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Immunopathology of Chronic Lentivirus-Induced Arthritis

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This study evaluated histopathology and mononuclear cell phenotypes in synovial lesions of chronic arthritis induced by experimental infection of Saanen goats with caprine arthritis-encephalitis lentivirus. Histological examination of carpal joint synovium of three infected goats with clinical arthritis revealed progressive lesions consisting of membrane villus hypertrophy with extensive angiogenesis and mononuclear cell infiltration and degenerative changes of membrane villus necrosis associated with loss of vasculature and infiltrates. Changes in synovial tissue of five age-matched infected goats without clinical arthritis were limited to moderate synovial membrane hyperplasia also noted in an age-matched uninfected goat. Immunohistochemistry identified CD45R$^+$ CD5$^-$ B lymphocytes as the principal component of most perivascular infiltrates in arthritic synovium. Other mononuclear cells included perivascular CD4$^+$ and CD8$^+$ T lymphocytes and macrophages with a prominent accumulation of CD8$^+$ T lymphocytes at the lining surface of inflamed villi. T lymphocytes and macrophages as well as synovial lining cells were activated with respect to MHC class II but not for interleukin-2 receptors. Inflamed villi also contained lymphoid aggregates comprised of B cell germinal centers and activated T-cell mantles. B cells expressing immunoglobulin occurred around follicles and throughout inflamed villi. These findings indicate that memory immune responses that favor expansion and maturation of B cells and immunoglobulin production contribute to the immunopathology of chronic arthritis. (Am J Pathol 1995, 146:1433–1443)

Caprine arthritis encephalitis virus (CAEV) is a lentivirus[1–4] that infects monocytes and macrophages and causes a complex of disease syndromes in domestic goats, including arthritis, mastitis, pneumonia, and encephalitis. Arthritis is the major clinical manifestation of infection.[5] CAEV-induced arthritis has been experimentally reproduced[6,7] and primarily affects radiocarpal joints in both experimentally and naturally infected goats.[6,8] Clinical signs of arthritis include periarticular swelling with excessive accumulation of synovial fluid (SF) containing inflammatory cells[6,9–11] and radiographic changes of soft tissue mineralization and erosion of articular surfaces.[10] The principal histological features of arthritis are synovial membrane villus hypertrophy with mononuclear cell infiltration and dense mononuclear cell aggregates resembling lymphoid follicles.[6,8,11] The histological lesions and progressive course of CAEV-induced arthritis are similar to rheumatoid arthritis (RA).[12]

Development of CAEV-induced arthritis is associated with dominance of immune responses that favor production of immunoglobulin (Ig).[9,13] Previous studies established that serum and SF antibody titers to CAEV gp135 surface protein correlate with the presence and severity of joint lesions.[13] Increased serum antibody titers to CAEV gp135 as early as 3 months postinfection predict the eventual development of chronic nonremittent arthritis.[14] Vigorous humoral immune reactivity to CAEV antigens includes pronounced production of polyclonal IgG1 in SF,[15] the presence of plasma cells within inflamed synovium[15] and formation of secondary B cell follicles in regional lymph nodes.[6] However, recent phenotype studies demonstrated that SF of arthritic carpal joints of CAEV infected goats contains few B lymphocytes and is enriched for major histocompatibility class (MHC) class II-activated CD8$^+$ T lymphocytes that express little IL-2 receptor (IL-2R).[10] These findings suggested that the pathogenesis of CAEV-induced arthritis could be more clearly defined by identifying specific im-

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mune cells infiltrating arthritic synovium. Accordingly, the present study utilized immunohistochemistry to identify the phenotypes of immune cells comprising mononuclear cell infiltrates in carpal joint synovial tissue of CAEV-infected goats with chronic arthritis. The results provide a histological description of progressive arthritis, establish that $CD5^-$ B lymphocytes are the principal component of synovial tissue infiltrates, and suggest specific mechanisms of immunopathogenesis of CAEV-induced arthritis.

Materials and Methods

Animals

Tissues used in this study were obtained from nine Saanen goats born in 1985. Eight goats were orally infected at birth with the prototype virus CAEV-63.[16] Tissues from an age-matched CAEV-free goat were used as the uninfected control. All CAEV-infected goats seroconverted for antibody to CAEV antigens by 3 months postinfection and maintained antibody titers throughout the period of infection.[14] At the time of necropsy (9 years postinfection), three of the persistently infected goats had long-standing chronic non-remitting arthritis of carpal joints. The development and progression of clinical arthritis in these goats has been described.[9,10] The other five infected goats had never shown clinical signs of arthritis.[9,10]

Tissue Preparation for Histopathology and Immunohistochemistry

Samples of carpal synovium and prescapular and axillary lymph nodes collected at necropsy were either fixed in 10% buffered formalin for light microscopy or embedded in ornithine carbamoyltransferase compound (Miles Diagnostics, Inc., Elkhart, IN) and snap frozen in liquid nitrogen for immunohistochemistry. Frozen tissue blocks were stored at −70 C.

Formalin-fixed samples were processed in paraffin, cut at 5 μ and stained with hematoxylin and eosin (H&E). For immunohistochemistry, 4-μ tissue sections were cut from the frozen blocks at −20 C using a Cryostat II (Miles Diagnostics), placed on silanized (3-aminopropyltrimethoxy-silane)-coated glass microscope slides (Probe On Plus) (Fisher Scientific, Pittsburgh, PA), and air dried for 10 minutes. The slides were fixed in acetone at room temperature for 10 minutes and stored at 4 C for less than 1 week.

