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http://www.sciencedirect.com/science/article/B6TD5-4BBHBMW-2/2/08c9b850e658a620968819994711ddc

Tumor necrosis factor (TNF) receptor-associated factors (TRAFs) were identified as signal transducers for the tumor necrosis factor receptor (TNFR) superfamily. In this study, we cloned and characterized two genes that encode chicken TNFR-II and TRAF5. The initial cDNA fragments were obtained by suppressive subtractive hybridization (SSH) of chicken spleen cells with or without lipopolysaccharide stimulation (Salmonella typhimurium SL1181 (RE-mutant)). The results showed that chicken TNFR-II is 1518 bp in length with an open reading frame (ORF) of 1386 bp having 31% homology with human TNFR-II. Expression analysis of chicken TNFR-II revealed that it is highly expressed in the spleen and bursa of Fabricius. The chicken cell lines IN24, MSB1 and 1104B express TNFR-II abundantly. The time course analysis of expression in spleen, bursa of Fabricius and IN24 cell line showed that TNFR-II is maximally expressed at 6 h after stimulation in bursa of Fabricius and after 8 h stimulation in the IN24 cell line. With regard to TRAF5, the complete sequence was 1936 bp in length with an ORF of 1671 bp that showed 71.3% homology with human TRAF5. Expression analysis showed that, among the tissues examined, TRAF5 was strongly expressed in spleen and bursa of Fabricius, while among the cell lines examined, it was maximally expressed in IN24. Thus, both genes were expressed in the same tissues and cell line among examined materials. These results suggest that chicken TNFR-II may interact with TRAF5 adaptor protein to complete its signal transduction pathway.


http://www.sciencedirect.com/science/article/B6TD5-40NMSK9-2/2/6a7b6e0c5d2dcbdb42c3545d8cb10012

Three canine cell lines, K1, K6 and DH82, derived from canine malignant neoplasms, were characterised. They were examined for expression of surface antigens, cytokines, neuropeptide receptors, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). The growth characteristics of the cell lines were established and bioassays used to detect production of TNF- [alpha], IL-1 and IL-6. In the DH82 cell line, production of TNF- [alpha] and IL-6 was readily detected. Neither K1 or K6 cell lines produced any measurable amounts of TNF- [alpha], IL-1 or IL-6. At a molecular level, using reverse transcription-polymerase chain reaction (RT-PCR) to detect specific mRNA, the DH82 cell line expressed TNF- [alpha], IL-1 and IL-6, whereas the K1 and K6 cell lines expressed TNF- [alpha]. Canine IL-5, IL-8 and IL-10 mRNA were
detected in the DH82 cell line but only IL-5 and IL-8 mRNA were detected in the K1 and K6 cell lines. Gelatin zymography was used for the detection of MMP-2 and MMP-9 and all three cell lines produced MMP-2 but only the DH82 cell line produced MMP-9. Reverse zymography was used to detect TIMP-1 and TIMP-2 and all three cell lines produced both proteins. The presence of these MMPs and TIMPs was confirmed at a molecular level using RT-PCR. Canine MMP-14 mRNA was detected in all three cell lines. For this investigation several genes for canine inflammatory molecules were cloned and sequenced for molecular detection; these included IL-1, IL-6, IL-8, TNF-[alpha], MMP-9, MMP-14, TIMP-1, TIMP-2 and [beta]-actin. Of all the cell surface antigens tested, only CD14 was expressed on the DH82 cell line although CD5 and CD45 was partially expressed. The K1 and K6 cell lines were negative for all of the CD markers tested. K1 and K6 were negative for Neurokinin 1 receptor (NK1-R) but positive for Calcitonin gene related peptide receptor type 1 (CGRP-1R) and Calcitonin gene related peptide receptor component protein (CGRP-RCP). The DH82 cell line expressed neither NK1-R or CGRP-1R; however, it did express CGRP-RCP. Generally the DH82 cell line exhibited considerable similarity to canine monocytes, but all three cell lines will be useful as standards and for the purification of various immunological and inflammatory mediators in the dog.


http://www.sciencedirect.com/science/article/B6TD5-3Y9HGF9-8/2/5bd66a91b390aecc1b03eed22a620615

Regulation of interleukin-1 (IL-1) mediated biological responses is complicated by the multiple ligands and receptors of the IL-1 family. Most studies of IL-1 receptors have used human or rodent cells. Here, we report that the coding region of the bovine type 1 interleukin-1 receptor (type 1 IL-1R) cDNA extends 1719 bp in length. Northern analysis of specific bovine cell and tissue RNA demonstrated a 4.5 kb transcript. Overall, the bovine type 1 IL-1R coding region exhibits approximately 81 and 76% similarity with the human type 1 IL-1R at the nucleotide and amino acid level, respectively, and somewhat less similarity with the mouse and rat sequences. Type 1 IL-1R transcripts were confirmed by RT-PCR in several bovine cell types, including peripheral blood mononuclear cells (PBMCs), neutrophils (PMNs), and fibroblast, peritoneal macrophage, and arterial endothelial cell lines. It is expected that molecular clones for the bovine type 1 and 2 IL-1 receptors will provide us with the tools needed to decipher species- and cell-specific regulation of IL-1 action in the bovine.


http://www.sciencedirect.com/science/article/B6TD5-4C0V78C1/2/574de5538a6093ef3f05765aef3a4401

Lymph node (LN) T cells from feline immunodeficiency virus (FIV)-infected cats have an increased expression of B7 co-stimulatory molecules as well as their ligand CTLA4, resembling an activation phenotype shown to induce anergy and apoptosis in activated T cells. In addition, LN T cells from FIV-infected cats also show increased spontaneous apoptosis compared to uninfected animals. The apoptosis observed in these animals occurs primarily in T cells expressing B7 and CTLA4, suggesting a role for B7 and CTLA4 interactions in the induction of anergy/apoptosis. In order to investigate the role of B7 and CTLA4 interactions on T cell apoptosis in LN T cells from FIV-infected cats, we performed blocking experiments by measuring T cell apoptosis in LN T cell cultures treated with anti-feline B7.1, B7.2, and CTLA4 specific
antibodies, as well as interleukin (IL)-2. The addition of IL2, the primary cytokine produced by B7/CD28 interactions, resulted in a significant decrease of T cell apoptosis in cultured LN cells as assessed by two-color flow cytometry and TUNEL assay. The addition of anti-B7.1 antibodies significantly inhibited T cell apoptosis in FIV-infected cats with low-level plasma viremia, while addition of anti-B7.2 and anti-CTLA4 antibodies had no effect. These results suggest a role of B7 signaling in the increased spontaneous apoptosis observed in LN T cells from FIV-infected animals.


http://www.sciencedirect.com/science/article/B6TD5-3RH6HB7-1/2/ad9fc0aebd4ca2834fc28346076b7bbf

Two monoclonal antibodies (MAb), HB65A (IgG 2a) and HB86A (IgG1), recognize a unique cell surface molecule on equine T-lymphocytes. The molecule, designated EqWC4, identified by these MAbs is present on a subpopulation of CD4+ equine lymphocytes (6.3-10.2% of Arabian lymphocytes CD4+ WC4+) and a smaller population of CD8+ lymphocytes (0.5% to 1.2% of Arabian lymphocytes CD8+ WC4+). EqWC4 is absent from B-lymphocytes, granulocytes, and macrophages. Both MAbs bound to a 46-kDa protein following immunoprecipitation reactions with lysates of surface labeled thymocytes. Immunoaffinity purification using HB65A yielded two molecules of 46 kDa and 52 kDa under reducing conditions and a third 92-kDa molecule was present in nonreduced conditions. Activation by mitogen did not increase expression of EqWC4 on equine lymphocytes. Lymphocytes from Arabian, Pony, and Thoroughbred breeds showed a common distribution of EqWC4 among leukocytes. However, there were significantly fewer Pony lymphocytes bound to HB65A and HB86A when compared to Arabian and Thoroughbred breeds. Using reverse transcriptase-polymerase chain reaction (RT-PCR), magnetically enriched populations (to 80% of cells isolated) of EqWC4+ lymphocytes expressed a cytokine RNA profile dominated by -interleukin2 (IL-2) and interferon-gamma (IFN-[gamma]) for unstimulated cells. Upon mitogen stimulation, IL-4 was also expressed at low levels while the IL-2 levels decreased and the IFN-[gamma] levels increased relative to unstimulated cells. EqWC4 is similar to CD28 in molecular weight and its formation of dimers and could therefore be the equine orthologue. However, because of the differences in CD28 expression, EqWC4 probably represents a previously uncharacterized equine lymphocyte marker.


