the pattern of cytokine expression in caprine arthritis was similar to that found in rheumatoid arthritis (RA), with a few cells expressing the lymphocyte-derived cytokines interferon (IFN)-γ and interleukin (IL)-2 and rather more cells expressing monocyte chemoattractant protein (MCP)-1, IL-6, and tumor necrosis factor (TNF)-α. IFN-γ mRNA expression in experimentally infected joints peaked at day 12 and was mostly detected in areas containing viral RNA. At later time points, no IFN-γ- or virus-expressing cells were found in inflamed joints but both were again detected in goats with severe arthritis. Interestingly, at the clinical stage of arthritis reflecting the chronic stage of infection, the inflammatory lesion was found to be immunologically compartmentalized. Humoral immune responses and cell-mediated immune responses appeared to concurrently occur in distinct areas of the synovial membrane. *(Am J Pathol 1997, 151:1053–1065)*

Caprine arthritis encephalitis virus (CAEV) belongs to the *Lentivirus* genus of retroviruses. It is related closely to the maedi visna virus of sheep and more distantly to the equine (EIAV), feline (FIV), simian (SIV), and human (HIV) immunodeficiency viruses. CAEV is widespread in the goat population and causes persistent infection,[1,2] yet only approximately one-third of the infected animals develop clinical symptoms.[1,3] Infection with CAEV leads to mononuclear infiltration of various tissues, in particular, the radiocarpal joints, mammary gland, lung, and in young animals, the brain.[4–6] The most prominent clinical sign of infection is carpitis characterized by periarticular swelling and accumulation of synovial fluid containing a large number of inflammatory cells. Histologically, synovial membranes show synovial lining hyperplasia, hypertrophy of membrane villi, and infiltration of intima and subintima with macrophages, lymphocytes, and plasma cells. In the advanced stage of the disease, necrosis and fibrosis of synovial tissue as well as mineralization and erosion of articular surfaces occur.[1,7,8]

In view of the histopathological similarity, caprine arthritis serves as a model for human rheumatoid arthritis (RA). The etiology of RA is unknown, but several infectious agents (bacterial or viral) have been implicated in initiating or perpetuating the disease.[9–11] Interestingly, CAEV transcripts and/or antigens remain difficult to detect in the synovium of infected goats before severe clinical arthritis has developed[8,12] (and this report), which suggests a possible involvement of immunopathological mechanisms in the persistence of chronic inflammation.

On the basis of the pattern of cytokines expressed, immune responses can be divided into Th1 and Th2 or, in more general terms, into type 1 and type 2 responses.[13–15] Type 1 cytokines interleukin (IL)-2 and interferon (IFN)-γ) are predominantly expressed during delayed-type hypersensitivity reactions and cell-mediated immune responses. Type 2 cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13) support humoral immune responses and are also expressed during allergic or anti-helminthic reactions.

Cytokines are thought to play an important role in RA by initiating or exacerbating the inflammatory process in the joint (for a review, see Feldmann et al[16]). A majority of studies indicate that RA can be characterized by a type 1 cytokine profile,[16–19] although some reports suggest otherwise.[20–23] Due to a shortage of available tissue samples from early stages of RA, cytokine expression and composition of inflammatory cells are poorly characterized at the time of the onset of the disease. It is conceivable, however, that this stage may be crucial for the

Supported by a grant by the Union Bank of Switzerland on behalf of a client. E. Peterhans was supported by the Swiss National Science Foundation (SNF) grant 3100–09733.93/1, and G. Bertoni was supported by SNF grant 3139–041859.94.

Accepted for publication July 23, 1997.

Address reprint requests to Dr. E. Peterhans, Institute of Veterinary Virology, University of Bern, Länggass-Strasse 122, CH-3012 Bern, Switzerland.

further development of the arthritic lesion. In this report, we have analyzed the expression of cytokines and viral RNA in caprine arthritis at both early and late time points after experimental CAEV infection as well as in naturally infected goats with clinical arthritis.

Materials and Methods

Animals and Tissue Samples

Eight 2- to 3-year-old Saanen goats were experimentally infected with the molecular clone CAEV CO[24] grown on goat synovial membrane fibroblast cells (GSMs). The goats were infected intravenously and intracarpally (left carpal joint) with 5×10^4 $TCID_{50}$ CAEV CO in a volume of 500 μl. The right carpal joint was mock-infected with 500 μl of GSM cell culture supernatant. Joint swelling was assessed by measuring the carpal/metacarpal circumference ratio.

Goats naturally infected with CAEV were purchased from infected flocks in Switzerland. These goats had shown clinical arthritis for over 2 years (except animal 1676 that showed no clinical signs of CAE). They were serologically CAEV positive as demonstrated by an enzyme-linked immunosorbent assay based on highly purified whole maedi visna virus antigen[25] and by Western blot.[26]

All goats were euthanized by an overdose of pentobarbital. Synovial membranes and subscapular lymph nodes were immediately removed, fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4), and then dehydrated and embedded in paraffin according to standard procedures. Similarly, tissue samples were obtained from two uninfected goats as controls for *in situ* hybridization experiments.

Paraformaldehyde-fixed tissues from two goats orally infected at birth with the biological clone CAEV-63 were kindly donated by Dr. W. P. Cheevers (Pullman, WA). These goats showed severe clinical arthritis and have been extensively described by Wilkerson et al.[8,27]

Virus Isolation from PBMCs or Synovial Fluid

Peripheral blood mononuclear cells (PBMCs) were isolated from citrate-buffered blood by centrifugation on Ficoll (Seromed, Munich, Germany). PBMCs were resuspended in RPMI 1640 medium (Seromed) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2.5 mmol/L), 2-mercaptoethanol (50 μmol/L), HEPES (10 mmol/L), and 10% heat-inactivated goat serum (Sigma Chemical Co., St. Louis, MO). The cells were cultured in Teflon bags made from hydrophobic Teflon foil (FEP 100A, Du Pont De Nemours International, Geneva, Switzerland) for 12 days to allow macrophages to mature. Teflon-bag-derived macrophages were added to semiconfluent GSM cultures grown in Earle minimal essential medium (MEM; Seromed) containing 7% fetal calf serum (FCS). Nonadherent cells were removed the next day and fresh medium was added. Cell cultures were passaged every week for at least 5 weeks or until a cytopathic effect appeared. Isolation of CAEV from synovial fluid was performed by adding 200 to 500 μl of freshly aspirated, unseparated synovial fluid to semiconfluent GSM cultures and passaging as above. Specificity of the cytopathic effect in GSM cultures was demonstrated by immunoblotting of viral antigens present in supernatants from freeze-thawed cell cultures. *In situ* hybridization of GSM cells infected with the isolated virus from the naturally infected goats showed specific staining with a CAEV CO *gag* probe.

Plasma Cell Detection

Paraffin-embedded tissue sections were dewaxed and rehydrated. The sections were treated with 0.2% (w/v) Trypsin (Difco, Detroit, MI), 14 mmol/L $CaCl_2$, pH 7.8, at 37°C for 30 minutes. After washing three times for 2 minutes each in Tris buffer (0.5 mol/L NaCl, 20 mmol/L Tris(hydroxymethyl)-aminomethane, pH 7.5, 0.25% Tween 20), the tissue was blocked for 30 minutes with 10 mg/ml human γ-globulins (Globuman Berna, Schweizerisches Serum- und Impfinstitut, Bern, Switzerland) in Tris buffer. A rabbit anti-goat IgG serum (made at our institute) was diluted 1:200 in Tris buffer and applied to sections for 60 minutes at room temperature. After washing three times for 2 minutes each in Tris buffer, an alkaline-phosphatase-conjugated mouse anti-rabbit antibody (Sigma) diluted 1:1000 in Tris buffer was added for 60 minutes at room temperature. After another washing step, the color substrate was added consisting of 2 mg/ml Fast Red TR (Chroma-Gesellschaft, Koengen, Germany), 1 mg/ml naphthol AS-MX phosphate (Sigma) and 0.5 mg/ml Levamisole (Sigma) in 0.1 mol/L Tris, pH 8.0. The color reaction was stopped by rinsing with tap water. The sections were counterstained with Mayer's hematoxylin and mounted in Glycergel (Dako, Carpinteria, CA).

Probes for in Situ Hybridization

The cDNAs of ovine IL-2,[28] IL-4,[29] IL-8,[30] IFN-γ,[31] IL-6, IL-10, TNF-α, and MCP-1 (H.F. Seow, unpublished) were cloned in pSPT19 (Boehringer Mannheim, Mannheim, Germany). CAEV CO *gag* (nucleotides 512 to 1858) was cloned in pBluescript (Stratagene, La Jolla, CA). Antisense probes and sense control pobes were transcribed *in vitro* from the T3, T7, or SP6 promoters in the presence of digoxigenin-UTP (Boehringer Mannheim). Labeled probes were sheared by alkaline hydrolysis to yield RNA of ~150 nucleotides in length and stored at −70°C.