Regions of the Synovium for Interpretation of Histopathology and Immunohistochemistry

Intimal, subintimal, and subsynovial connective tissue regions corresponding to human synovium[17] were identified in the H&E-stained synovial tissue sections. The intima consisted of multiple membrane villi supported by the subintima, a well-vascularized connective tissue matrix of loosely arranged collagen fibers, fibroblasts, and variable amounts of fat. The subsynovium is defined as a dense collagenous tissue containing sparse vascular structures lined by an adventitial surface contiguous with tendon attachments.

Antibodies Used for Immunohistochemistry

Rabbit anti-human factor VIII-related antigen (DAKO Corp., Carpinteria, CA) was used to identify vascular endothelium. Rabbit anti-human CD3 (DAKO) and monoclonal antibodies GR60A (anti-CD5),[18] GC1A (anti-CD4),[19] and CACT80C (anti-CD8)[19] were used to identify T lymphocytes and T cell subsets. γδ T lymphocytes were identified with monoclonal antibody GB21A, which immunoprecipitates the δ chain of the γδ T cell receptor (W.C. Davis, unpublished observation). B lymphocytes were identified by positive staining with monoclonal antibody GS5A to CD45R[20] and negative staining by monoclonal antibody GR60A (anti-CD5) or rabbit anti-human CD3. B lymphocytes expressing membrane or surface Ig were localized by a cocktail of two monoclonal antibodies (BIG43A and BIG501E) to Ig κ and λ light chains[21] and monoclonal antibody PIG45A2 specific for the heavy chain of IgM.[22] Macrophages were identified with monoclonal antibody DH59B.[18] Germinal center B lymphocytes and follicular dendritic cells were identified with monoclonal antibody GB25A to the caprine ortholog of CD21 (W.C. Davis, unpublished observations). Expression of two specific leukocyte activation markers (IL-2R-α chain and MHC class II DRα) were detected using monoclonal antibodies CACT116A[23] and TH14B.[24]

Single and Dual Immunohistochemistry

Cytospin preparations of SF and cryostat tissue sections were immunolabeled using the MicroProbe manual staining system (FisherBiotech, Pittsburgh,

PA). Acetone-fixed slides were rehydrated in Tris-buffered saline (TBS) (0.125 mol/L TRIS, pH 7.6, containing 0.15 mol/L NaCl) before labeling with antibodies. An indirect avidin-biotin-complex immunoperoxidase procedure[25] was applied with modifications. Endogenous peroxidase activity was quenched in TBS containing 0.3% H_2O_2 and 0.1% azide.[26] The slides were washed in TBS containing 0.025% Triton X-100 (TBS-X) (Sigma Chemical Co., St. Louis, MO). To block nonspecific binding, the sections were incubated with 3% normal serum corresponding to the species of the secondary antibody. Tissue sections were incubated 60 minutes with an appropriate concentration of primary antibody (diluted in TBS-X containing 1.5% normal serum) followed by the appropriate biotinylated anti-IgG (Vector Laboratories, Burlingame, CA) (diluted 1:700 in TBS-X containing 1.5% normal serum and 2.0% goat serum) and ABC-peroxidase conjugate (Vector Laboratories). Each incubation step was preceded by a 10-minute wash in TBS-X. Color was developed with 0.5% diaminobenzidine tetrahydrochloride substrate (DAB) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) and 0.01% H_2O_2 in 0.1 mol/L TRIS-HCL, pH 7.6. Sections were counterstained with Mayer's hematoxylin (Sigma Chemical Co.). Each antibody was tested on caprine lymph node sections as a positive control. Negative controls consisted of substituting irrelevant antibody of analogous concentration, subclass, and isotype for the specific antibodies. These controls included *Escherichia coli* S 69A (IgG1), *E. coli* S 205A (IgG2a), and *E. coli* S 169A (IgG2b).[27] Rabbit immunoglobulin fraction X 903 (DAKO) were used as the negative control for rabbit anti-human factor VIII-related antigen and rabbit anti-human CD3.

Dual immunostaining for simultaneous visualization of two leukocyte markers within the same tissue section was performed by a modified indirect sequential peroxidase/alkaline phosphatase method.[25,28] After immunoperoxidase staining, the slides were rinsed in TBS-X for 60 minutes, blocked with 3.0% horse serum for 30 minutes, and incubated with a second primary antibody followed by anti-species-specific biotinylated secondary IgG and an avidin-biotin-alkaline phosphatase complex (Vector Laboratories). Immunostaining by the second sequence antibody was visualized with an alkaline phosphatase substrate containing naphthol phosphate (Fast Red substrate) (DAKO) diluted in 0.1 mol/l Tris buffer, pH 8.2. Counterstaining with Mayer's hematoxylin was applied after the development of the Fast Red substrate.

Quantitative Immunohistochemistry

Mononuclear cell subsets in synovial perivascular infiltrates were enumerated by counting immunoperoxidase-positive cells surrounding five randomly selected vessels within the subintimal layer of carpal synovium. Each perivascular infiltrate was counted within a constant area of 640 μ^2 using an ocular micrometer. The numerical counts were evaluated by analysis of variance for differences between experimental groups.