http://www.sciencedirect.com/science/article/B6TD5-430WX9F-2/2/b2ab6a0cf16309f288fa95ba649f51ee

Previous work using Southern analysis of genomic DNA detected a polymorphism at the 5’ end of the sheep IgE gene. Identical length differences found between fragments following digestion with restriction enzymes indicated that the basis for the polymorphism was an insertion/deletion event. To characterise the polymorphism, the entire cattle and sheep C[epsiv] genes were sequenced including 668 bp of 5’ untranslated DNA. Sequence comparison revealed a high degree of similarity between the ovine and bovine genes at both the nucleotide and amino acid level. A feature of the 5’ untranslated DNA was the presence of an 87 bp repeat starting at -365 upstream of the C[epsiv] start site. PCR primers were designed to span most of the 5’ untranslated sequence, including the repeat unit, and used to amplify genomic DNA from a panel
of 40 sheep. Three alleles were found with frequencies of 0.7, 0.29, 0.01 which were identical to the Southern analysis results. Sequencing of the two commonest alleles revealed the basis for the polymorphism was a 36 bp deletion from the 87 bp repeat. Association studies in a sheep selection flock phenotypically assessed for parasite resistance found a highly significant association between one of the IgE alleles and resistance to the intestinal nematode parasite *Trichostrongylus colubriformis* (P=0.005). Attempts to confirm this finding in two other flocks using linkage analysis and genotype association failed to find any significant associations between the IgE polymorphism and resistance to either *T. colubriformis* or *Haemonchus contortus*.


http://www.sciencedirect.com/science/article/B6TD5-3WM5540-B/2/0b31ec0454f0a4794f423743f8e100b1

The effects of IL-12 on the responses of cattle peripheral blood mononuclear cells (PBMC) to bovine respiratory syncytial virus (BRSV) antigen and ovalbumin (OVA) were tested, in vitro. IL-12 did not affect the proliferative responses of PBMC to these antigens but markedly accelerated and augmented the level of IFN[gamma] secreted. When tested on lymphoblasts rather than resting T-cells IL-12 also enhanced proliferation. In contrast IL-4 and, to greater extent, IL-10 inhibited the response. The effect of IL-12 on IFN[gamma] synthesis was confirmed at the level of IFN[gamma] mRNA expression using Taqman(R) PCR. CD4 and CD8 T-cell populations produced IFN[gamma], however, CD4 T-cells comprised the largest contributors to the IFN[gamma] production. [gamma]/[delta] T-cells did not contribute markedly. A comparison of the species cross-reactivity showed bovine IL-12 was also active in the human system. This study shows that antigen-driven responses in cattle can be significantly influenced by exogenous cytokines and suggests the IL-12/IL-10 balance is crucial for regulation of IFN[gamma].


http://www.sciencedirect.com/science/article/B6TD5-3S130WJ-6/2/dbb159346b55701e596bc9bf84065ca

Peripheral blood leukocytes of 11 normal cows, 7 cows heterozygous and 2 heifers homozygous for bovine leukocyte adhesion deficiency (BLAD) were analysed by flow cytometry for the intensity of their [beta]2 integrin expression (LFA-1(CD11a/CD18), CR3 (CD11b/CD18) and CR4 (CD11c/CD18)). BLAD-homozygotes revealed no or a very weak expression of the [beta]2 integrins and had a 10-fold and 4- to 5-fold increase in absolute number of neutrophils and monocytes, respectively, whereas the absolute number of lymphocytes remained normal. The mean fluorescence intensity (MFI) of the [beta]2 integrins (CD18) in heterozygous animals was 56 to 90% of this in the normal cows (MFI between 14 and 512). The difference in the expression level was most pronounced for LFA-1 on the small cluster of lymphocytes with the highest MFI for LFA-1. Repeated analysis and phorbol myristate acetate stimulation revealed that the LFA-1 expression on this high-expressing cell population of the peripheral blood allowed a ready identification of BLAD-heterozygotes by flow cytometry.


http://www.sciencedirect.com/science/article/B6TD5-48M7X5H-1/2/3a6ffcc85399c98e6668c7ee58ad0af856

The genetic immunodeficiency disease canine leukocyte adhesion deficiency (CLAD) was originally described in juvenile Irish Setters with severe, recurrent bacterial infections. CLAD was subsequently shown to result from a mutation in the leukocyte integrin CD18 subunit which prevents leukocyte surface expression of the CD11/CD18 complex. We describe the development of a mixed-breed CLAD colony with clinical features that closely parallel those described in Irish Setters. We demonstrate that the early identification of CLAD heterozygotes and CLAD-affected dogs by a combination of flow cytometry and DNA sequencing allows the CLAD-affected animals to receive life-saving antibiotic therapy. The distinct clinical phenotype in CLAD, the ability to detect CD18 on the leukocyte surface by flow cytometry, and the history of the canine model in marrow transplantation, enable CLAD to serve as an attractive large-animal model for the investigation of novel hematopoietic stem cell and gene therapy strategies.


http://www.sciencedirect.com/science/article/B6TD5-4BS0FTF-2/2/443489947d000d8ed0b218ae1bba3d

[beta]-Defensins are cysteine-rich endogenously produced antimicrobial peptides that play an important role in innate immune defense. Although, previous investigations have identified [beta]-defensins in several mammalian species, no reports have identified equine [beta]-defensins. Using a strategy of database searching for expressed sequence tags (EST) we identified putative expression of equine [beta]-defensins in hepatic tissue. Based on this information, sequence specific primers were designed for the equine gene enabling the identification of the full-length cDNA sequence of equine [beta]-defensin-1. Comparative analyses showed that equine [beta]-defensin-1 has 46-52% amino-acid identity with other [beta]-defensins, sharing the greatest identity with porcine [beta]-defensin-1. Complete conservation of cysteine residues was maintained between the species evaluated, and RT-PCR analysis revealed diverse mRNA tissue expression for equine [beta]-defensin-1. These data extend the repertoire of equine antimicrobial peptides and expand our understanding of equine innate immunity.


http://www.sciencedirect.com/science/article/B6TD5-4F924G1-2/2/c1463dd522c5c160852179b155703b3e

NK-lysin is an antimicrobial peptide of cytotoxic and NK lymphocytes that has powerful antibacterial properties as well as antitumor activity. Here we report the full-length cDNA and deduced amino acid sequence for equine NK-lysin. Equine NK-lysin is 67% identical to porcine NK-lysin, 53% identical to bovine NK-lysin and 41% identical to granulysin in amino acid sequence. Complete conservation of cysteine residues between equine, bovine and porcine NK-lysin suggests similar disulfide bonding patterns among these peptides. Equine NK-lysin has the most positive surface charge when compared with other homologues. Similar to expression profiles in other species, equine NK-lysin is constitutively transcribed in various lymphocytes that include CD4+ and CD8+ staining cells. These findings suggest that equine NK-lysin, similar in
cDNA sequence to the porcine, bovine and human homologues may play a role in antimicrobial defense.


http://www.sciencedirect.com/science/article/B6TD5-408BJFM-C2/885ff6f31c0ba8c6a9f4585b9d576b6

The effect of a CXC-chemokine, stromal cell derived factor-1 (SDF-1), on the replication of divergent strains of feline immunodeficiency virus (FIV) was examined in order to identify the mechanism of cell entry of FIV. A chemotaxis assay, using a modified Boyden chamber method, confirmed the biological activity of recombinant human (rh) SDF-1 for a feline T-lymphoid cell line (Kumi-1). The viral replication of FIV, as measured by the reverse transcriptase (RT) activity in the culture supernatant, was significantly suppressed by addition of rhSDF-1 in a dose-dependent manner in Kumi-1 cells. Furthermore, PCR analysis of the FIV proviral genome indicated that the inhibitory effect of rhSDF-1 on the replication of FIV in Kumi-1 cells was due to the inhibitory effect in the early event of replication. The inhibitory effect on viral replication by exogenous rhSDF-1 was shown for four divergent FIV isolates of subtypes A, B, and D in Kumi-1 cells.