In Situ Hybridization

Paraffin-embedded tissue sections were dewaxed and hybridized as described previously.[32,33] Tissue sections were treated with 1.5 μg/ml proteinase K (Boehringer Mannheim) for 15 minutes at 37°C, acetylated, and then prehybridized for 2 hours at 52°C. Hybridization was performed overnight at 52°C in a solution containing 50% formamide (v/v), 4X SSC (sodium chloride/sodium citrate

Table 1. Infection of Goats with CAEV

Goat number	Duration of infection	Mode of infection	C/MC (left/right)	Clinical arthritis*	Histology† (left/right)	Isolation of virus‡	
						PBMCs	SFs (left/right)
38	6 days	Experimental	1.64/1.66	No	2/0–1	+	+/+
1053	6 days	Experimental	1.72/1.72	No	1–2/0–1	+	+/+
25	12 days	Experimental	1.66/1.6	No	3/1	+	+/−
17	12 days	Experimental	1.63/1.6	No	3–4/1	+	+/−
15	33 days	Experimental	1.77/1.69	Weak	4/1	+	+/−
33	33 days	Experimental	1.73/1.65	No	3–4/3	+	+/+
8	1 year	Experimental	1.69/1.63	No	2/3–4	−	−(pool)§
9	1 year	Experimental	1.74/1.64	No	3–4/0	−	−(pool)
1676	>2 years	Natural	1.64/1.64	No	2/2	+	+/+
53	>2 years	Natural	1.79/1.73	Weak	4/3	+	+(pool)
957	>2 years	Natural	1.61/1.89	Yes	1/4	+	+(pool)
1007	>2 years	Natural	1.92/1.64	Yes	4/0–1	+	+/+
85–14	>2 years	Experimental at birth	2.32/2.36	Yes	5/5	ND	+(pool)
85–17	>2 years	Experimental at birth	2.79/2.59	Yes	5/5	ND	+(pool)

C/MC, carpal/metacarpal circumference ratio on the day of euthanasia; SFs, synovial fluids; ND, not done.
*An arbitrary threshold for clinical arthritis was set at C/MC = 1.80, although some animals with lower ratios clearly showed swelling of carpal joints.
†Evaluation of hematoxylin and eosin (H&E)-stained tissue sections of synovial membranes was performed independently by two examiners. 0, no inflammation; 1, some inflammatory cell aggregates, synovial lining layer hyperplasia; 2, dispersed mononuclear infiltrates in villi, with few perivascular infiltrates; 3, infiltrates in villi, subintima, and around vessels, with few small lymphoid-like follicles; 4, same as 3, with lymphoid-like follicles and large plasma cell aggregates; 5, intense mononuclear infiltration of intima and subintima, numerous plasma cells, and necrosis/fibrosis.
‡Isolation of virus from PBMCs and synovial fluids from left and right carpal joints was performed on the day of euthanasia.
§Pooled synovial fluids of the left and right carpal joints of some animal were tested.

buffer), 2X Denhardt's reagent, 10% dextran sulfate (w/v), 500 μg/ml blocking RNA (calf liver RNA, Sigma), and 1 μg/ml digoxigenin-labeled RNA probe. Unbound labeled RNA probe was removed by an RNAse treatment for 30 minutes at 37°C (200 U/ml RNAse T1 (Boehringer) and 0.2 μg/ml RNAse, DNAse-free (Boehringer Mannheim) and by washing in 0.2X SSC twice for 15 minutes each at 50°C. Bound probe was detected with anti-digoxigenin antibody Fab fragments conjugated with alkaline posphatase (Boehringer), and color reaction was performed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

As a control, labeled sense RNA probes were included in each *in situ* hybridization experiment.

From each animal, several cross sections of synovial membranes and subscapular lymph nodes were hybridized with digoxigenin-labeled riboprobes and, where applicable, all stained cells per tissue section were counted using a light microscope. The area of the tissue analyzed was determined using a video-based computer system and the Datasys software (Datasys version 3, Datalab, Thoeringen, Switzerland). Photographs were taken with a differential interference contrast microscope (Carl Zeiss, Zurich, Switzerland).

After *in situ* hybridization, some tissue sections were stained with an antibody specific for major histocompatibility complex (MHC) class II DR (VPM 54,[34] kindly donated by Dr. B. Blacklaws (Edinburgh, UK). The sections were rinsed and then blocked as described for plasma cell detection (see above). Incubation with VPM 54 antibody was performed overnight at 4°C followed by incubation with a biotinylated goat anti-mouse antibody (Jackson ImmunoResearch, West Grove, PA) for 60 minutes at room temperature. After the addition of avidin/biotin-peroxidase complexes (Vector Laboratories, Burlingame, CA), the sections were stained with 3,3′-diaminobenzidine tetrahydrochloride (Sigma).

Results

Experimental Infection of Goats

Eight Saanen goats were infected intravenously and intracarpally (left carpal joint) with the molecularly cloned CAEV CO virus. The right carpal joint was mock-infected and served as a control. Two goats were euthanized at 6, 12, or 33 days or 1 year after infection, and synovial membranes and lymph nodes were fixed in paraformaldehyde. Four goats naturally infected with CAEV and two goats orally infected at birth with the CAEV-63 isolate were also included in this study.[8,27]

In goats infected intracarpally, first joint swelling with increased carpal/metacarpal circumference ratio and tenderness of the joint were detected between 12 and 15 days after infection (Table 1). In all animals analyzed, antiviral antibodies became detectable by an enzyme-linked immunosorbent assay between 20 and 28 days after infection. At 6 days after infection, CAEV was isolated by co-cultivation with indicator cells from PBMCs of some, but not all, experimentally infected animals (not shown), whereas at 12 days after infection, virus was isolated from PBMCs of all animals tested (Table 1). No virus was isolated from two antibody-positive animals that had been experimentally infected for 1 year.

Interestingly, 6 days after experimental infection, CAEV was isolated by co-cultivation of synovial fluid not only from infected left carpal joints but also from mock-infected right carpal joints, suggesting that nonspecific

Table 2. Detection of Plasma Cells, Viral RNA, and IFN-γ mRNA in Caprine Arthritis

Goat number	Duration of infection	Plasma cells (% of inflammatory cells)	*In situ* hybridization (positive cells/mm²)		
			IFN-γ RNA in synovium	Viral RNA (*gag*) in synovium	Viral RNA (*gag*) in lymph nodes
38	6 days	19	0/0	7.1/0	0/0
1053	6 days	4	0/0	2.3/0	0/0
25	12 days	21	1.4/0	10.0/0.2	0.1/0
17	12 days	18	0.7/0	8.1/0.1	0.1/0
15	33 days	45	0/0	0/0	0/0
33	33 days	40	0/0	0.5/0	1.0/0
8	1 year	50	0/0	0/0	0/0
9	1 year	52	0/0	0/0	0/0
1676	>2 years	13	0/0	0/0	0/0
53	>2 years	62	0/0	0/0	2.2/0.1
957	>2 years	53	0/0.5	0/0	0.4/0.9
1007	>2 years	63	0.1/0	3.3/0	0.1/1.0
85–14	>2 years	71	1.2/ND	22.2/ND	0.3/0.2
85–17	>2 years	73	0.2/ND	14.1/ND	1.4/1.9

The percentage of plasma cells was determined by counting the total number of inflammatory cells and the cells stained with an anti-goat IgG antibody in five areas of the inflamed carpal synovium (results of the carpal joint with the more severe inflammation are shown). For *in situ* hybridization, four to eight cross sections of synovial membranes were hybridized with a viral *gag* RNA probe or with a probe specific for IFN-γ mRNA. Two to six cross sections of prescapular lymph nodes were hybridized with a viral *gag* RNA probe. Results of the left and right carpal joints and prescapular lymph nodes are shown. ND, not done.

irritation of the synovium led to local CAEV replication (Table 1).

Results of histological examination and characterization of the inflammatory cells in the synovium at different times after infection are described in detail elsewhere[8,27] (U. von Bodungen, F. Lechner, H. Pfister, HR. Vogt, WP. Cheevers, G. Bertoni, T. Jungi, E. Peterhans, submitted). Briefly, intracarpal infection led to the rapid development of mononuclear cell infiltration in the synovial membrane. At day 6, hyperplasia of the lining synovial membrane layer and mononuclear cell aggregates in sublining areas were noted. At 12 days, extensive mononuclear infiltration of synovial villi and subintima was observed. A few lymphoid-like follicle of a small size were also found. Infection for 33 days led to severe inflammation of the synovium with abundant lymphoid follicles, some exhibiting germinal-center-like structures. Additionally, angiogenesis was noted in villi and subintima. At this stage, the histological appearance of the synovium was similar to that seen in the synovium of naturally infected goats with clinical arthritis[8] (von Bodungen et al, submitted).