Results

Gross Pathology

Three CAEV-infected goats (85-14, -17, -28) had periarticular soft tissue swelling of carpal joints, whereas the remaining five infected goats (85-16, -18, -19, -24, -27) were clinically indistinguishable from the uninfected control goat (85-49).[10] Postmortem dissection of carpi revealed fibrosis and mineralization of the soft tissue covering the tendon of the extensor carpi radialis and enlarged bursae containing synovial fluid, fibrin clots, and fragments of cartilage. The bursae were lined by proliferative synovial membrane villi contiguous with the lining at the articular space. All articular surfaces within the carpal joints were roughened. The synovial membrane proliferation was most extensive in goat 85-17 in which the joint space was nearly obstructed by proliferative membrane villi (Figure 1). Collectively, these macroscopic changes corresponded with carpal/metacarpal ratios of >2.0 and

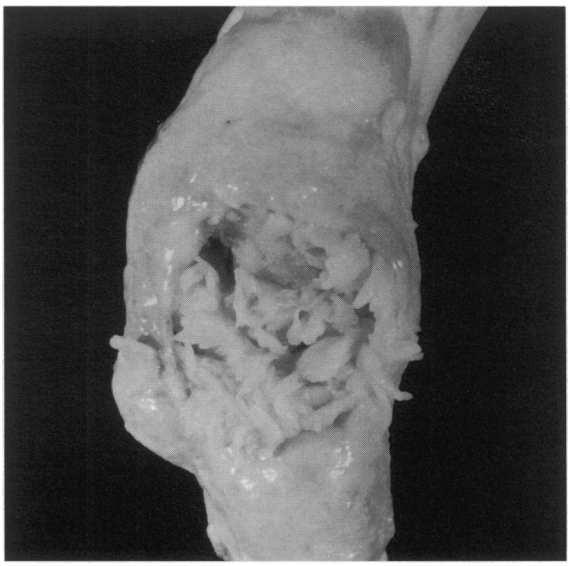

Figure 1. *Carpus of arthritic goat 85-17. Note soft tissue thickening and prominent synovial membrane villi.*

radiographic evidence of soft tissue swelling and mineralization with degenerative changes of the articular cartilage.[10] These features were absent in the carpal joints of the asymptomatic goats and the age-matched uninfected control.

Histopathology

The morphological changes in carpal synovium of asymptomatic goats included minimal accumulation of mononuclear cells and moderate synovial membrane hyperplasia (Figure 2A). Similar morphological features were noted in carpal synovium obtained from the age-matched uninfected goat (not shown). These findings indicate that minimal changes in asymptomatic synovium are nonspecific and age-related.

The most consistent finding in arthritic goats was hyperplasia of the lining cells and hypertrophy of membrane villi. In arthritic goat 85-14, this villus hypertrophy was due to expansion by diffuse mononuclear cell infiltrates (Figure 2B). Immunostaining of vascular endothelium for factor VIII-related antigen revealed numerous capillaries within hypertrophied villi (Figure 2C). These observations established that angiogenesis contributes to villus hypertrophy and that the inflammatory membrane lesion represents a coalescence of perivascular infiltrates. Although similar findings were noted in arthritic goat 85-28, comparative histological evaluation of carpal synovium from this goat and arthritic goat 85-17 demonstrated that synovial membrane hypertrophy was not always associated with angiogenesis and mononuclear cell infiltration. Membrane villi were less cellular in goat 85-28. Infiltrates were replaced in some areas of the tissue by a pale hyaline to fibrillar eosinophilic matrix representative of degenerative collagen or fibrin. Almost all hypertrophied villi in goat 85-17 were necrotic, lacked vasculature and lacked inflammatory cells (Figure 2D). Some of the hyaline material, particularly that deposited in vessel walls, was identified as amyloid. Polarizing microscopy of Congo red-stained sections imparted a green birefringence to stained amyloid deposits. The affinity of the Congo red stain was sensitive to potassium permanganate,