http://www.sciencedirect.com/science/article/B6TD5-48B01MX-32/2bb2d2b105d472184cd5e5ba345daf2

Japanese cedar (Cryptomeria japonica, CJ) pollinosis is mediated by type-I hypersensitivity and induces seasonal rhinitis and conjunctivitis in humans. Previous studies showed that dogs could be experimentally sensitized with CJ pollen. In this study, we carried out quantitative analysis of mRNA levels of various cytokines in the peripheral blood mononuclear cells (PBMC) of 12 dogs experimentally sensitized to Japanese cedar pollen. Experimental sensitization was carried out by injection of crude CJ pollen extract with aluminium hydroxide gel. The expression levels of interleukin (IL)-1[beta], IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, interferon (IFN)-[gamma], transforming growth factor (TGF)-[beta]1, and tumor necrosis factor (TNF)-[alpha] mRNAs in the PBMC were quantified using a real-time sequence detection system. In the PBMC tested without culture, the expression levels of IL-8 and TNF-[alpha] mRNAs in experimentally sensitized dogs were significantly higher than those in control dogs. The expression level of IFN-[gamma] mRNA in the sensitized group was significantly lower than that in the control group. When the PBMCs were cultured in the presence of CJ pollen extract, the level of IL-4 mRNA expression was markedly increased in the PBMC from the experimentally sensitized dogs. In the PBMC stimulated with the CJ pollen extract, the expression level of IL-2 mRNA in the sensitized group was also significantly higher than that in the control group. Our data indicated that a Th2 response and proliferation of PBMC occur in response to the sensitizing antigen in dogs experimentally sensitized with CJ pollen, and revealed the presence of antigen-specific Th2 cells in this canine model. In addition, the expression levels of the mRNAs encoding proinflammatory cytokines were shown to be elevated after CJ pollen sensitization, indicating the activation of monocytes and macrophages.

http://www.sciencedirect.com/science/article/B6TD5-3VJ3DKH-1/2/9844aec300dbcab520eb551ccf31e714

A reverse transcription-competitive polymerase chain reaction (RT-cPCR) method was developed to quantitate equine interleukin (IL)-1[alpha], IL-1[beta], IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p35, IL-12 p40, interferon-[gamma] (INF-[gamma]), tumor necrosis factor-[alpha] (TNF-[alpha]), and [beta]-actin mRNA expression. Using primers based on equine-specific sequences, these cytokines could be detected in concanavalin A-stimulated peripheral blood mononuclear cells. The specificity of the amplified product was confirmed by sequencing. For each cytokine, the assay was made quantitative by generating competitor DNA fragments (mimic) containing the same primer template as a equine cytokine, but differing in size to make them distinguishable on an agarose gel. Serial dilutions of the mimic were added to PCR reactions containing constant amount of equine cDNA. Following gel electrophoresis and ethidium bromide staining, densitometric analysis of the bands corresponding to the target and mimic were used to construct a standard curve from which the amount of target cDNA was derived. Quantitation of IL-6 gene expression from a cDNA sample on four different days gave a coefficient of variation or 6.6%. Sample-to-sample variation in the efficiency of the reverse transcription as well as in the quantity of quality of starting RNA was considerably attenuated by normalizing the results to [beta]-actin mRNA expression used as a house-keeping gene. Small differences (2-fold) in cytokine mRNA expression were reliably detected. The sensitivity and reproducibility of this technique will make it valuable in following changes in equine cytokine gene expression in vitro and in vivo. In addition, the RT-cPCR technique described will have broad applicability for quantitation of cytokine gene expression in other animal species of veterinary interest.


http://www.sciencedirect.com/science/article/B6TD5-44SK852-1/2/98134b202f844a8928f61cfb3c80f7af1

Work in humans and laboratory animals has identified a central role for cytokines and chemokines in development and persistence of lower airway inflammation. The objectives of this study were to determine interleukin (IL)-1[beta], IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, interferon (IFN)-[gamma] and tumor necrosis factor (TNF)-[alpha] induction in bronchoalveolar lavage (BAL) of control horses and horses with heaves both during remission and exacerbation of the disease, and to determine the effect of therapy with inhaled fluticasone propionate on the cytokine profile of horses with heaves. IL-1[beta] and TNF-[alpha] mRNA expression was significantly higher in horses with heaves after exposure to moldy hay compared to either values obtained during clinical remission or to healthy controls. IL-8 mRNA expression and protein concentrations were significantly higher in horses with heaves than in controls. Both IL-4 and IFN-[gamma] mRNA expression was increased at various times in heaves-susceptible horses compared to controls. IL-2, IL-5 and IL-10 mRNA expression was not detected in BAL cells of either group. Therapy with inhaled fluticasone propionate after induction of a severe heaves exacerbation resulted in complete resolution of clinical signs, normalization of pulmonary function tests, and significant decrease in BAL neutrophilia. This was associated with a significant decrease in IL-4 mRNA expression and increase in IFN-[gamma]/IL-4 ratio in horses with heaves. These results demonstrate the clinical efficacy of inhaled fluticasone propionate for the treatment of heaves and suggest a role for cytokines in the development of lower airway inflammation in heaves-susceptible horses.

http://www.sciencedirect.com/science/article/B6TD5-43G3056-D/2/17a35e9b335e6f3edbab17856947fe4c

Partial transcripts of the homologues in cattle, of the genes encoding the NKR-P1 and NKG2-D natural killer cell lectin-like receptor families, were cloned by reverse transcriptase-PCR from bovine spleen. Three different cDNAs were partially sequenced for the NKG2-D homologue, and two for the NKR-P1 homologue. Identity to human nucleotide sequences was of 90 and 75%, respectively, and all structural residues of C-type lectin carbohydrate recognition domains were conserved. The identification of two of its members allows to hypothesise the existence of a bovine NK gene complex, prospectively located on chromosome 5.


http://www.sciencedirect.com/science/article/B6TD5-4DS805H-2/2/6c63e804bf4ef095359b0ea360a67dbc

Bovine respiratory syncytial virus (BRSV) has been identified worldwide as an important pathogen associated with acute respiratory disease in calves. An infection model has been developed reflecting accurately the clinical course and the development of pathological signs during a natural BRSV-infection. In the experiments described in the present study, calves were infected at 13-21 weeks of age and reinfeected 14 weeks later. Blood samples from the entire infection period were analysed for acute phase protein (haptoglobin) by ELISA and for expression (mRNA level in peripheral blood mononuclear cells) of the cytokines interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6) and interferon-[gamma] (IFN[gamma]) by quantitative real-time reverse transcribed polymerase chain reaction (RT-PCR). IFN[gamma], interleukin-6 and haptoglobin were markedly induced together with development of clinical signs in response to the first infection with BRSV. The IFN[gamma] response was biphasic, with an early peak at day 1-3 post infection (p.i.) and a later increase between day 5 and 8 p.i. Reinfection also resulted in an induction of IFN[gamma], but without induction of clinical signs, IL-6 and haptoglobin. These results indicate that early mediators connected with the innate responses are induced on a first encounter with the pathogen, but not on a second encounter (reinfection) where the adaptive immune system may act as the first line defence.


http://www.sciencedirect.com/science/article/B6TD5-3V7JFMB-D/2/57480a2e56d750bb2d3bbcc90363840

Two recombinant FIPV spike proteins were assessed for their immunogenic properties in 8-week-old kittens, which were then challenged intranasally with FIPV 79-1146. Humoral responses were assessed by ELISA and serum neutralisation test. Changes in PBMC cytokine mRNA levels were detected by a reverse transcription, semiquantitative polymerase chain reaction assay (RT-
sqPCR), assessing IL-2, IL-4, IL-6, IL-10, IL-12 and IFN[gamma]. All of the kittens developed clinical signs typical of FIP, which were confirmed on gross post mortem examination. The recombinant proteins induced little or no specific antibody response prior to challenge, and failed to alter the course of disease compared to controls. One week after virus challenge, the stimulated PBMCs showed small increases in the expression of IL-6 and IFN[gamma] mRNA, which correlated with a transient pyrexia. After this time expression of IL-6 mRNA remained unaltered but, as FIP developed, mRNA levels of IL-2, IL-4, IL-10, IL-12 and IFN[gamma] became markedly depressed.


http://www.sciencedirect.com/science/article/B6TD5-47DD8FF-C/2/6f2ef730d3065d403bdb512c345df5df