Plasma Cells in the Synovium

Caprine arthritis is characterized by large numbers of plasma cells in the synovial membrane.[8,35] We investigated whether plasma cell accumulation in the synovium is a feature of late-stage arthritis or whether these cells appear early after CAEV infection. Paraffin sections of synovial membranes were stained with a polyclonal rabbit anti-goat IgG serum. Surprisingly, considerable numbers of plasma cells were found in the synovium as early as 6 days after intracarpal infection (Table 2). The numbers of plasma cells steadily increased between 6 days and 1 year after experimental infection. In naturally infected goats with severe clinical arthritis, plasma cells accounted for up to 73% of all inflammatory cells in the synovial membranes. Plasma cell clusters were predominantly found around lymphoid follicles and sometimes as a band below the synovial lining layer (see Figure 4b).

Detection of Viral RNA in the Synovial Membrane and Subscapular Lymph Nodes

Six days after infection, viral RNA was detected in synovial membranes of experimentally infected goats by *in situ* hybridization with a probe specific for the viral *gag* RNA (Table 2). Cells of the synovial lining layer and mononuclear cells in the inflamed sublining layer stained positive with the *gag* probe. The number of virus-replicating cells peaked at 12 days after intracarpal infection (8 to 10 positive cells per 10-mm² synovial tissue; Table 2). At this stage, viral RNA was found in the synovial lining layer and in subintimal areas infiltrated by large numbers of mononuclear cells (Figure 1a). In the mock-infected carpal joint, very few cells (zero to three cells per tissue section) stained positive for viral RNA and no or only weak inflammation of the synovium was noted (Tables 1 and 2). At later time points (33 days or 1 year after infection), levels of viral RNA in synovial membranes were in most cases below the limit of detectability for the *in situ* hybridization technique, although histologically, the synovial membranes appeared inflamed. Accordingly, no virus-expressing cells were detected by *in situ* hybridization in synovial fluid cells of animals infected for longer than 12 days (not shown). In goats with severe clinical arthritis, however, viral RNA was again detected in the synovial membrane (Table 2). In most cases, cells expressing viral *gag* RNA were found in restricted foci at the rim of or outside inflamed areas. Viral RNA was often detected in cells located between collagen fibers in fibrotic areas almost devoid of inflammatory cells (see Figure 3b).

Double-staining experiments revealed that some of the cells expressing viral RNA also strongly expressed MHC

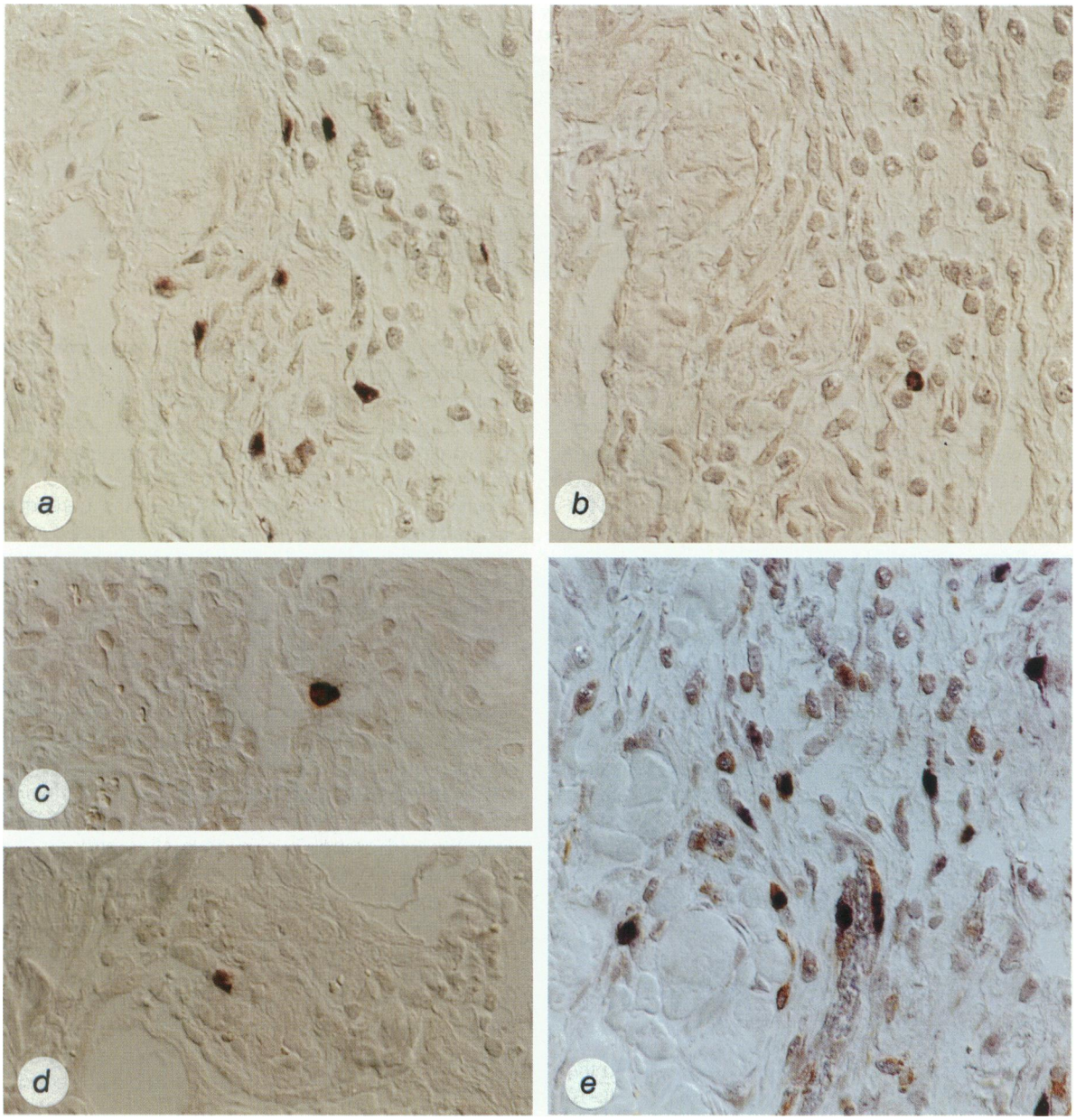

Figure 1. Detection of viral RNA and cytokine mRNA in the joint of a goat experimentally infected with CAEV at 12 days after infection. Synovial membrane tissue section of the left carpal joint of goat 17 was hybridized with a probe specific for viral *gag* RNA (**a**), IFN-γ mRNA (**b**; subsequent tissue section to **a**), IL-2 mRNA (**c**), and IL-10 mRNA (**d**). **e**: Double staining of viral *gag* RNA (black) and MHC class II DR (brown). Original magnification, ×400.

class II DR molecules, irrespective of the duration of infection (Figure 1e). Morphologically, most of these double-stained cells resembled macrophages. Fibroblast-like cells and very rare endothelial cells were also found to express viral RNA.

We did not find substantial numbers of virus-expressing cells in draining subscapular lymph nodes of goats experimentally infected for 6 days to 1 year (zero to three positive cells per lymph node cross section; Table 2). Most subscapular lymph nodes of goats with clinical arthritis, however, contained cells expressing viral RNA (7 to 50 positive cells per cross section). Stained cells were found either scattered and/or in clusters in the paracortex, in follicles, and in the medulla. Occasionally, viral RNA was detected in large foamy cells with multiple nuclei. We were not able to detect diffuse follicular staining originating from virions trapped on follicular dendritic cells as has been described for HIV, SIV, and FIV,[36–38] although various proteinase K concentrations were tested for *in situ* hybridization.[37]

Detection of IFN-γ mRNA in Synovial Membranes

IFN-γ mRNA was not detected in the synovial membrane 6 days after experimental infection although mononuclear inflammatory cells were present at this stage. At day 12,

Table 3. Expression of Cytokine mRNA in Synovial Membranes

Duration of CAEV infection	TNF-α	MCP-1	IFN-γ	IL-2	IL-6	IL-8	IL-10
6 days	++	+/++	−	−	−/+	−	−
12 days	+++	+++	+	+	++/+++	−	+
33 days	++	++	−	−	+/++	−	+
>2 years (clinical arthritis)	++	+++	−/+	−/+	++	−	+

In situ hybridization of cytokine mRNA in synovial membranes of CAEV-infected goats. The left carpal joints of experimentally infected goats (days 6, 12, and 33 after infection) were analyzed. Both carpal joints of naturally infected goats were analyzed. −, not detected; −/+, not detected in every goat; +, occasional positive cells (<1%); ++, areas with up to 20% positive cells; +++, areas with more than 20% positive cells.

IFN-γ mRNA-expressing cells with a lymphocyte morphology were detected in mononuclear infiltrates. By analyzing subjacent tissue sections of goats infected for 12 days, we noted that IFN-γ-expressing cells always co-localized with areas in which viral RNA was detected (Figure 1, a and b). Single IFN-γ-positive cells were found within mononuclear infiltrates, and virus-expressing cells were located adjacent to the infiltrate, often in cells between collagen fibers. No IFN-γ mRNA was found in synovial membranes of goats infected for longer than 12 days or of goats with subclinical or mild clinical arthritis (Table 2). Analogously, these goats had no, or very little, detectable viral RNA in the inflamed synovium. In synovial membranes of goats with more severe clinical arthritis, however, IFN-γ-expressing cells were found, although not in large numbers (Table 2). In most cases, these cells again co-localized with areas with detectable virus expression (see Figure 3, a and b). Lymphoid-like follicles only very rarely contained single IFN-γ-mRNA-positive cells. Hence, at all stages studied, IFN-γ mRNA and CAEV RNA were found to be co-expressed in the synovium, which suggests that CAEV stimulates local IFN-γ expression or *vice versa*.