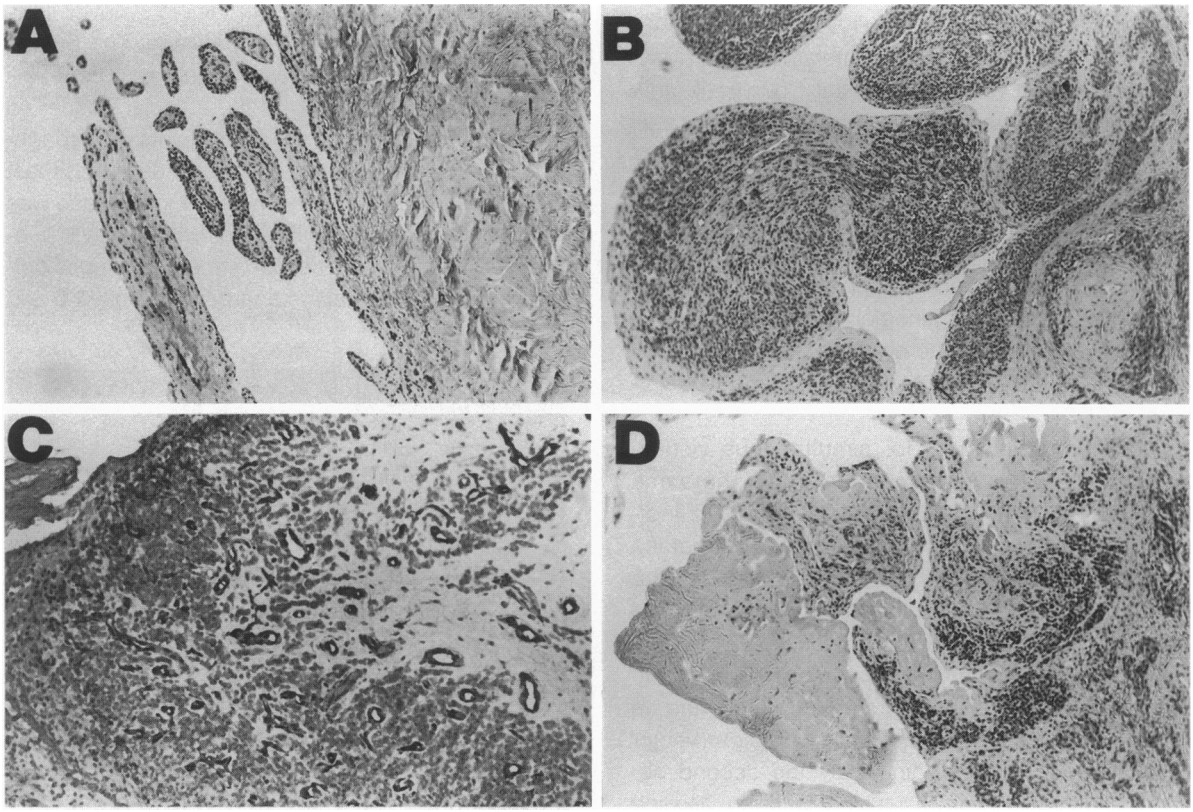

Figure 2. *Histopathology of carpal synovium of CAEV-infected goats.* (**A**) *Formalin-fixed carpal synovium of asymptomatic goat 85-18 shows moderate age-related synovial lining hyperplasia (H&E, 45×).* (**B**) *Formalin-fixed carpal synovium of arthritic goat 85-14 shows marked villus hypertrophy with dense mononuclear cell infiltration (H&E, 45×).* (**C**) *Cryostat section of synovial membrane villus of arthritic goat 85-14 labeled with antibody to factor VIII-related antigen and counterstained with Mayer's hematoxylin (71×). Immunoperoxidase staining (DAB) highlights membrane angiogenesis.* (**D**) *Formalin-fixed carpal synovium of goat 85-17 shows subintimal perivascular infiltrates and necrosis of hypertrophied villi (H&E, 45×).*

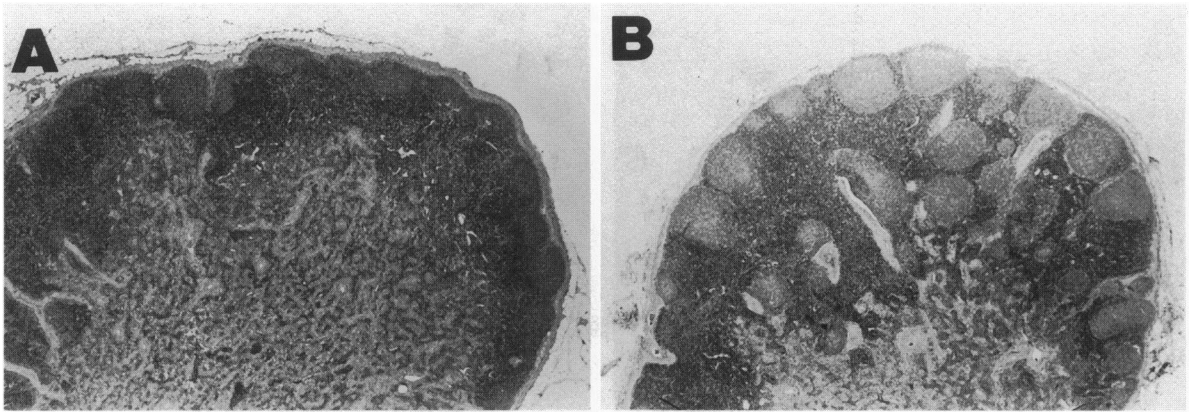

Figure 3. *Histology of regional lymph nodes.* (**A**) *Formalin-fixed prescapular lymph node from asymptomatic goat 85-18 shows minimal secondary follicle formation (H&E, 11×).* (**B**) *Formalin-fixed axillary lymph node of arthritic goat 85-28 shows numerous secondary follicles, paracortical hyperplasia, and expansion of medullary cords (H&E, 11×).*

indicating that the deposits represent reactive systemic amyloid. Perivascular infiltration of the subintimal layers was essentially equivalent in all three arthritic goats. In addition, the subintima contained focal cellular infiltrates not associated with vessels. The variability observed in synovial membrane pathology among the arthritic goats suggest that progressive synovial membrane degeneration and necrosis are secondary to inflammation and angiogenesis.

Prescapular and axillary lymph nodes of arthritic goats had prominent follicular hyperplasia, including development of numerous secondary follicles extending from the cortex into the medulla, hyperplasia of the paracortical regions, and expansion of the medullary cords (Figure 3B). In contrast, lymph nodes from the infected asymptomatic and uninfected control goats contained primary follicles with secondary follicle formation being restricted to the cortex (Figure 3A).

Mononuclear Cell Phenotypes in Perivascular Infiltrates

Subintimal vessels of arthritic tissue had significantly higher numbers of mononuclear infiltrates than subintimal vessels of asymptomatic goats and the control goat (Figure 4). The phenotypes of perivascular leukocytes were not significantly different between the uninfected control goat and the asymptomatic goats. In contrast, all mononuclear subsets except the γδ T cell population were significantly increased in arthritic synovium compared with the uninfected control and asymptomatic goats (monocytes, $P = 0.0024$; $CD45R^+$ cells, $P = 0.0013$; $CD4^+$ cells, $P = 0.0009$; $CD8^+$ cells, $P = 0.00001$; γδ T cells, $P = 0.2513$). Only arthritic synovial perivascular infiltrates contained Ig-producing cells.