Bovine cytokine-specific primers and the reverse transcription-polymerase chain reaction (RT-PCR) were used to clone cDNA fragments that were specific for bovine IL-1[alpha], IL-1[beta], IL-2, and IFN-[gamma]. Specificity of the cDNA fragments was verified by sequence analysis based on known bovine IL-1[alpha], IL-1[beta], IL-2, and IFN-[gamma] gene sequences. In addition, RT-PCR was used to monitor cytokine mRNA expression in concanavalin A (Con A) and lipopolysaccharide (LPS)-stimulated bovine peripheral blood mononuclear cells (PBMC), and the results were compared with those obtained by measuring PBMC cytokine secretion using biologic assays. IL-1 activity in LPS-stimulated PBMC cultures was similar at 12 h and 24 h, although the activity decreased by approximately 40% at 48 h. IL-2 and IFN-[gamma] activity in supernatants of Con A-stimulated PBMC cultures was low at 12 h and reached maximum levels at 48 h. RT-PCR transcript analysis detected an increase in IL-1[alpha], IL-1[beta], IL-2, and IFN-[gamma] mRNA expression that was usually correlated with the detection of these soluble cytokines by the bioassays. These results indicate that RT-PCR is a sensitive and effective method of obtaining cDNA probes and that this technique can be used to monitor bovine cytokine mRNA expression.


http://www.sciencedirect.com/science/article/B6TD5-435CRHC-5/2/b409880bb322e3dbedc6be58f44e93a

Interleukin-18 (IL-18) is a cytokine with potent interferon-[gamma]-inducing activity, and plays an important biologic role in the enhancement of the activity of natural killer cells and cytotoxic T-lymphocytes. In this study, feline IL-18 cDNA was cloned and characterized to establish a basis for the prospective cytokine therapy in small animal practice. The nucleotide sequence of feline IL-18 cDNA obtained in this study was 712 bp long and contained its entire open reading frame encoding 192 amino acid residues. The predicted amino acid sequence of feline IL-18 cDNA showed 77.2, 84.8, 60.2 and 62.6% similarity with those of human, dog, rat and mouse counterparts, respectively. The feline IL-18 cDNA included a putative cleavage site of IL-1[beta]-converting enzyme (ICE) and IL-1 signature-like sequences identified in human and mouse IL-18 cDNAs. Expression of IL-18 mRNA was detected in various tissues including spleen, liver and cerebrum in the cat.
CCL27 (also named CTACK, ALP, ILC and ESkine) is a CC chemokine primarily expressed by keratinocytes of the skin. The cognate receptor of CCL27 named CCR10 (GPR-2), is also expressed in skin-derived cells, and in addition by a subset of peripheral blood T-cells and in a variety of other tissues. In this paper, we report the cloning of porcine CCL27 cDNA and investigation of CCL27 mRNA expression in Staphylococcus hyicus infected piglets. At the protein level, 77% and 74% homology was found to human and mouse CCL27 sequences, respectively. The results of the expression analyses show that CCL27 mRNA is upregulated in the skin of infected piglets and to a lesser extent in piglets recovered from disease and without clinical signs of infection, indicating a role for CCL27 both during inflammation and after recovery from an infection.

Reliable housekeeping gene controls are critical for measuring and comparing gene expression at the transcription level by Northern blot and RT-PCR. In order to develop such controls for studying cytokine mRNA expression in dogs, DNA sequence encoding a full-length canine HPRT protein has been obtained. Numerous primer pairs derived from the canine HPRT sequence have been tested on canine genomic DNA as well as cDNA. The data from the present study suggest that there may be processed HPRT pseudogenes in dogs. Three pairs of canine HPRT primers designed and tested in the present study were able to differentiate between cDNA and genomic DNA under specific PCR conditions. These primers would be useful controls for measurement of mRNA expression by RT-PCR in the dog.

Johne's disease progresses through distinct stages including a protracted subclinical stage in which the infection appears to be controlled; followed by a more acute stage in which the host animal demonstrates clinical signs such as diarrhea and weight loss. Little is known about the dynamics of the host immune response during these two phases of disease, however, it is possible that immune modulation in the early stages of disease may play an important role in disease progression. We hypothesized that the clinical stage of Johne's disease is mediated by the expression of cytokines such as transforming growth factor-beta (TGF-[beta]) and interleukin-10 (IL-10) that may be accompanied by the downregulation of IFN-[gamma] gene expression. In the present study, tissue samples were collected from the ileum, ileocecal junction, ileocecal lymph node, and mesenteric lymph nodes of healthy, subclinically or clinically infected cows. The
expression of TGF-[beta], IL-10, and IFN-[gamma] genes in these tissues was determined by quantitative competitive RT-PCR. The results demonstrate that TGF-[beta] and IL-10 mRNA levels are higher in cows that have progressed to the clinical stage of disease compared to subclinically infected or healthy cows. In contrast, IFN-[gamma] gene expression was significantly higher in subclinically infected cows. These results suggest that a change in the balance of cytokines at the site of infection may contribute to the ability of the host to control Mycobacterium avium subsp. paratuberculosis infection.


http://www.sciencedirect.com/science/article/B6TD5-42PBYFW-7/2/166730adc5e0a180c9d50a3792d2b8c0

Real-time PCR systems were developed to quantitate cytokine expression in short-time cultivated feline monocytes. Feline-specific interleukin-[beta] (IL-[beta]), IL-6, and tumor necrosis factor-[alpha] (TNF-[alpha]) primers as well as TaqMan probes were designed and were adapted to a quantitative PCR system which had been previously established for feline IL-10 and IL-12 p40. Quantitative analysis of cytokine messenger RNA (mRNA) transcription based on the comparison of the cytokine with the housekeeping gene feline glyceraldehyde-3-phosphate dehydrogenase (GAPDH), providing universally expressed mRNA. GAPDH mRNA was readily detectable in cDNA prepared from short-time cultivated peripheral blood monocytes. Cytokine mRNA was demonstrated in all samples at variable amounts. IL-[beta] and TNF-[alpha] mRNA was constitutively expressed whereas IL-6, IL-10 and IL-12 p40 mRNA was generally expressed at a lower level and was occasionally not detected. There was a great variability of cytokine production between individual cats and at different time points in the same cat.


Here we present a novel methodology to quantitate bovine cytokines and growth factors contributing to immunity against bacterial infections of the mammary gland in cattle. Real-time TaqMan(R) PCR systems were developed to overcome limitations of conventional quantitative PCR methods. The TaqMan(R) method is based on the cleavage of fluorescent dye-labeled probes by the 5'-3’ exonuclease activity of the Taq DNA polymerase during PCR and measurement of fluorescence intensity by an automated spectrophotometer integrated in a sequence detection system (Applied Biosystems, Foster City, CA). The bovine-specific TaqMan(R) probes were designed to encompass an intron, thus allowing differentiation between complementary DNA (cDNA) and genomic DNA (gDNA) amplification products. Quantitative analysis of cytokine cDNA was performed in comparison to bovine glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Messenger RNA (mRNA) from the universally expressed housekeeping gene GAPDH proved to be useful as an amplification control and allowed for correction of variations in different numbers of cells in the starting material, in the efficiencies of RNA extraction and reverse transcription. With this method, high-throughput analysis of large numbers of samples was possible within a short time. In addition, decreasing the numbers of working steps shortened the time for analysis and increased accuracy. Profiles of cytokines (interleukin (IL)-2, IL-6, IL-8, IL-12 p40, TNF-[alpha], IFN-[gamma]) and granulocyte-macrophage colony stimulating factor (GM-CSF) were established in normal lactating cattle. Differences of
cytokine profiles obtained with the real-time TaqMan(R) PCR system and conventional methods are discussed.