Detection of IL-2 and IL-10 mRNA in Synovial Membranes

Only few cells (less than 1% of inflammatory cells) in the inflamed synovium were found to express IL-2 or IL-10 message using the *in situ* hybridization method (Table 3). Both at early and late time points, single cells with lymphocyte morphology were expressing IL-2 or IL-10 mRNA (Figure 1, c and d; Figure 4, c and d). These cells were mostly located in perivascular infiltrates or close to the synovial lining layer. As numbers of stained cells were small, no correlation with viral RNA expression could be established.

Detection of TNF-α, IL-6, and MCP-1 mRNA in Synovial Membranes

Cells expressing TNF-α, MCP-1, and IL-6 mRNA were more abundant in caprine arthritis than cells expressing IL-2, IL-10, and IFN-γ mRNA. Interestingly, cytokine expression was not uniform throughout the inflamed synovium but confined to certain inflammatory areas, whereas other areas, although histologically identical, did not stain with any of the cytokine probes tested. A subset of mononuclear infiltrates were highly expressing MCP-1, TNF-α, IL-6, and in some instances, IFN-γ mRNA as well as viral RNA, whereas other areas did not contain detectable cytokine message, suggesting that not all mononuclear cell aggregates were in the same activation state. Due to the heterogeneity of cytokine expression in the synovial membrane, the percentage of stained cells in positive mononuclear aggregates instead of total numbers of stained cells was determined for each time point (Table 3).

In an earlier report, we demonstrated the presence of TNF-α mRNA in joints of goats with clinical arthritis.[33] These studies have now been extended to earlier time points of CAEV infection. TNF-α mRNA was detected in the infected synovium as early as 6 days after infection, mostly in areas infiltrated by mononuclear cells and also in cells of the synovial lining layer. MCP-1 mRNA was detected in the same regions as TNF-α. At this stage, only very few inflammatory cells (less than 1%) stained with the IL-6 probe. Twelve days after intracarpal infection, TNF-α was expressed in infiltrated subintimal layers and in infiltrated synovial villi (Figure 2c). A few cells of the synovial membrane lining layer were also stained. Morphologically, most TNF-α-expressing cells resembled macrophages that were abundant in perivascular infiltrates (von Bodungen et al, submitted). MCP-1 mRNA was expressed in synovial lining layer cells and in perivascular infiltrates as well as in scattered cells throughout the subintima. Some fibroblast-like cells also expressed MCP-1 mRNA. IL-6 mRNA was detected in cells within or close to the synovial lining layer and in perivascular infiltrates. Morphologically, these cells resembled macrophages, lymphocytes, and fibroblasts.

In synovial membranes of goats with clinical arthritis, TNF-α-expressing cells were less abundant than in goats infected intracarpally for 12 days. In clinical arthritis, only few regions contained TNF-α-expressing cells. The pattern of MCP-1 expression, however, was similar to that found at 12 days after infection. Abundant MCP-1 message was found in synovial lining layer cells, scattered inflammatory cells in the subintima and in perivascular infiltrates (Figure 3c). IL-6 mRNA was mostly detected in cells close to the lining layer (sometimes adjacent to plasma cell aggregates) and in infiltrated villi whereas perivascular infiltrates in the deeper layers of the tissue did not contain many IL-6 mRNA-expressing cells (Figure 4a). Plasma cell aggregates did not express detectable message for IFN-γ, TNF-α, MCP-1, or IL-6.

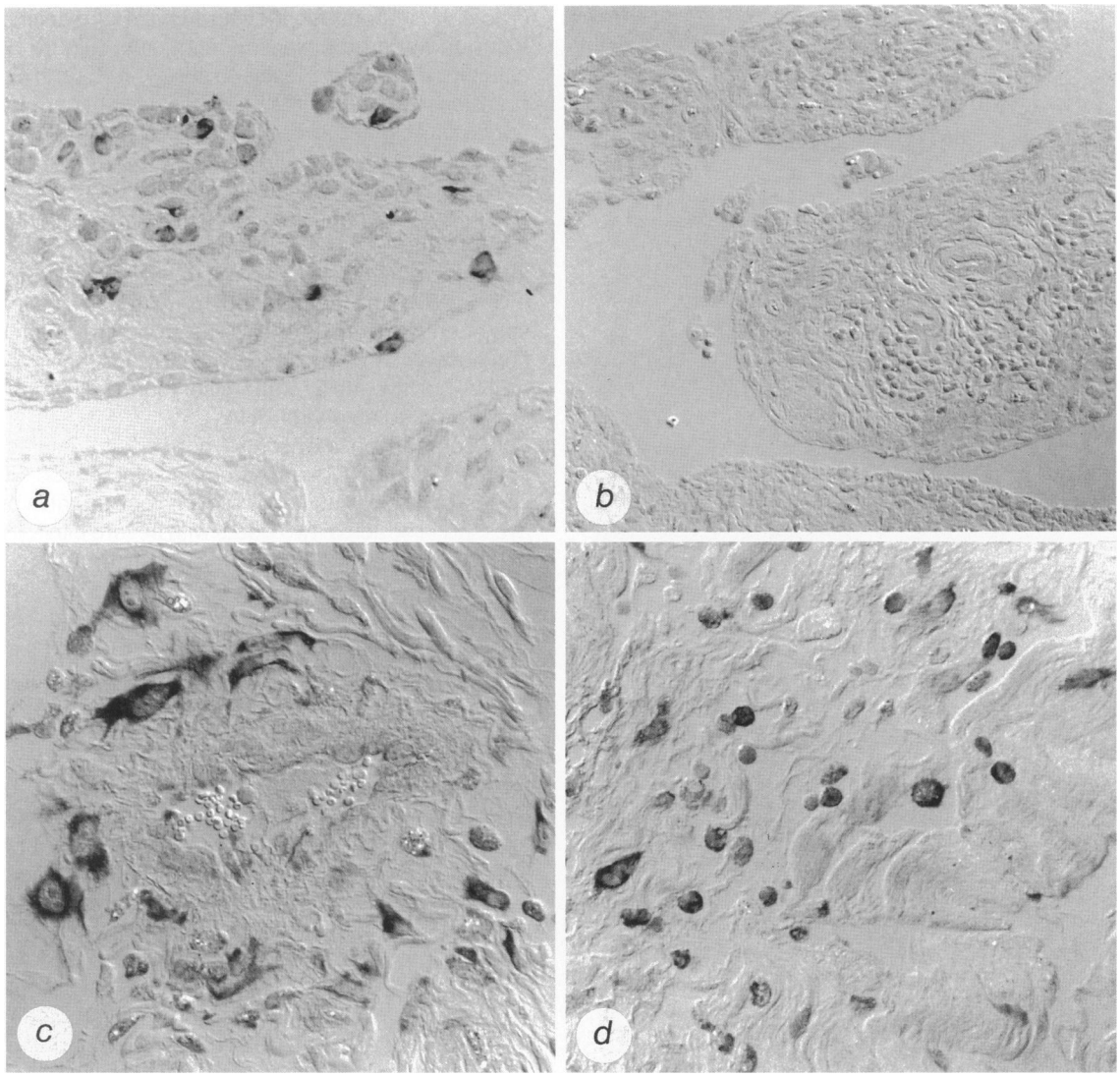

Figure 2. Detection of IL-6, TNF-α, and MCP-1 mRNA in the joint of a goat experimentally infected with CAEV at 12 days after infection. Synovial membrane tissue sections of the left carpal joint of goat 17 hybridized with probes specific for IL-6 mRNA (**a**), TNF-α mRNA (**c**), and MCP-1 mRNA (**d**). **b**: Control *in situ* hybridization with a sense IL-6 RNA probe. Original magnification, ×400 (**a**, **c**, and **d** and ×100 (**b**).

A few MCP-1 and TNF-α mRNA-expressing cells but no cells expressing IFN-γ, IL-2, IL-6, or IL-10 mRNA were detected in synovial membranes of mock-infected carpal joints at days 6 or 12 after infection as well as in synovial membranes from uninfected goats (not shown).

Control *in situ* hybridization experiments with sense cytokine probes revealed no specific staining (Figure 2b).

Subjacent tissue sections were analyzed to study a possible correlation between viral replication and cytokine expression in infected cells. MCP-1 mRNA was always present in areas containing virus-expressing cells, although it was also found in some areas lacking detectable viral RNA. TNF-α- and IL-6-expressing cells were not consistently found in areas in which viral RNA was detected, which makes it unlikely that CAEV directly induces these two cytokines *in situ*. We were unable to detect IL-8 message in caprine arthritis, irrespective of the duration of infection or the presence of viral transcripts in the joint. To check whether this was due to insufficient sensitivity of the IL-8 hybridization probe, granulomatous tissue from a goat that had been subcutaneously injected with complete Freund's adjuvant was analyzed. Numerous IL-8 mRNA-expressing cells were readily detected in areas infiltrated by macrophages, lymphocytes, and neutrophils (not shown), which thus confirms the sensitivity of the hybridization method.