Dual immunostaining identified the distribution of mononuclear cell perivascular infiltrates of arthritic

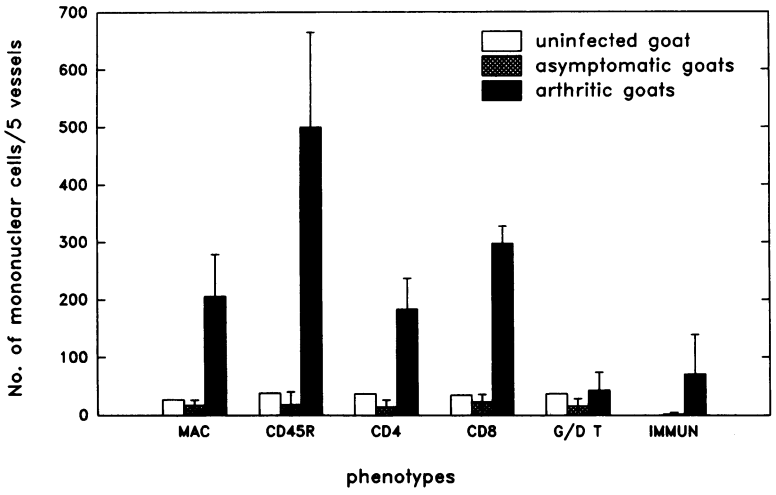

Figure 4. *Enumeration of mononuclear cell phenotypes surrounding five randomly selected subintimal vessels. Mean ± 1 SD.*

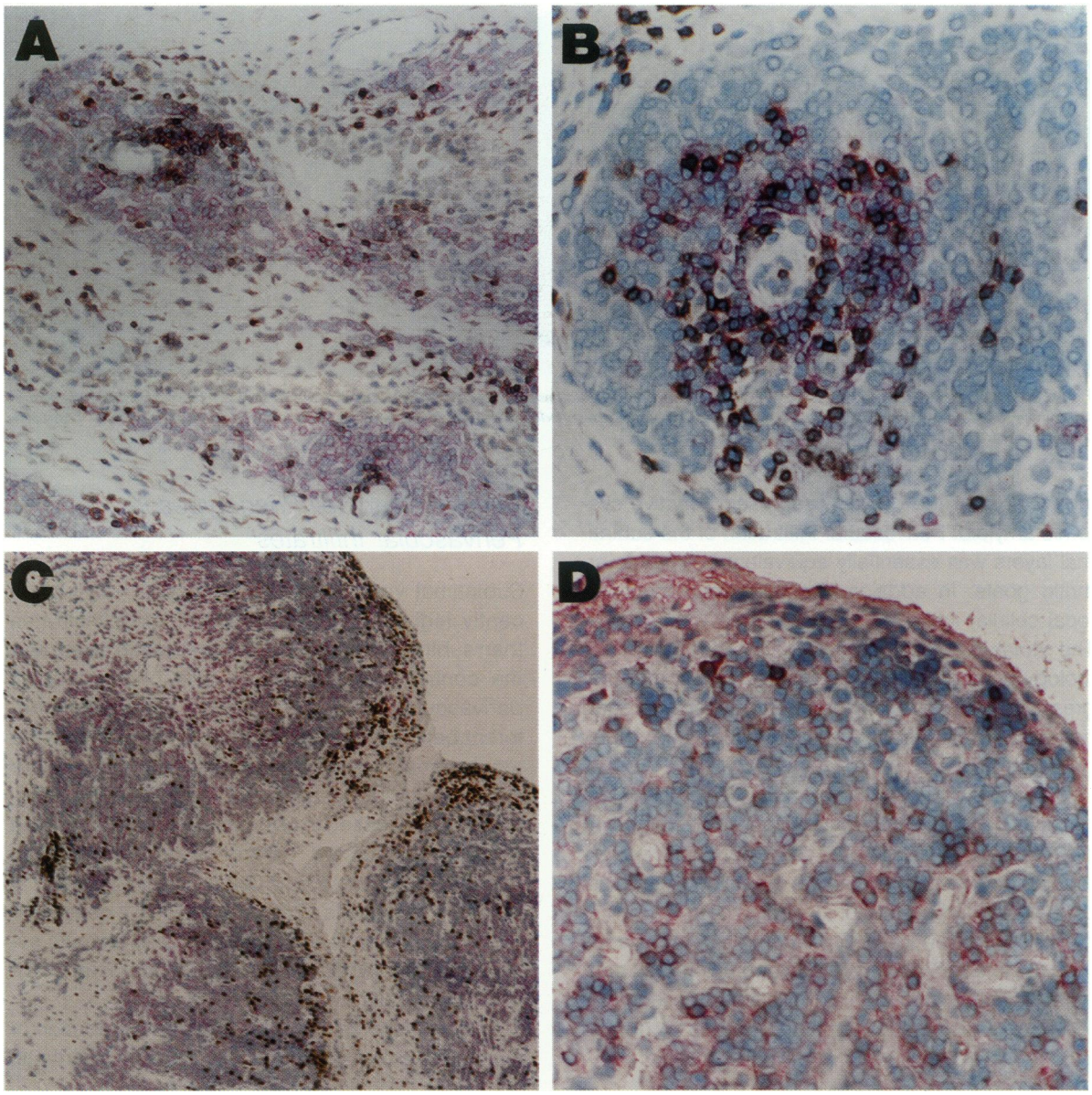

Figure 5. *Phenotypes of perivascular mononuclear infiltrates in cryostat sections of arthritic synovium.* (**A**) *Subintimal perivascular distribution of CD5$^+$ T lymphocytes (DAB) and CD45R$^+$ B lymphocytes (Fast Red) (96.9×).* (**B**) *Subintimal perivascular distribution of CD8$^+$ (DAB) and CD4$^+$ (Fast Red) T lymphocytes (241.8×).* (**C**) *Distribution of CD3$^+$ T lymphocytes (DAB) and CD45R$^+$ B lymphocytes in hypertrophied synovial membrane villi (Fast Red) (38.1×).* (**D**). *Distribution of B lymphocytes expressing Ig κ and λ light chains in a hypertrophied membrane villus (193.8×).*