Leutenegger, C. M., C. N. Mislin, et al. (1999). "Quantitative real-time PCR for the measurement of feline cytokine mRNA." Veterinary Immunology and Immunopathology 71(3-4): 291.

http://www.sciencedirect.com/science/article/B6TD5-3Y9HGF9-D/2/f621b091ad022739ce5eafee7e4a20f8

We have developed real-time PCR systems to quantitate feline cytokine gene expression. The method is based on the cleavage of fluorescent dye-labelled probes by the 5’-3’ exonuclease activity of the Taq DNA polymerase during PCR and measurement of fluorescence intensity by a Sequence Detection System. The feline-specific TaqMan probes were designed to encompass an intron, thus allowing differentiation of complementary DNA versus genomic DNA amplification products. Quantitative analysis of cytokine cDNA concentrations was performed in comparison to feline GAPDH. Messenger RNA (mRNA) from the universally expressed housekeeping gene GAPDH proved to be useful as an amplification control and allowed for correction of variations in the efficiencies of RNA extraction and reverse transcription. GAPDH mRNAs were readily detectable in cDNAs prepared from unstimulated feline peripheral blood mononuclear cells (PBMCs) and from frozen cell pellets, while cytokines (Interleukin (IL)-4, IL-10, IL-12 p35, IL-12 p40, IFN[gamma], IL-16) were expressed at variable amounts. IFN[gamma] transcription was found to be upregulated in stimulated PBMCs and feline cell lines. The synthesis of cDNA and the performance of the PCR in separate tubes proved to be of superior sensitivity compared to a single-tube based system. The assays described are highly reproducible, require no post-PCR manipulation of the amplicons and permit the analysis of several hundred PCR reactions per day. With this method it is possible to detect and quantify cytokine mRNA expression reliably in small amounts of cells even after storage of samples for at least 5 years.


http://www.sciencedirect.com/science/article/B6TD5-44HXJY5-8/2/a8cccb86768f88ef4e596ab26d738579a

Melanoma antigens (MAGE) are regarded as inducing tumor-specific immune response and thought to be potential therapeutical agents for cancer immunotherapy. We hereby report the cloning of feline MAGE cDNA obtained from a lymphoma cell line derived from cat malignant lymphoma, and its expression pattern in tumor and normal tissues. The cDNA encoding the MAGE is 1668 base pairs (bp) in length, and contains an open reading frame (ORF) of 936 bp encoding a protein of 311 amino acids. The predicted amino acid sequence has 29-46% of homology with other MAGE proteins from human and mouse. mRNA transcripts for the feline MAGE were detected in certain tumors, but not in adult cat normal tissues except in testis, by reverse transcription polymerase chain reaction (RT-PCR) analysis. This indicates that the expression pattern of feline MAGE mRNA is similar to those of other MAGE family genes in tumors and normal tissues.

In this study, we investigated the mRNA expression of a chemokine, thymus and activation-regulated chemokine (TARC), and cytokines including IL-1[beta], IL-4, IFN-[gamma] and TNF-[alpha] in skin samples obtained from both dogs with atopic dermatitis (AD) and healthy dogs. TARC mRNA was found to be selectively expressed in lesional skin of the dogs with AD, but not in non-lesional skin of the dogs with AD or the normal skin of the healthy dogs. The expression levels of IL-1[beta], IFN-[gamma] and TNF-[alpha] in the lesional skin were also significantly higher than those in the non-lesional skin of the dogs with AD. However, IL-4 mRNA was not detected in any of the skin samples in this study. The present results suggest that TARC and inflammatory cytokines such as IL-1[beta], IFN-[gamma] and TNF-[alpha] may play roles in the pathogenesis of canine AD as well as that of human AD.


A competitive PCR assay (cPCR) was used to quantify swine cytokine responses to parasite infection. Internal standards (deleted cDNA competitor molecules [DcDNA mimics]) were produced and tested for swine interleukin-12 (IL-12), interleukin-10 (IL-10) and hypoxanthine phosphoribosyltransferase (HPRT) from PCR generated cDNA cloned in plasmid vectors. Deletion clones for the cDNA competitor molecules (DcDNA mimics) were generated for IL-10, IL-12 and HPRT by PCR in a single step and verified by (1) amplification of the expected smaller PCR product with the original primers, (2) appropriate fragment size released by restriction digestion of the deleted clone, and (3) correct sequence of the new DcDNA insert. DcDNA mimics were used to quantitate cytokine gene mRNA production during experimental and natural infections of swine with the gastrointestinal nematode parasite Trichuris suis. Mesenteric lymph node cells were collected from control and infected pigs at the time of maximal pathogenicity (35 days after infection) and snap frozen. After RNA extraction, samples were reverse transcribed (RT) to cDNA. cPCR was performed using the housekeeping gene HPRT DcDNA mimic and HPRT specific primers to insure RNA integrity and concentration. Cytokine cDNA content in these samples was then quantitated using cytokine mimics and gene specific primers. IL-10 gene expression in MLN draining the colon of pigs experimentally infected with T. suis increased 10-20 fold at day 35 compared to control pigs. IL-12 gene expression was not detectable in MLN of these pigs, but was detectable in MLN of pigs exposed naturally to T. suis on a contaminated dirt lot that also exhibited signs of secondary bacterial invasion. Swine IL-10 and IL-12 gene expression can be quantitated in local mesenteric tissues. This cPCR assay will enable scientists to quantitate cytokine gene expression in swine and determine the nature of immune responses to important infectious diseases.


In order to isolate a part of the immunoglobulin E (IgE) heavy chain cDNA of the horse, primers...
have been designed based upon well conserved sequences in humans, sheep and rats. The PCR resulted in a 500 bp fragment which hybridised with a human IgE constant region probe. The fragment was cloned and sequenced and its derived protein sequence compared with the corresponding sequences in humans, sheep and mice. Most amino acids common to these three species are also shared by the horse.


Mammary-associated serum amyloid A 3 (M-SAA3) was secreted at highly elevated levels in bovine, equine and ovine colostrum and found at lower levels in milk 4 days postparturition. N-terminal sequencing of the mature M-SAA3 protein from all the three species revealed a conserved four amino acid motif (TFLK) within the first eight residues. This motif has not been reported to be present in any of the hepatically-produced acute phase SAA (A-SAA) isoforms. Cloning of the bovine M-Saa3 cDNA from mammary gland epithelial cells revealed an open reading frame that encoded a precursor protein of 131 amino acids which included an 18 amino acid signal peptide. The predicted 113 residue mature M-SAA3 protein had a theoretical molecular mass of 12,826 Da that corresponded with the observed 12.8 kDa molecular mass obtained for M-SAA3 in immunoblot analysis. The high abundance of this extrahepatically produced SAA3 isoform in the colostrum of healthy animals suggests that M-SAA3 may play an important functional role associated with newborn adaptation to extrauterine life and possibly mammary tissue remodeling.


Real-time polymerase chain reaction (PCR) assays were developed for woodchuck leukocyte cluster of differentiation (CD) and cytokine mRNA expression. Plasmid DNA standards of each marker (CD3, CD4, CD8, IL-2, IFN-[gamma], TNF-[alpha], IL-4, IL-10), and RNA standards from mitogen-stimulated woodchuck peripheral blood mononuclear cells (PBMCs) were used to validate and optimize the assays for TaqMan 7700(R) and iCycler(R) PCR instruments. The complementary DNAs (cDNAs) produced by reverse transcription (RT) of RNA were quantified by real-time PCR against the plasmid DNA standards (6-8 log range) with detection of as few as 10-50 copies of amplicon cDNA per reaction. Analysis of unstimulated and concanavalin A-stimulated woodchuck PBMC demonstrated increased CD and cytokine mRNA expression following mitogenic activation. A liver sample from a woodchuck hepatitis virus (WHV) infected woodchuck with histologically confirmed acute hepatitis had increased intrahepatic CD and cytokine mRNAs compared to liver from an uninfected control woodchuck. The real-time PCR assays were highly specific for the woodchuck markers in PBMC and liver samples and were equally applicable for use in alternate real-time PCR instrumentation. These assays will enable the high-throughput analyses of mRNA markers during WHV infection, and thereby facilitate continued modelling of the immunopathogenesis and immunotherapy of human hepatitis B virus (HBV) infection.
Mizuno, T., Y. Goto, et al. (2003). "Quantitative analysis of Fas and Fas ligand mRNAs in a feline T-lymphoid cell line after infection with feline immunodeficiency virus and primary peripheral blood mononuclear cells obtained from cats infected with the virus." *Veterinary Immunology and Immunopathology* 93(3-4): 117.

http://www.sciencedirect.com/science/article/B6TD5-48S4NPC-5/2/9eb09e7beead23de93591ce43344cb