Detection of Cytokine mRNA in Draining Lymph Nodes

As IL-2, IL-10, and IFN-γ mRNA-expressing cells were rare in the synovium and therefore difficult to quantify, we decided to additionally analyze the subscapular lymph nodes draining the carpal joint. Three to six cross sections of subscapular lymph nodes of experimentally and naturally infected goats were analyzed by *in situ* hybrid-

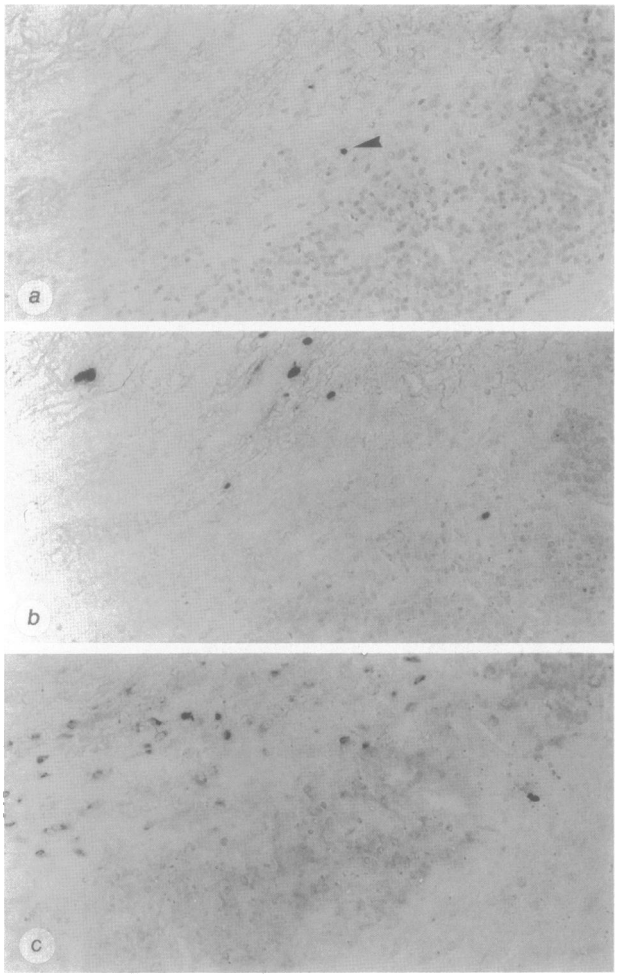

Figure 3. Detection of viral RNA, IFN-γ mRNA, and MCP-1 mRNA in the joint of a goat with clinical arthritis. Subsequent synovial membrane tissue sections of goat 85–14 were hybridized with probes specific for IFN-γ mRNA (**a**), viral *gag* RNA (**b**), and MCP-1 mRNA (**c**). The **arrow** denotes a single cell stained with the IFN-γ mRNA probe. Original magnification, ×100.

ization, and stained cells were counted. For comparison, subscapular lymph nodes draining the mock-infected joints and subscapular lymph nodes from two uninfected goats were included (Figure 5). Enhanced cytokine expression in the draining lymph nodes was found between 6 and 12 days after experimental infection as well as in chronically infected animals with clinical arthritis. At days 6 or 12, expression of IFN-γ, IL-10, and to a lesser degree, IL-2 mRNA in the left subscapular lymph nodes was increased compared with the right subscapular lymph nodes draining the mock-infected joints. Increased cytokine expression in the left nodes may well have reflected an ongoing local immune response against CAEV inoculated into the left carpal joint. Morphologically, cells expressing IFN-γ, IL-2, or IL-10 message resembled lymphocytes and were located in the paracortex, in the medulla adjacent to the paracortex, and to a lesser degree, in follicles. In long-term infected animals without clinical arthritis, no increased levels of IFN-γ, IL-2, or IL-10 mRNA were found in subscapular lymph nodes compared with control lymph nodes, although a histological examination of the carpal joints revealed considerable inflammation (Table 1). In contrast, elevated levels of cytokines were found in nodes draining arthritic joints (Figure 5). Interestingly, the highest levels of IFN-γ, IL-2, and IL-10 expression were found in one of the two subscapular lymph nodes of animal 53. This lymph node also exhibited the highest viral load of all lymph nodes tested (Table 2). Analogous to the synovium, IFN-γ expression in the lymph node appeared to be stimulated by local expression of CAEV. In most cases, areas of the nodes containing detectable viral RNA also contained cells expressing IFN-γ mRNA as determined by *in situ* hybridization of subjacent sections (not shown). In contrast to the situation observed in the synovial membranes, however, IFN-γ mRNA-positive cells were also found in lymph nodes lacking detectable viral RNA.

In comparison with control nodes from uninfected animals, IL-2 mRNA expression was increased in lymph nodes of two goats with mild clinical arthritis but suppressed in nodes of two goats with severe clinical arthritis (Figure 5).

Discussion

The arthritis caused by CAEV is the result of persistent infection and develops slowly over a period of months to years. The histopathological lesions of caprine arthritis are similar to those of RA in humans, a condition for which an infectious cause has been postulated but not demonstrated.[9–11] With respect to a possible role played by viruses in RA, studies of the very early stages of the disease would appear particularly relevant because viruses may trigger inflammation that may subsequently develop in an autonomous fashion. However, for obvious reasons, invasive studies of inflamed articular tissue of early-stage RA are difficult to carry out in humans. Such investigations are more feasible in CAEV-induced arthritis. The typically slow course of pathogenesis can be speeded up by injection of virus directly into the synovial cavity.[12,39] Using this mode of infection, the histological appearance at 1 month after infection closely resembled that after 1 to 2 years of natural infection[12] (von Bodungen et al, submitted). We observed that the relative proportions of synovial macrophages, $CD4^+$, $CD8^+$, and γδ T cells were essentially the same in experimentally infected goats at 33 days after infection as in naturally infected goats with chronic arthritis[8] (von Bodungen et al, submitted). In this study, we have investigated the relationship between the kinetics of viral replication and cytokine expression. Our key findings may be summarized as follows. First, CAEV RNA was readily detected early after experimental infection (days 6 and 12), but at later time points, no or only very few cells expressing viral RNA were found in the joint by *in situ* hybridization even though the inflammation persisted. Significant numbers of viral RNA-expressing cells were found only in naturally infected goats with severe clinical arthritis. Second, the pattern of cytokine expression in goat lentiviral arthritis resembled that seen in RA, with few cells expressing IFN-γ and IL-2 mRNA and a larger number of cells expressing MCP-1, IL-6, and TNF-α mRNA. Third, IFN-γ