synovium. The principal component of perivascular infiltrates was a CD5$^-$ CD45R$^+$ subset of B lymphocytes (Figure 5A). CD4$^+$ and CD8$^+$ T lymphocytes were distributed close to vessel walls (Figure 5B). Additional findings not shown were that 1) the unstained cells peripheral to the perivascular T lymphocytes in Figure 5B were CD5$^-$ CD45R$^+$ B lymphocytes in a serial section; 2) γδ T cells were few and exhibited a scattered distribution among the perivascular infiltrates, whereas macrophages were more numerous and found closely associated with lymphocytes; 3) moderate numbers of macrophages and CD8$^+$ T lymphocytes were in zones between the lymphocyte-rich perivascular areas, and 4) very few cells in perivascular infiltrates stained for IL-2R.

Dual immunostaining was also used to characterize the distribution of the mononuclear cell phenotypes comprising the coalescing perivascular infiltrates in hypertrophied synovial membrane villi. Sequential immunolabeling with antibodies to CD3 and CD45R demonstrated that these infiltrates were predominately CD3$^-$ CD45R$^+$ B lymphocytes (Figure 5C). CD3$^+$ T lymphocytes were distributed as small aggregates associated with membrane vessels simi-

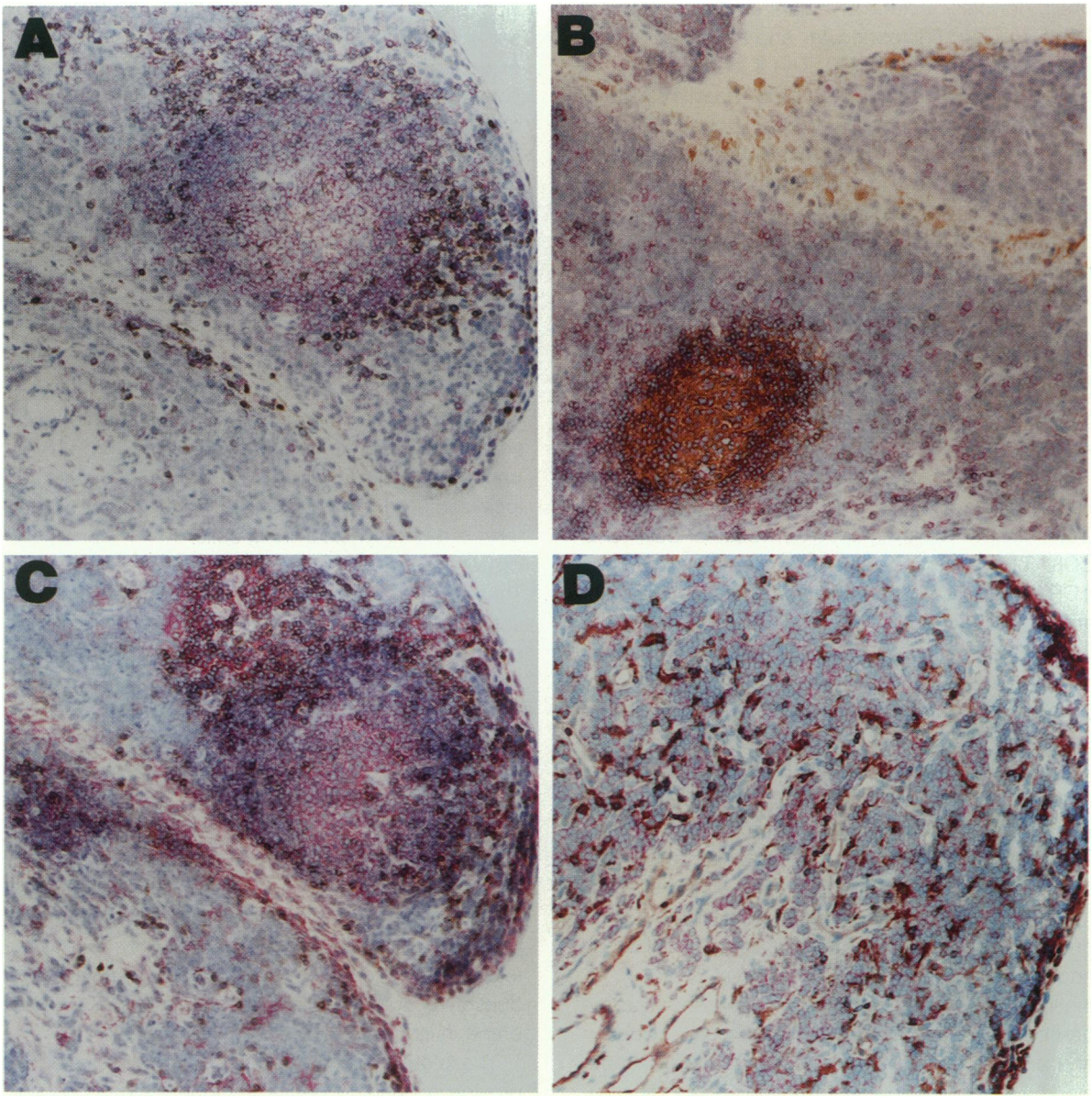

Figure 6. *Phenotypic composition and activation status of intimal lymphoid aggregates.* (**A**) *Peripheral distribution of CD5$^+$ T lymphocytes (DAB) and central distribution of CD45R$^+$ B lymphocytes (Fast Red) (96.9×).* (**B**) *Germinal center containing CD21$^+$ cells (DAB) with peripheral distribution of CD45R$^+$ B lymphocytes (Fast Red) (96.9×).* (**C**) *Germinal center containing MHC class II activated cells (Fast Red) with peripheral distribution of CD5$^+$ T lymphocytes (DAB) (96.9×) and other class II activated cells (Fast Red).* (**D**) *Extrafollicular distribution of class II$^-$ CD45R$^+$ B lymphocytes (Fast Red) and class II$^+$ mononuclear cells and endothelium (DAB) (96.9x).*

lar to that seen in Figure 5B and arranged as a band of cells near the apex of the hypertrophied villi (Figure 5C). Similar distributions of T and B lymphocytes were detected with CD5 and CD45R antibodies (not shown). Dual immunolabeling with CD4- and CD8-specific antibodies identified the T cell perivascular aggregates as a mixture of CD4$^+$ and CD8$^+$ T lymphocytes, whereas the T cells near the intimal surface were CD8$^+$ T lymphocytes (not shown). Immunostaining for Ig κ and λ light chains demonstrated numerous Ig-producing cells in the hypertrophied villus membrane (Figure 5D). Serial sections immunostained for surface IgM showed that very few Ig-producing cells expressed IgM (not shown).

Organization of Lymphoid-Like Follicles in Synovial Tissue

Dual immunolabeling studies defined the composition of lymphoid aggregates in the synovial intima. Organizational features resembling secondary lym-

phoid follicles included a central aggregation of $CD45R^+$ $CD5^-$ B lymphocytes and a mantle of $CD5^+$ T lymphocytes (Figure 6A). The T cell mantle contained a mixture of $CD4^+$ and $CD8^+$ T cells similar to that of the lymph node paracortex (not shown). The central cores resembling germinal centers were less densely populated and consisted of cells expressing a high level of the C3d complement receptor (CD21) (Figure 6B). B lymphocytes within the center of synovial lymphoid follicles were more strongly positive for MHC class II DRα than lymphocytes outside of these structures (Figure 6C). Immunostaining for class II DR and CD45R confirmed that extrafollicular $CD45R^+$ B lymphocytes were negative for class II expression, whereas synovial lining cells intermixed with activated $CD8^+$ T lymphocytes, stellate cells throughout the membrane stroma, and some vascular endothelium stained strongly for class II (Figure 6D). Other class II^+ cells included perivascular $CD4^+$ and $CD8^+$ T lymphocytes and macrophages, and all class II DR^+ cells also stained with antibodies to class II DP and DQ (not shown).