Apoptosis is frequently observed in feline lymphocytes in association with feline immunodeficiency virus (FIV) infection. In this study, to investigate the mechanism of FIV-induced apoptosis, levels of Fas and Fas ligand mRNAs were measured by real-time reverse transcription-PCR. In a feline T-lymphoid cell line the amounts of Fas ligand mRNA increased along with the induction of apoptosis after in vitro infection with FIV. In PBMC collected from 10 cats naturally infected with FIV, Fas ligand mRNA levels were significantly higher than those in PBMC from five uninfected cats. These results indicate that the increased expression of Fas ligand may be involved in the induction of apoptosis of lymphocytes in FIV infection.


http://www.sciencedirect.com/science/article/B6TD5-41JTNY3-9/2/8b00da064e2c9c5bc119b8d4f439f29f

We examined expression of TGF-[beta]s in chicken thymic stromal cells and thymocytes and roles of TGF-[beta]s in thymocyte development within the thymus. Thymic stromal cells expressed TGF-[beta] 2 and 3 genes but not TGF-[beta] 4 gene. Thymocytes showed expressions of TGF-[beta] 2, 3 and 4 genes and each TGF-[beta] gene was expressed more strongly in CD3- than CD3+ thymocytes. When anti-TGF-[beta] antibody was added with supernatants of stromal cells into thymocyte culture, only proliferative activity of CD3- thymocytes was enhanced and the cell in S and G2/M compartments of cell cycle increased. These results suggest that TGF-[beta] which is expressed in the thymus may regulate the ability of immature thymocytes to progress through the cells cycle and to differentiate to CD3+ thymocytes.


http://www.sciencedirect.com/science/article/B6TD5-3VXYR2M-3/2/b446b53e91ea4163d9b2a8c6c34e9230

We have studied the ability of thymic stromal cells (TSC) and thymocytes to produce cytokines and the involvement of cytokines in intrathymic T cell development. When thymocytes were co-cultured with thymic stromal cells in absence of direct contact and mitogenic stimulation, induction of thymocyte proliferation was observed. Supernatants of cultured stromal cells (TSC-CS) promoted a high proliferative response on CD3- thymocytes but had little effect on CD3+ thymocytes. These results indicate that stromal cells have produced a cytokine which can induce immature thymocyte proliferation. Moreover, stromal cells express the mRNA for stem cell factor (SCF) and c-kit (the receptor for SCF) was detected on CD3- thymocytes but not on CD3+
thymocytes. Since SCF can enhance the proliferation of immature thymocytes in synergy with IL-7 in mammals, there is a possibility that chicken stromal cells may produce a IL-7-like factor. Thymocytes have clearly expressed interferon (IFN)-[gamma]. In contrast, thymic stromal cells showed no detectable expression of IFN-[gamma]. CD3+ thymocytes express IFN-[gamma] mRNA more strongly than CD3- thymocytes, suggesting that IFN-[gamma] from thymocytes may operate on stromal cells and then may indirectly induce clonal elimination of CD3+ cells on stromal cells. The expression of these cytokines and receptors by thymic stromal cells and thymocyte subpopulations suggests that these cytokines participate in paracrine interactions between these cell populations during thymocyte differentiation.


Tumour necrosis factor (TNF) is well recognised for its role in mediating innate immune responses. However, the mechanisms of TNF that influence the adaptive immune response to viral infections are poorly understood. Over recent years, there has been evidence to suggest a role for TNF in the early phase of infection of ruminants with bovine leukaemia virus (BLV). In this study, we infected TNF-/- mice with a plasmid encoding infectious BLV to further elucidate the role of TNF in BLV infection. TaqMan quantitative PCR showed that proviral DNA was present in genomic DNA isolated from spleen cells of TNF-/- mice 4 weeks post-infection, whereas it was not detected in wild-type mice. We were not able to detect differences in serum IgM or IgG levels between the TNF-/- and wild-type mice, or antibodies to BLV after this short period. In showing that the lack of TNF enables the plasmid encoded BLV to persist longer, and therefore rendering the mice more susceptible to an infection with BLV, the data suggest an important defence function of TNF in the early phase of BLV infection.


The extracellular domains of swine leukocyte antigen class I (SLA-I, major histocompatibility complex protein class I) were cloned and sequenced for two haplotypes (H4 and H7) which do not share any alleles based on serological typing, and which are the most important in Danish farmed pigs. The extracellular domain of SLA-I was connected to porcine [beta]2 microglobulin by glycine-rich linkers. The engineered single-chain proteins, consisting of fused SLA-I and [beta]2 microglobulin, were overexpressed as inclusion bodies in Escherichia coli. Also, variants were made of the single-chain proteins, by linking them through glycine-rich linkers to peptides representing T-cell epitopes from classical swine fever virus (CSFV) and foot-and-mouth disease virus (FMDV). An in vitro refold assay was developed, using a monoclonal anti-SLA antibody (PT85A) to gauge refolding. The single best-defined, SLA-I restricted porcine CD8+ T-cell epitope currently known is a 9-residue peptide from the polyprotein of CSFV (J. Gen. Virol. 76 (1995) 3039). Based on results with the CSFV epitope and two porcine haplotypes (H4 and H7), the in vitro refold assay appeared able to discriminate between peptide-free and peptide-occupied forms of SLA-I. It remains to be seen whether the rapid and technically very simple in vitro refold
assay described here will prove generally applicable for the screening of virus-derived peptides for SLA-I binding.


http://www.sciencedirect.com/science/article/B6TD5-40NMSK9-4/2/e0227e1add71562479dd3333430c3b97

Until recently, it was presumed that Bartonella vinsonii only infected voles, a species of North American rodents. In April of 1993, however, our laboratory isolated a novel subspecies of B. vinsonii (B. vinsonii subsp. berkhoffii) from the blood of a dog diagnosed with vegetative valvular endocarditis. Subsequently, based on a seroepidemiologic survey of dogs from North Carolina and Virginia presenting for a variety of medical problems, we found evidence supporting a potentially important association between B. vinsonii and *Ehrlichia canis* co-infection in dogs. In the following study, eight dogs were infected with B. vinsonii: four specific pathogen free dogs and four dogs that had previously been infected with *E. canis*. Flow cytometric analysis of peripheral blood lymphocytes revealed a cyclic elevation of the CD4/CD8 T-cell ratio that correlated with cyclic CD8+ lymphopenia in all dogs infected with B. vinsonii, regardless of prior exposure to *E. canis*.


http://www.sciencedirect.com/science/article/B6TD5-430WX9F-5/2/23e6e22bab241b943b05766a906088e7

The goal of this study was to identify a strain of feline immunodeficiency virus (FIV) that would be more virulent for adult cats than the prototype FIV-Aetalula and, thereby, enhance the FIV infection model for HIV-1 related research. Diehl et al. reported that one clade C strain of FIV, FIV-Cammar, was more virulent than other known FIV isolates. Mortalities from 58 to 100% were reported for kittens 12 weeks of age and less following intravenous inoculation. A more variable and somewhat less virulent disease course was observed in neonatal to 8-10-week-old kittens infected orally, intravaginally or intrarectally with this same isolate (Obert and Hoover, 2000). However, no studies have been done with FIV-Cammar in adult cats. Therefore, the virulence of FIV-Cammar for young adult cats was compared to that of FIV-Aetalulma, the original FIV isolate. One group of five cats were inoculated intraperitoneally with 470 TCID50 of FIV-Cammar in the form of pooled plasma from acutely infected cats, while a second group was infected with plasma containing the 750 TCID50 of FIV-Aetalulma. The cats were observed for 20 weeks for gross signs of disease, hematologic abnormalities, time of antibody appearance, and plasma and peripheral blood mononuclear cell (PBMC) associated virus levels. Viral RNA and proviral DNA were measured by a real-time PCR, sensitive to 50 copies per milliliter. The only outward sign of disease was lymphadenopathy, which occurred at a similar time and intensity in both groups of cats. Cats infected with FIV-Cammar were more likely to be neutropenic and lymphopenic during the first 10-12 weeks of infection than cats infected with FIV-Aetalulma. Both groups of cats showed similar overall declines in absolute mean CD4 cell counts and identical concomitant increases in CD8 cells. CD4/CD8 cell ratios were also similar. Antibody, as measured by an ELISA against recombinant FIV-TM antigen, appeared in all cats by 4 weeks post-infection. The most significant differences were in plasma viral RNA and PBMC proviral DNA levels. Cats infected with FIV-Cammar had up to 100 times higher mean levels of viral RNA during the first few weeks of infection than cats infected with FIV-Aetalulma. This difference was also mirrored in
levels of proviral DNA in PBMC, which were significantly higher in the FIV-Cammar infected cats. Plasma viral RNA and PBMC proviral DNA levels were virtually identical in both groups of cats at 20 weeks post-infection. However, proviral DNA in tissues such as thymus and popliteal lymph nodes was 10-fold or so higher in FIV-Cammar infected cats at 20 weeks and histopathologic lesions were more severe. Based on these various parameters, we concluded that FIV-Cammar was more virulent than FIV-Aetaluma in young adult cats during the 20-week study period. However, we were not able to recreate the severe and rapidly progressive disease previously reported for kittens, suggesting an age-related resistance similar to that observed previously for FIV-Aetaluma (George et al., 1993).