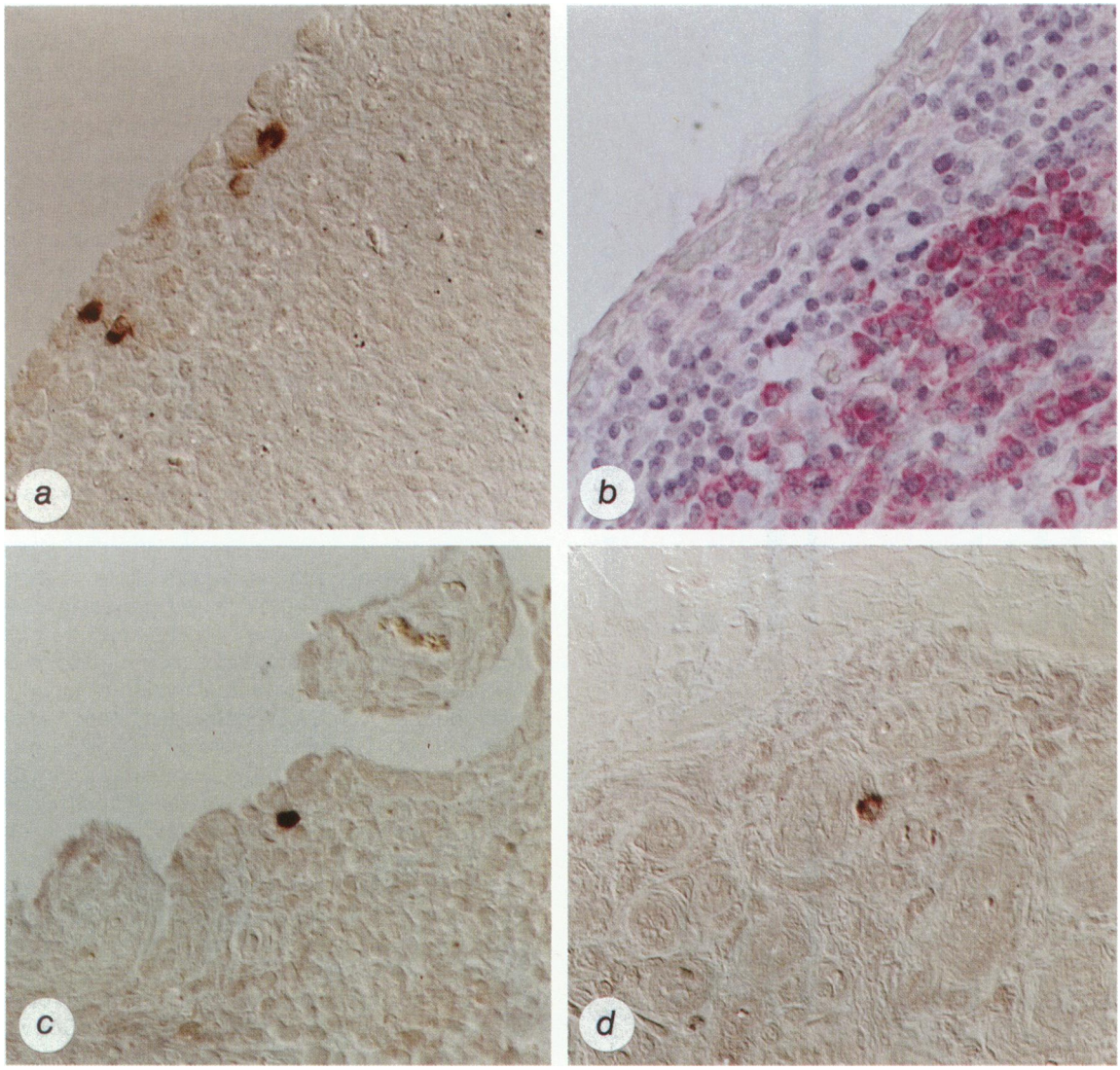

Figure 4. Detection of cytokines and plasma cells in the joint of a goat with clinical arthritis. Synovial membrane tissue sections of goat 85–14 hybridized with a probe specific for IL-6 mRNA (**a**), IL-2 mRNA (**c**), and IL-10 mRNA (**d**). **b:** Similar region as in **a** stained with an anti-goat IgG antibody (Fast red). Original magnification, ×400.

mRNA was always co-localized with viral RNA. And fourth, inflammation developed in a highly compartmentalized fashion, with cellular and humoral immune responses occurring concurrently but in different regions of the synovial membrane.

The mechanisms leading to rapid inhibition of productive virus expression in the joint after experimental infection are currently unknown. Large numbers of $CD8^+$ lymphocytes as well as $CD4^+$ lymphocytes were found in synovial membranes at 12 days after infection (von Bodungen et al, submitted), and shortly afterwards, antiviral antibodies became detectable in the serum. Hence, cellular as well as humoral antiviral immune responses and/or soluble antiviral mediators may be involved in the suppression of CAEV replication. In many cases, the specific hybridization signal for viral RNA was located in the nucleus, but not in the cytoplasm, of infected cells, which argues in favor of a possible restriction of virus replication.

In all except two animals, virus was successfully isolated from synovial fluid (Table 1), which suggests that, even in goats lacking detectable viral RNA-expressing cells, the virus had not been eliminated from the joint. This is indicative of either the persistence of provirus in synovial fluid cells or a presence of low titers of cell-free virus in the synovial fluid.

At 6 days after infection, virus was also isolated from the synovial fluid of contralateral, mock-infected carpal joints but not from that of tarsal joints (Table 1, not shown). Nonspecific irritation associated with mock infection may favor the homing of virus to the joint. This interpretation is supported by experiments showing that virus can readily be demonstrated also in tarsal joints 12 days after intravenous and intracarpal infection if the tarsal joints had been mock infected (not shown).

The numbers of virus-expressing cells in subscapular lymph nodes observed between 6 days and 1 year after infection were surprisingly small (Table 2). This is clearly

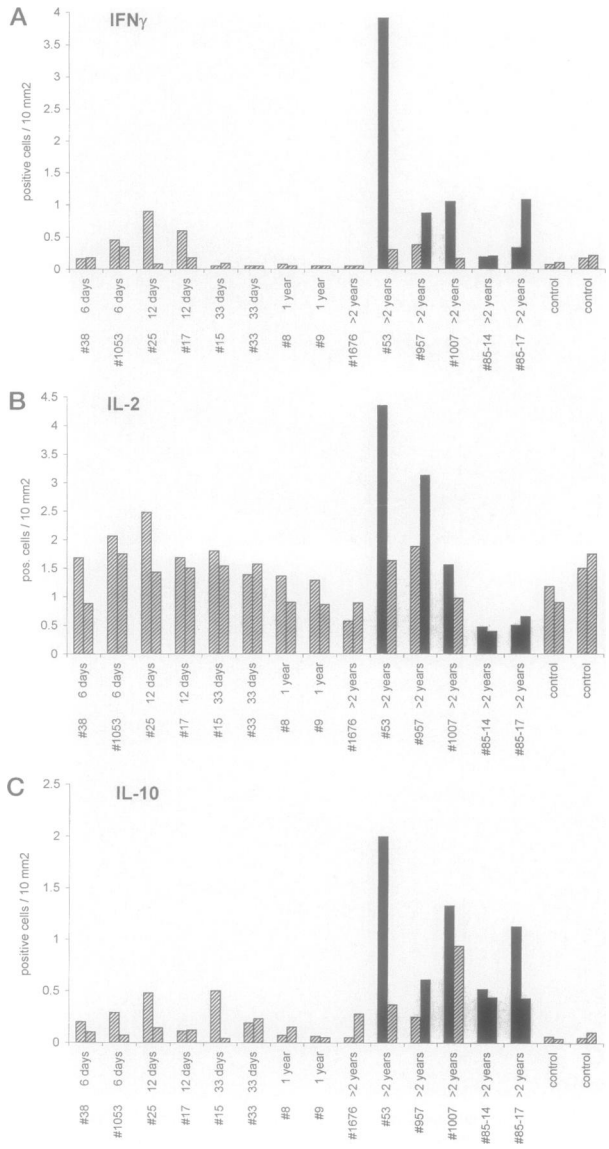

Figure 5. Expression of cytokines in prescapular lymph nodes of CAEV-infected goats. Prescapular lymph nodes of experimentally and naturally infected goats (see Table 1) were analyzed. Three to six cross sections of the left and right prescapular lymph nodes of each goat were hybridized with IFN-γ, IL-2, or IL-10 mRNA probes, and stained cells were counted. Results of the left and right nodes are shown. Prescapular lymph nodes of two uninfected goats were included as a control. ▨, lymph nodes draining joints without clinical arthritis; ■, lymph nodes draining joints with clinical arthritis.

different from the situation observed in lymph nodes of HIV-infected humans,[40] SIV-infected macaques,[38,41,42] and FIV-infected cats,[42,43] where high viral loads are found in the acute stage of infection and, in some cases, also in the asymptomatic stage.

Our short-term model of CAEV infection permitted us to follow the expression of cytokines in the joint from the onset of inflammation. The pattern of expression of some, but not all, cytokines investigated was similar to that observed in RA. In CAEV-induced arthritis, the T-lymphocyte-derived cytokines IL-2 and IFN-γ were detected in only a few cells in the synovial membrane whereas MCP-1, IL-6, and TNF-α were expressed more abundantly at all stages (Table 3). Surprisingly, IL-2 and IFN-γ expression was not only relatively low in late-stage arthritis but also at early time points, eg, at day 12 when virus expression was at its peak. There has been a controversy regarding the contribution of T lymphocytes to the pathogenesis of RA due to low expression of IL-2 and IFN-γ mRNA and protein.[44] Recently, it has been shown that IL-15 is expressed at relatively high levels in RA, which might contribute to the chemoattraction and activation of T lymphocytes in the absence of abundant IL-2 and IFN-γ expression.[45] IL-15 might therefore be an attractive candidate for future studies on the role cytokines play in goat lentiviral arthritis.