Discussion

This study extends previous reports of pathological changes in carpal synovium of CAEV-induced arthritis.[6,8,10,29] Histological changes in the synovium of CAEV-infected goats without clinically apparent arthritis were limited to moderate age-related synovial membrane hyperplasia with a normal distribution of primary B cell follicles in the cortex of regional lymph nodes. Conversely, synovial tissue of clinically arthritic joints had marked synovial membrane villus hypertrophy mediated by mononuclear cell infiltration and angiogenesis. These changes were accompanied by secondary follicle formation, paracortical hyperplasia, and expansion of medullary cords in regional lymph nodes. Areas of degenerative necrosis in addition to inflammatory changes in arthritic synovial tissue indicated that lesion progression involves necrosis of membrane villi with loss of infiltrates and vasculature. These degenerative changes correlate well with previous radiographic studies of these goats showing periarticular soft tissue swelling and mineralization with erosion of articular surfaces.[10] Similar degenerative changes in progressive RA[30] are related to the production of collagenases and the affects of proinflammatory monokines released by activated synoviocytes.[31,32]

This study also demonstrated that perivascular infiltrates and lymphoid aggregates in arthritic synovium predominantly consist of a $CD5^-$ $CD45R^+$ subset of B lymphocytes. CD45 is a leukocyte surface glycoprotein that exits in multiple isoforms designated CD45R that define leukocyte subsets based on differentiation status.[33] The lowest molecular mass isoform CD45R0 is expressed by memory T cells, whereas the highest molecular mass isoform CD45RA defines naive T cells.[33] CD45 expression by B lymphocytes has not been characterized in detail; however, limited studies indicate that the CD45RA isoform is involved in B cell expansion and maturation.[34] Infiltrates also contain MHC class II activated macrophages and class II activated $CD4^+$ and $CD8^+$ T lymphocytes that express little or no IL-2R. $CD4^+$ T lymphocytes mainly occur in close association with vessel walls and in the mantle zone of synovial lymphoid aggregates, whereas $CD8^+$ T lymphocytes are localized predominantly at the membrane surface intermixed with synovial lining cells. $\gamma\delta$ T lymphocytes do not constitute a significant proportion of infiltrates. These observations parallel many RA reports describing the distribution and phenotypes of mononuclear cell infiltrates in rheumatoid synovium.[12,35–39]

Three subsets of $CD45R^+$ B lymphocytes with specific regional distributions were identified: 1) $CD45R^+$ B cells that coexpress MHC class II and CD21 were localized to the central core of synovial lymphoid aggregates. Follicular dendritic cells (FDC) in addition to B lymphocytes probably account for intense CD21 expression localized to central zones of lymphoid aggregates.[40] 2) $CD45R^+$ B cells that lack class II and CD21 expression were localized to membrane perivascular infiltrates. 3) $CD45R^+$ B lymphocytes that produced κ and λ Ig light chains with little production of IgM were distributed throughout membrane infiltrates.