http://www.sciencedirect.com/science/article/B6TD5-476TWCF-8Y/2/f0eab0e0ca821d2149b388cb7d41a0679

To determine the efficacy of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) as a prophylactic chemotherapeutic agent for the treatment of lentivirus infections, three groups of specific pathogen free cats were treated with 0, 3, or 6 mg kg-1 twice daily doses of PMEA beginning 24 h prior to virus challenge with feline immunodeficiency virus Petaluma strain. Treatment was continued for 7 weeks post challenge. During this time cats were monitored for drug toxicity, virus specific antibody response, circulating viral antigen and infectious recoverable virus. To determine the long-term influence of PMEA therapy the cats were monitored for 1 year following the cessation of treatment. The low levels of infectious virus present in blood prompted the development of quantitative polymerase chain reaction assay to enumerate viral DNA burdens in the peripheral blood mononuclear cells of the infected cats and thereby assess drug efficacy. The results indicate that, although prophylactic PMEA did not prevent infection, it did substantially limit feline immunodeficiency virus replication. Furthermore, viral DNA levels remained low in the cats receiving drug a full year (the duration of the study) after cessation of treatment.


http://www.sciencedirect.com/science/article/B6TD5-445RJM3-X/2/92f235d75d29a90058141376e9ace7a6

The superficial inguinal lymph nodes of 10 piglets which had died spontaneously of post-weaning multisystemic wasting syndrome (PMWS), in which the porcine circovirus type II (PCV-II) genome was revealed by PCR, were submitted to immunohistochemical investigation for CD4, CD8, IgM, MAC387, S-100 protein, vimentin and F-VIII-RA and compared with three normal cases. The lymph node reaction was graded as initial, intermediate and end stage according to histological criteria. In the initial and intermediate stages, absence of follicles and depletion of lymphocytes were evident. Associated with this was a reduction in numbers of interfollicular dendritic cells and interdigitating cells and a reduction/absence of B cells and mainly CD4+ T lymphocytes. In the end stage the reduced expression of high endothelial venules and the prevalence of the stromal component of the lymph node was prominent, as well as the above changes. It is concluded that more than one mechanism is involved in the immunosuppressive ability of PCV-II: reduction of the antigen presenting ability and reduction of B cells and CD4+ T cell function.
Ovine tumour necrosis factor-alpha (OvTNF-[alpha]) was cloned by reverse transcription-polymerase reaction using RNA isolated from lipopolysaccharide (LPS)-stimulated alveolar macrophages and primers based on the human TNF-[alpha] cDNA sequence. An expression vector carrying the coding sequence of the mature form of ovine TNF was constructed. The recombinant Ov-TNF[alpha] (rOvTNF-[alpha]) was expressed as a glutathione-S-transferase (GST) fusion protein. It was cleaved with thrombin to yield rOvTNF free of the GST moiety. Growth at a lower temperature of 30[deg]C and use of Escherichia coli strains AM207, AM305, E392 and NM522 did not improve the recovery of rOvTNF-[alpha] from the soluble fraction to a significant extent. Purification of recombinant proteins was achieved rapidly and easily by affinity chromatography using glutathione-Sepharose. Yields of pure rOvTNF-[alpha] achieved in E. coli JM109 and AM207 were approximately 1 mg L-1. Both rOvTNF-[alpha] and recombinant human TNF-[alpha] (rhTNF-[alpha]) exerted cytotoxicity on L929 cells. However, rOvTNF-[alpha] but not rhTNF-[alpha] stimulated proliferation of ovine thymocytes. Maximum levels of TNF-[alpha] mRNA expression by LPS-stimulated ovine alveolar macrophages were detected at approximately 4 h post-stimulation.

Studies of immune correlates of disease outcome associate humoral immune response mediated by T-helper 2 cytokines (IL-4, IL-10) with more virulent disease relative to a cell-mediated response driven by T-helper 1 cytokines (IL-2, IFN-gamma), particularly in viral and other intra-cellular infections. Specifically, the kinetics of both human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) infection are closely associated with Type 1 versus Type 2 cytokine profiles. Puma (Felis concolor) lentivirus (PLV) is closely related to FIV, but based on phylogenetic and clinical studies, is more ancient and less pathogenic. The aims of this study were to validate feline real-time PCR primer/probe systems for puma cytokines and PLV as sensitive, quantitative assays for use in investigations of PLV pathogenicity. We demonstrate that primer/probe systems for IL-4, IL-10, IFN-gamma, TNF-alpha, GAPDH, and the pol region of PLV-1695 amplify puma cytokines and PLV-1695 with high amplification efficiency and sensitivity. Detection of PLV-1695 provirus in experimentally inoculated domestic cats proved to be of equivalent sensitivity, specificity, and positive and negative predictive value to co-culture of one million peripheral blood mononuclear cells (PBMC). Evaluation of cytokine induction during naturally occurring PLV infection will allow insight into mechanisms of host control associated with apathogenic infection. In addition, determination of viral loads during different stages of PLV infection or in different tissues from domestic cats or pumas will further elucidate capacity of these viruses to replicate and establish infection.
This communication reports the cloning of cDNAs encoding two canine IL-13 receptor [alpha] chains (caIL-13R[alpha]1 and caIL-13R[alpha]2). As described for the members of type-I cytokine receptors, both caIL-13R[alpha]1 and caIL-13R[alpha]2 were found to contain the highly conserved motifs, such as cysteine and tryptophan residues in their N-terminal portion and the WSXWS at C-terminus. The isolated caIL-13R[alpha]1 cDNA contains 1547 nucleotides with an open reading frame that encodes 405 amino acid residues. Canine IL-13R[alpha]1 is 82.0 and 69.3% identical to human and mouse IL-13R[alpha]1s, respectively, at the amino acid level. Canine IL-13R[alpha]1 has an almost identical cytoplasmic domain to its human and mouse counterparts. The isolated caIL-13R[alpha]2 cDNA contains 1454 nucleotides and encodes an open reading frame of 386 amino acid residues. Canine IL-13R[alpha]2 is 62.6 and 47.5% identical to its human and mouse counterparts, respectively, at the amino acid level. Using RT-PCR with caIL-13R[alpha]1 and caIL-13R[alpha]2 specific primers, mRNAs of caIL-13R[alpha]1 and caIL-13R[alpha]2 were detected in most dog tissues. In addition, RT-PCR detected caIL-13R[alpha]1 mRNA in one of two canine mastocytoma (C2 but not Br) cell lines and in a canine macrophage-derived cell line (DH82). CaIL-13R[alpha]2 mRNA was detected in all three canine cell lines.


http://www.sciencedirect.com/science/article/B6TD5-43G3056-5/2/9cea06a5afa391cc60b4250f3d5eb604

cDNAs encoding four different canine immunoglobulin G (calgG) [gamma] chains were identified in this study. One of these IgG [gamma] chain cDNAs, (calgG-A), represents 92.5% of the IgG [gamma] chain cDNAs in a dog spleen cell cDNA library; a second partial IgG [gamma] chain cDNA (calgG-B) was also identified in the library. The other two IgG [gamma] chain cDNAs (calgG-C and calgG-D) were RT-PCR amplified from canine lymphoma samples. Comparison of the four different canine IgG [gamma] chain cDNAs showed homologies from 83.6 to 89.2% and from 73.1 to 81.8% at nucleotide and amino acid sequence levels, respectively. Despite the high similarity in CH1, CH2 and CH3 domains among the different calgG [gamma] chains, the hinge regions were distinct, sharing only 19.0-35.2% homology at the amino acid level. No multiple duplication of the hinge region, as reported for human IgG1 and IgG3, was detected in any of the canine IgG [gamma] chains. The numbers of cysteines in the putative hinge regions were found to be 3, 2, 7 and 3 for the four canine IgG heavy [gamma] chains (A, B, C and D), respectively. Specific primers were designed based on calgG [gamma] chain hinge region DNA sequences and were used in RT-PCR for measuring different calgG [gamma] chain mRNA levels in canine PBMC samples.