Differences between CAEV-induced arthritis and RA were found with respect to IL-8 and IL-10. In caprine arthritis, IL-10 message was detected only in few lymphocyte-like cells, whereas in RA, IL-10 protein was demonstrated in numerous lymphocytes as well as macrophages by immunohistochemistry.[46,47] Gene expression, as assayed by us, may not directly reflect the presence of protein, as assayed in the studies of RA. It seems less likely that IL-8[48] can be explained in the same way. IL-8 is a strong chemoattractant for neutrophils, a cell type that is present in RA but uncommon in caprine arthritis. We have recently shown that infection of blood-derived macrophages with CAEV leads to increased expression of MCP-1 and IL-8 mRNA *in vitro* (F. Lechner, J. Machado, G. Bertoni, H.F. Seow, D.A.E. Dobblelaere, E. Peterhans: Caprine arthritis encephalitis dysregulates the expression of cytokines in macrophages. J Virol (in press).) Similarly, infection of alveolar macrophages *in vitro* with the related maedi visna virus leads to enhanced IL-8 expression.[49] However, we failed to detect IL-8 mRNA-expressing cells in synovial membranes at any stage of caprine arthritis, even when cells expressing viral RNA were present. Interestingly, *in vitro*, induction of IL-8 was observed only in macrophages replicating CAEV at maximal levels. Such high levels may not be achieved *in vivo*, due to a rapid elimination of infected cells by the immune system. Alternatively, the expression of IL-8 may be regulated differently *in vitro* compared with *in vivo*. In contrast to IL-8, MCP-1 transcripts were consistently detected in those regions of the synovium containing virus-expressing cells (Figure 3c), which suggests that local CAEV replication may directly increase the expression of this cytokine *in vitro* (Lechner et al, submitted) as well as *in vivo*. As MCP-1 transcripts were also observed in synovial membranes lacking detectable viral RNA, expression of this cytokine may, in addition, be regulated by nonviral mechanisms. MCP-1 was the most abundantly expressed cytokine in joints of goats with clinical arthritis (Table 3). Similar to RA,[50] this cytokine was strongly expressed in cells in the synovial lining layer as well as in mononuclear infiltrates in the subintima. MCP-1 acts as a chemoattractant for monocytes/macrophages as well as lymphocytes[51–53] and may therefore contribute to a mononuclear infiltration of the synovium. Furthermore, MCP-1 activates monocytes to express adhesion molecules and secrete other cytokines.[54] Interestingly, MCP-1 has recently been shown to support Th2 immune responses in a murine model.[55] It is therefore tempting to

speculate that MCP-1 may be involved not only in the accumulation of inflammatory cells but also in supporting type 2 immune responses.

Six days after intracarpal infection of goats, viral RNA, but no IFN-γ mRNA, was detected in the joint (Table 3). Twelve days after infection, viral RNA and IFN-γ mRNA were expressed in a co-localized fashion, and at 33 days after infection, neither were detected any longer. The role of IFN-γ in the immune response against CAEV is at present unclear. On the one hand, in many viral models, IFN-γ was found to play an important antiviral role by stimulating both the innate and the adaptive immune system.[56–58] On the other hand, IFN-γ may activate viral transcription by binding to IFN-γ-responsive elements (γ-activated sites) present in the long terminal repeat of the CAEV genome.[59] It is also conceivable that IFN-γ-mediated virus activation in latently infected cells may subsequently lead to recognition and elimination of these cells by the immune system.

IL-6, which was highly expressed in caprine arthritis, has been shown to co-stimulate T lymphocytes[60] and act as a B cell differentiation factor.[61,62] Interestingly, IL-6-positive cells were often located adjacent to plasma cell aggregates in the synovium (Figure 4, a and b), which suggests that IL-6 may contribute to local maturation of B cells into plasma cells in the synovial membrane. It remains to be shown whether these plasma cells are the source of the high titer of antiviral antibodies found in the synovial fluid.[63] It has previously been proposed that a shift from a predominant type 1 to a type 2 immune response may correlate with the severity of arthritis.[8,27,64] However, the pattern of cytokine expression observed in this study suggests that the situation may well be more complex. The fact that synovial membranes and draining lymph nodes contained cells expressing IFN-γ (a cytokine typical of a type 1 immune response) both during acute infection (day 12) and in goats with severe clinical arthritis, demonstrates that goats with clinical arthritis do not exhibit a general deficiency in IFN-γ expression in response to CAEV. Local IFN-γ mRNA expression depended more on the presence of viral transcripts than on the stage of arthritis. However, preliminary experiments revealed that IL-10 expression (supporting type 2 immune responses) appeared to be increased in lymph nodes of goats with chronic arthritis (not shown). We were unable to detect IL-4 transcripts in the synovium or in lymph nodes of CAEV-infected goats. We do not know to date whether this is due to a lack of sensitivity of the *in situ* hybridization method or whether, similarly to RA,[16,17] IL-4 is not significantly expressed in CAEV-induced arthritis.

The fact that we used *in situ* hybridization to study the expression of cytokines and viral replication enabled us to make an observation that we believe may add an important extension to the general concept of the type 1/type 2 paradigm; we observed a striking compartmentalization of the inflamed synovium at the level of both cytokine expression and virus replication. Taking into account the histological and *in situ* hybridization results obtained in the synovial membrane makes it difficult to classify caprine arthritis as a type 1 or a type 2 disease. On the basis of the finding that IFN-γ mRNA and viral RNA were expressed in a restricted and co-localized fashion, we hypothesize that areas with a predominant expression of this type 1 cytokine may represent sites with an ongoing cellular immune response against the virus. In contrast, areas with mainly plasma cell aggregates and lymphoid-like follicles may be indicative of a type-2-centered, mainly humoral, response. Both types of response occur concurrently, but in different regions, in the same arthritic joint. Over the period of observation, areas with a predominantly humoral immune response tended to outweigh those with an ongoing cellular immune response. The transition from a predominantly cellular to a predominantly antibody-centered immune response during the course of infection may be irreversible, thus leading ultimately to an inflammation characterized by an extensive accumulation of plasma cells. As outlined above, it will be interesting to determine the specificity of the antibodies released from these plasma cells.

Acknowledgments

We thank Pablo Cordano for the help with tissue sample collection and Hedwig Pfister, Ingrid Strasser, and Marie-Louise Zahno for excellent technical assistance. We are grateful to Carmen Cardozo and Karin Beck for preparing the tissue sections, to Marc Vandevelde for helpful discussions, and to Ruth Parham for linguistic improvements. We are indebted to Barbara Blacklaws for the VPM 54 antibody.

References

1. Crawford TB, Adams DS: Caprine arthritis-encephalitis: clinical features and presence of antibody in selected goat populations. J Am Vet Med Assoc 1981, 178:713–719
2. Adams DS, Oliver RE, Ameghino E, DeMartini JC, Verwoerd DW, Houwers DJ, Waghela S, Gorham JR, Hyllseth B, Dawson M, Trigo FJ: Global survey of serological evidence of caprine arthritis-encephalitis virus infection. Vet Rec 1984, 115:493–495
3. East NE, Rowe JD, Madewell BR, Floyd K: Serologic prevalence of caprine arthritis-encephalitis virus in California goat dairies. J Am Vet Med Assoc 1987, 190:182–186
4. Cork LC, Hadlow WJ, Crawford TB, Gorham JR, Piper RC: Infectious leukoencephalomyelitis of young goats. J Infect Dis 1974, 129:134–141
5. Crawford TB, Adams DS, Cheevers WP, Cork LC: Chronic arthritis in goats caused by a retrovirus. Science 1980, 207:997–999
6. Cork LC, Narayan O: The pathogenesis of viral leukoencephalomyelitis-arthritis of goats. I. Persistent viral infection with progressive pathologic changes. Lab Invest 1980, 42:596–602
7. Woodard JC, Gaskin JM, Poulos PW, MacKay RJ, Burridge MJ: Caprine arthritis-encephalitis: clinicopathologic study. Am J Vet Res 1982, 43:2085–2096
8. Wilkerson MJ, Davis WC, Baszler TV, Cheevers WP: Immunopathology of chronic lentivirus-induced arthritis. Am J Pathol 1995, 146: 1433–1443
9. Moreland LW, Koopman WJ: Infection as a cause of arthritis. Curr Opin Rheumatol 1991, 3:639–649
10. Wilder RL: Hypothesis for retroviral causation of rheumatoid arthritis. Curr Opin Rheumatol 1994, 6:295–299
11. Kalden JR, Gay S: Retroviruses and autoimmune rheumatic diseases. Clin Exp Immunol 1994, 98:1–5
12. Adams DS, Crawford TB, Klevjer Anderson P: A pathogenetic study of the early connective tissue lesions of viral caprine arthritis-encephalitis. Am J Pathol 1980, 99:257–278

13. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986, 136:2348–2357
14. Romagnani S: Human TH1 and TH2 subsets: doubt no more. Immunol Today 1991, 12:256–257
15. Clerici M, Shearer GM: The Th1-Th2 hypothesis of HIV infection: new insights. Immunol Today 1994, 15:575–581
16. Feldmann M, Brennan FM, Maini RN: Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 1996, 14:397–440
17. Simon AK, Seipelt E, Sieper J: Divergent T-cell cytokine patterns in inflammatory arthritis. Proc Natl Acad Sci USA 1994, 91:8562–8566
18. Miltenburg AM, van Laar JM, de Kuiper R, Daha MR, Breedveld FC: T cells cloned from human rheumatoid synovial membrane functionally represent the Th1 subset. Scand J Immunol 1992, 35:603–610
19. Bucht A, Larsson P, Weisbrot L, Thorne C, Pisa P, Smedegard G, Keystone EC, Gronberg A: Expression of interferon-γ (IFN-γ), IL-10, IL-12 and transforming growth factor-β (TGF-β) mRNA in synovial fluid cells from patients in the early and late phases of rheumatoid arthritis (RA). Clin Exp Immunol 1996, 103:357–367
20. Chen E, Keystone EC, Fish EN: Restricted cytokine expression in rheumatoid arthritis. Arthritis Rheum 1993, 36:901–910
21. Ulfgren AK, Lindblad S, Klareskog L, Andersson J, Andersson U: Detection of cytokine producing cells in the synovial membrane from patients with rheumatoid arthritis. Ann Rheum Dis 1995, 54:654–661
22. Schlaak JF, Pfers I, Meyer Zum Buschenfelde KH, Marker Hermann E: Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies. Clin Exp Rheumatol 1996, 14:155–162
23. al Janadi N, al Dalaan A, al Balla S, Raziuddin S: $CD4^+$ T cell inducible immunoregulatory cytokine response in rheumatoid arthritis. J Rheumatol 1996, 23:809–814
24. Saltarelli M, Querat G, Konings DAM, Vigne R, Clements JE: Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology 1990, 179:347–364
25. Zanoni RG, Vogt HR, Pohl B, Bottcher J, Bommeli W, Peterhans E: An ELISA based on whole virus for the detection of antibodies to small-ruminant lentiviruses. J Vet Med B 1994, 41:662–669
26. Zanoni R, Krieg A, Peterhans E: Detection of antibodies to caprine arthritis-encephalitis virus by protein G enzyme-linked immunosorbent assay and immunoblotting. J Clin Microbiol 1989, 27:580–582
27. Wilkerson MJ, Davis WC, Cheevers WP: Peripheral blood and synovial fluid mononuclear cell phenotypes in lentivirus induced arthritis. J Rheumatol 1995, 22:8–15
28. Seow HF, Rothel JS, Radford AJ, Wood PR: The molecular cloning of ovine interleukin 2 gene by the polymerase chain reaction. Nucleic Acids Res 1990, 18:71–75
29. Seow HF, Rothel JS, Wood PR: Cloning and sequencing an ovine interleukin-4-encoding cDNA. Gene 1993, 124:291–293
30. Seow HF, Yoshimura T, Wood PR, Colditz IG: Cloning, sequencing, expression, and inflammatory activity in skin of ovine interleukin-8. Immunol Cell Biol 1994, 72:398–405
31. Radford AJ, Hodgson ALM, Rothel JS, Wood PR: Cloning and Sequencing of the ovine gamma-interferon gene. Aust Vet J 1991, 68:82–84
32. Graber HU, Meyer RK, Fatzer R, Vandevelde M, Zurbriggen A: In situ hybridization and immunohistochemistry for prion protein (Pr P) in bovine spongiform encephalopathy (BSE). J Vet Med Assoc 1995, 42:453–459
33. Lechner F, Vogt HR, Seow HF, von Bodungen U, Bertoni G, Zurbriggen A, Peterhans E: Expression of TNF α in arthritis caused by caprine arthritis encephalitis virus. Vet Immunol Immunopathol 1996, 54:281–289
34. Dutia BM, Hopkins J, Allington MP, Bujdoso R, McConnell I: Characterization of monoclonal antibodies specific for α- and β-chains of sheep MHC class II. Immunology 1990, 70:27–32
35. Johnson GC, Adams DS, McGuire TC: Pronounced production of polyclonal immunoglobulin G1 in the synovial fluid of goats with caprine arthritis-encephalitis virus infection. Infect Immun 1983, 41: 805–815
36. Biberfeld P, Chayt KJ, Marselle LM, Biberfeld G, Gallo RC, Harper ME: HTLV-III expression in infected lymph nodes and relevance to pathogenesis of lymphadenopathy. Am J Pathol 1986, 125:436–442
37. Fox CH, Tenner Racz K, Racz P, Firpo A, Pizzo PA, Fauci AS: Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA (published erratum appears in J Infect Dis 1992, 165:1161). J Infect Dis 1991, 164:1051–1057
38. Chakrabarti L, Isola P, Cumont MC, Claessens Maire MA, Hurtrel M, Montagnier L, Hurtrel B: Early stages of simian immunodeficiency virus infection in lymph nodes: evidence for high viral load and successive populations of target cells. Am J Pathol 1994, 144:1226–1237
39. Klevjer-Anderson P, Adams SD, Anderson W, Banks KJ, McGuire TC: Sequential study of virus expression in retrovirus-induced arthritis of goats. Gen Virol 1984, 65:1519–1525
40. Pantaleo G, Menzo S, Vaccarezza M, Graziosi C, Cohen OJ, Demarest JF, Montefiori D, Orenstein JM, Fox C, Schrager LK, Margolick JB, Buchbinder S, Giorgi JV, Fauci AS: Studies in subjects with long term nonprogressive human immunodeficiency virus infection. N Engl J Med 1995, 332:209–216
41. Reimann KA, Tenner Racz K, Racz P, Montefiori DC, Yasutomi Y, Lin W, Ransil BJ, Letvin NL: Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. J Virol 1994, 68:2362–2370
42. Hurtrel B, Chakrabarti L, Hurtrel M, Bach JM, Ganiere JP, Montagnier L: Early events in lymph nodes during infection with SIV and FIV. Res Virol 1994, 145:221–227
43. Beebe AM, Dua N, Faith TG, Moore PF, Pedersen NC, Dandekar S: Primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets. J Virol 1994, 68:3080–3091
44. Firestein GS, Zvaifler NJ: How important are T cells in chronic rheumatoid synovitis? (published erratum appears in Arthritis Rheum 1990, 33:1437). Arthritis Rheum 1990, 33:768–773
45. McInnes IB, Leung BP, Sturrock RD, Field M, Liew FY: Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-α production in rheumatoid arthritis. Nature Med 1997, 3:189–195
46. Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M: Immunoregulatory role of interleukin 10 in rheumatoid arthritis. J Exp Med 1994, 179:1517–1527
47. Cohen SBA, Katsikis PD, Chu CQ, Thomssen H, Webb LMC, Maini RN, Londei M, Feldmann M: High level of interleukin-10 production by the activated T cell population within the rheumatoid synovial membrane. Arthritis Rheum 1995, 38:946–952
48. Deleuran B, Lemche P, Kristensen M, Chu CQ, Field M, Jensen J, Matsushima K, Stengaard Pedersen K: Localisation of interleukin 8 in the synovial membrane, cartilage-pannus junction and chondrocytes in rheumatoid arthritis. Scand J Rheumatol 1994, 23:2–7
49. Legastelois I, Cordier G, Cozon G, Cadore JL, Guiguen F, Greenland T, Mornex JF: Visna-maedi virus-induced expression of interleukin-8 gene in sheep alveolar cells following experimental in vitro and in vivo infection. Res Virol 1996, 147:191–197
50. Harigai M, Hara M, Yoshimura T, Leonard EJ, Inoue K, Kashiwazaki S: Monocyte chemoattractant protein-1 (MCP-1) in inflammatory joint diseases and its involvement in the cytokine network of rheumatoid synovium. Clin Immunol Immunopathol 1993, 69:83–91
51. Rollins BJ, Walz A, Baggiolini M: Recombinant human MCP-1/JE induces chemotaxis, calcium flux, and the respiratory burst in human monocytes. Blood 1991, 78:1112–1116
52. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA: Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proc Natl Acad Sci USA 1994, 91:3652–3656
53. Baggiolini M, Dewald B, Moser B: Interleukin-8 and related chemotactic cytokines: CXC and CC chemokines. Adv Immunol 1994, 55: 97–179
54. Jiang Y, Beller DI, Frendl G, Graves DT: Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocytes. J Immunol 1992, 148:2423–2428
55. Chensue SW, Warmington KS, Ruth JH, Sanghi PS, Lincoln P, Kunkel SL: Role of monocyte chemoattractant protein-1 (MCP-1) in Th1 (mycobacterial) and Th2 (schistosomal) antigen-induced granuloma formation. J Immunol 1996, 157:4602–4608
56. Orange JS, Wang B, Terhorst C, Biron CA: Requirement for natural killer cell-produced interferon γ in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration. J Exp Med 1995, 182:1045–1056
57. Muller U, Steinhoff U, Reis LF, Hemmi S, Pavlovic J, Zinkernagel RM, Aguet M: Functional role of type I and type II interferons in antiviral defense. Science 1994, 264:1918–1921

58. Young HA, Hardy KJ: Role of interferon-γ in immune cell regulation. J Leukocyte Biol 1995, 58:373–381
59. Tong Starksen SE, Sepp T, Pagtakhan AS: Activation of caprine arthritis-encephalitis virus long terminal repeat by γ interferon. J Virol 1996, 70:595–599
60. Lotz M, Jirik F, Kabouridis P, Tsoukas C, Hirano T, Kishimoto T, Carson DA: B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. J Exp Med 1988, 167:1253–1258
61. Akira S, Hirano T, Taga T, Kishimoto T: Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB J 1990, 4:2860–2867
62. Kishimoto T: The biology of interleukin-6. Blood 1989, 74:1–10
63. Johnson GC, Barbet AF, Klevjer-Anderson P, McGuire TC: Preferential immune response to virion surface glycoproteins by caprine arthritis-encephalitis virus-infected goats. Infect Immun 1983, 41:657–665
64. Perry LL, Wilkerson MJ, Hullinger GA, Cheevers WP: Depressed $CD4^+$ T lymphocyte proliferative response and enhanced antibody response to viral antigen in chronic lentivirus-induced arthritis. J Infect Dis 1995, 171:328–334