These observations together with previous studies provide a basis to define immune effector mechanisms that perpetuate chronic arthritis. CAEV-induced arthritis is associated with pronounced production of polyclonal IgG1 in SF[15] and markedly increased serum and SF antibody titers to CAEV antigen.[13] Thus, arthritis is associated with dominant immune responses that promote B cell proliferation and Ig production rather than cell-mediated immunity. As expected, recent studies demonstrated that enhanced humoral immune responses in arthritic goats are associated with a dominant population of CAEV-reactive $CD4^+$ T lymphocytes analogous to murine and human Th2 cells.[14] The present results extend these findings and provide evidence that secondary germinal centers in regional lymph nodes and lymphoid follicles in synovial lesions are sites of prolific

memory B cell expansion that accounts for B cell infiltration of synovial tissue and production of high levels of Ig.

This interpretation implicates the alternative pathway of antigen presentation by memory B cells.[41] The alternative pathway predominates over the classical pathway of antigen presentation by macrophages and dendritic cells in perpetuating memory antibody responses, especially in antibody-enriched environments with relatively low antigen levels.[42,43] This is due to efficient internalization of antigen by subpopulations of antigen-specific memory B cells via high avidity membrane Ig receptors.[43] Regarding CAEV antigen levels in arthritic goats, indirect immunoperoxidase staining using a monoclonal antibody to the CAEV p28 capsid protein[44] detected viral antigen in <1% of SF macrophages and type A synovial membrane cells in the goats used for the present study. Virus-infected macrophages were also detected infrequently in regional lymph node sinuses and subintimal perivascular infiltrates of synovial tissue; however, infected cells were not detected in synovial lymphoid aggregates. These observations confirm that viral antigen levels are low in chronic arthritis and support the hypothesis that the classical pathway of CAEV antigen presentation is relatively inoperative in secondary B cell germinal centers of arthritic lesions.

We hypothesize that high Ig concentrations in arthritic joints favor the formation of immune complexes with CAEV and possibly other antigens not identified and that these immune complexes mediate B cell expansion via the alternative pathway of antigen processing in secondary lymph node follicles and synovial lymphoid aggregates. Current knowledge of the alternative pathway in T-cell dependent memory humoral immune responses indicates that immune complexes are retained on Fc receptors of cytoplasmic processes (iccosomes) of $CD21^+$ FDC in germinal centers.[40,42,45] FDC probably account for intense centralized CD21 staining of germinal centers in the present study. This microenvironment establishes interactions between FDC and memory B cells necessary for maintenance of germinal centers[42,46] and the transfer of antigen from FDC iccosomes to memory B cells via Ig receptor mediated endocytosis.[41] Antigen processing transforms germinal center memory B cells into plasmacytoid blasts[45,46] that present antigenic peptides to memory T cells in the context of MHC class II molecules.[41] Antigen presentation by memory B cell blasts to T lymphocytes at the germinal center-T cell mantle zone junction[41] perpetuates activation of both cell types[40,47] and stimulates lymphokine production by memory T cells.[48] In this regard, the Th2 cytokine IL-4 may play a pivotal role in maintaining antigen presentation by antigen-activated B cells by increasing class II expression on these cells.[48] Specific memory antibody responses are maintained by repeated B cell antigen presentation,[48] and the Th2 cytokines IL-4 and IL-5 may function collectively to induce terminal differentiation of B cells into antibody-secreting cells as well as Ig isotype class switching associated with B cell memory.[33,48,49] Moreover, the antagonist effect of IL-4 on IL-2 production[33] may explain the paucity of IL-2R expression by synovial lymphocytes in CAEV arthritis. Our results indicate that clonal expansion and differentiation of antibody-specific B cells to plasma cells occurs in the extrafollicular areas of arthritic synovium. The $CD21^-$ class II^- Ig^+ B cell phenotype likely represents plasma cells derived from memory B cells, given that CD21 expression is lost upon maturation of B cells to plasma cells.[50] The scarcity of IgM-expressing cells in synovial membrane together with pronounced production of polyclonal IgG1 in SF[15] is indicative of Ig class switching mediated by IL-4 and IL-5[49] released by activated Th2 cells.[51]

In summary, our results indicate that memory B cell expansion in regional lymph nodes and lymphoid aggregates in synovial tissue is a prominent immune effector mechanism in chronic arthritis caused by CAEV and that memory B cell proliferation occurs in the context of Th2 cell activation via the alternative B cell pathway of antigen presentation. The synovial tissue changes and phenotypes of perivascular infiltrates identified in long-standing chronic CAEV-induced arthritis share many features with chronic RA. Unlike RA, the inducing agent in CAEV arthritis has been well characterized. Moreover, immune effector functions in this model can be studied without the complicating impact of therapeutic modalities often associated with human studies. Therefore, further studies of immune interactions involved in the pathogenesis of CAEV-induced arthritis have the potential to define mechanisms of joint destruction in RA.

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