The acceptance of the fetal allograft by pregnant women and mice seems to be associated with a shift from a Th 1 dominated to a Th 2 dominated immune response to certain infectious agents. The goal of this study was to examine cytokine expression in peripheral blood mononuclear cells (PBMCs) from cattle immune to bovine viral diarrhea virus (BVDV) to determine whether pregnancy also has an influence on the type of immune response in this species. Forty-six heifers and cows between 14 months and 13 years of age were included in this study. Twenty-four were seropositive and 22 seronegative for BVDV. Eleven of the seropositive animals and 11 of the seronegative animals were in the eighth month of gestation, the remaining animals were virgin heifers. PBMC from these animals were analyzed for Interferon (IFN)-[gamma] and Interleukin (IL)-4 mRNA expression by real-time RT-PCR after stimulation with a non-cytopathic strain of BVDV. Additionally, an ELISA was performed to measure IFN-[gamma] in the supernatants of stimulated cell cultures. In BVDV seropositive animals, IFN-[gamma] mRNA levels were significantly higher than in BVDV seronegative animals and there was a significant positive correlation between the changes in IFN-[gamma] and IL-4 mRNA expression. There was, however, no significant difference in IFN-[gamma] and IL-4 mRNA levels between pregnant and non-pregnant animals. These results are inconsistent with BVDV inducing a Th1 or Th2 biased immune response. Furthermore, a shift in the cytokine pattern during bovine pregnancy was not evident.


Standard therapies including administration of potent antibiotics, aggressive fluid resuscitation and metabolic support have not been successful in relieving symptoms and reducing mortality associated with acute coliform mastitis. It is important to understand the pathophysiological response of the mammary gland to coliform infections when designing preventive or therapeutic regimens for controlling coliform mastitis. Our laboratory has previously shown that macrophages and polymorphonuclear neutrophils in milk express CD14 on their cell surface. In this study, we found that soluble CD14 (sCD14) is present in milk whey as a 46 kDa protein reacted with anti-ovine CD14 antibody. Additional functional studies found that: (1) under serum-free condition, complexes of LPS-recombinant bovine soluble CD14 (rbosCD14) induced activation of mammary ductal epithelial cells (as measured by changes in interleukin-8 (IL-8) mRNA level by competitive RT-PCR) at low concentrations of LPS after 6 or 24 h incubation (1-1000 ng/ml), whereas LPS alone did not induce activation of mammary ductal epithelial cells at the same concentrations, and (2) intramammary injection of low concentrations of LPS did not increase concentration of leukocytes in milk. In contrast, LPS-rbosCD14 complex containing the same concentration of LPS increased the concentration of leukocytes in the injected mammary gland at 12 and 24 h post-injection. These results indicate that rbosCD14 sensitizes mammary epithelial cells to low concentrations of LPS in vitro and in vivo. Endogenous sCD14 in milk may be important in initiating host responses to Gram-negative bacterial infections.

A feline splenic cDNA library was screened with a 32P-labelled cDNA probe encoding the canine IgE epsilon heavy chain subunit. A cDNA sequence of 1614 nucleotides encoding the complete feline IgE heavy chain, as well as a portion of a variable region, was identified. A search of the GenBank database revealed an identity of 82% at the nucleotide level and 76% at the amino acid level between the feline epsilon heavy chain sequence and the canine homologue. In a separate study, feline genomic DNA, isolated from whole feline embryo cells, was subjected to PCR amplification using primers based on known partial genomic DNA sequences for the feline C[epsilon] gene. Following removal of an intron from the 683 bp PCR product, the coding sequence yielded an ORF of 506 bp. The DNA sequence of this PCR clone differed by a single nucleotide from the cDNA clone. This difference is silent, and therefore the proteins encoded by the two sequences are identical over the regions cloned and sequenced. Phylogenetic analysis of the constant regions of nine immunoglobulin epsilon genes revealed that the feline cDNA is most similar to the canine homologue.


http://www.sciencedirect.com/science/article/B6TD5-46MBHFW-2/2/4e33fbdc4cb75064e8d501acfab93aa0

Type I interferons (IFN) are important mediators of the host defense against viral infections in mammals. In humans multiple subtypes of IFN-[alpha] exist, most of which possess antiviral activity. Little is known about the type I IFN genes in cats and the role they may play in feline immunological responses to viruses. We have isolated cDNAs encoding five feline IFN-[alpha] (feIFN) subtypes that share from 95 to 99% amino acid sequence identity. FeIFN-[alpha]5 has five additional amino acids inserted at position 139, which are not present in the other four subtypes. Sequence identity of the feIFN proteins encoded by the five clones compared to human IFN-[alpha]2 is approximately 60%. Unlike most of the human subtypes, each of the five feline IFN sequences has an N-glycosylation recognition site. Expression of all five feIFN-[alpha] subtypes in Chinese hamster ovary (CHO) cells was confirmed by Western blot analysis, and all resulting proteins were glycosylated. The antiviral activity of each feIFN-[alpha] subtype produced in transiently transfected CHO cell cultures was tested in vitro. In addition, subtype feIFN-[alpha]6 was expressed in the yeast, Pichia pastoris. The resulting secreted mature recombinant protein was purified and demonstrated significant antiviral activity and induction of 2',5'-oligoadenylate synthetase activity in vitro.


http://www.sciencedirect.com/science/article/B6TD5-3T176MDF-6/2/51c37cf13ab5653e80693f83c3f893

The cDNAs encoding bovine macrophage colony-stimulating factors [alpha] and [beta] (M-CSF[alpha] and M-CSF[beta]) were cloned and recombinant bovine M-CSF[alpha] (rbM-CSF[alpha]) in its dimeric form was expressed by using a recombinant baculovirus/insect cell system. The predicted amino acid sequence of rbM-CSF[alpha] and rbM-CSF[beta] shared 83.3 and 75.9% ([alpha]) and 75.3 and 65.9% ([beta]) similarity with the sequence for human and murine M-CSFs, respectively. The biological activity of rbM-CSF[beta] was confirmed by the colony-
forming assay using mouse bone marrow cells. SDS-PAGE under a reducing condition showed that the molecular weight of rbM-CSF[beta] was approximately 34 kDa. On the other hand, Western blot analysis under a non-reducing condition revealed that this rbM-CSF[beta] was secreted in dimeric form into the cell supernatant.


http://www.sciencedirect.com/science/article/B6TD5-46MBHFW-4/2/b28599b08da9c94cd1d94ae4524b1ab

The CD40 molecule is a member of the tumour necrosis factor receptor (TNFR)-like supergene family and plays a major role as a co-stimulatory molecule in the activation of T cells in response to antigens presented by dendritic cells. In this study, reverse transcription-PCR cloning was used to derive the sequence encoding ovine CD40. The ovine CD40 sequence demonstrated a similarity of 97, 76 and 64% with the bovine, human and murine sequences, respectively, at the nucleic acid level. The cysteine residues characteristic of the TNFR family and N-linked glycosylation sites are conserved. Furthermore, RNA analysis confirmed expression of CD40 mRNA in both ovine dendritic cells from lymphatic drainage and dermal fibroblasts in culture. In addition, cDNA encompassing the extracellular region of ovine CD40 (CD40e) was fused 'in-frame' with the enhanced green fluorescent protein (EGFP) to generate a fusion protein upon the transfection of Chinese hamster ovary (CHO) cells. Immunoprecipitation with an anti-EGFP monoclonal antibody of a 78 kD protein from conditioned medium of CHO transfectants confirmed that the CD40e-EGFP was secreted in the supernatant. All experiments were controlled with a pEGFP-N1 vector-blank construct. Moreover, the biological activity of ovine CD40e-EGFP was demonstrated by its ability to inhibit a two-way mixed lymphocyte reaction. Thus these observations confirm that ovine CD40 blockade inhibits co-stimulation mediated by CD40-CD40L (CD154) interactions as has been reported in murine and human studies.