Cessation of feline calicivirus shedding coincident with resolution of chronic gingivostomatitis in a cat

Feline calicivirus (FCV) shedding and oral bacterial flora were monitored over a period of 22 months in a case of feline gingivostomatitis (FGS). The cat was treated daily with 50 mg thalidomide capsules by mouth, and 200 mg lactoferrin powder was applied directly to the lesions. Clinical signs began to resolve after 11 months when, in addition to treatment, the diet had been changed to an additive-free cat food supplemented with antioxidant vitamins A, D_3 and E. Resolution of clinical signs of FGS coincided with the cessation of FCV shedding, and this is the first report documenting such an association. Which part of the treatment, if any, contributed to the cure requires further investigation.

D. D. Addie, A. Radford*, P. S. Yam and D. J. Taylor

Journal of Small Animal Practice (2003) **44**, 172–176

Department of Veterinary Pathology, University of Glasgow, Bearsden Road, Glasgow G61 1QH

*University of Liverpool, Veterinary Teaching Hospital, Leahurst, Chester High Road, Neston CH64 7TE

INTRODUCTION

Feline gingivostomatitis (FGS) is a condition of unknown, but probably multifactorial, aetiology that is frequently refractory to treatment. Feline calicivirus (FCV) has been implicated in the pathogenesis of the disease and is present in up to 100 per cent of cases (Knowles and others 1991, Reubel and others 1992). However, FCV may simply be an opportunistic infection, rather than the cause. Although acute FGS can be induced experimentally by infecting cats with a FCV strain from a case of FGS, chronic FGS cannot (Knowles and others 1991, Reubel and others 1992). Nevertheless, the prevalence of FCV in cats with chronic FGS is very much higher than in the general cat population (Knowles and others 1991).

The mouths of normal cats generally contain between seven and 16 species of cultivable bacteria (Love and others 1990), and others which have not yet been cultivated may also occur. Gingivitis in the cat has been associated with an increased anaerobic population compared to the normal feline mouth (Mallonnee and others 1988, Love and others 1990). However, it is widely accepted that antibiotics alone will not usually cure FGS (Williams and Aller 1992).

There is an immune-mediated component to FGS. Examination of the cytokine messenger RNA (mRNA) content of biopsies of FGS lesions by Harley and others (1999) showed a tendency towards a mixed T helper 1 (Th1) and Th2-type cytokine profile rather than the predominantly Th1-type cytokine profile found in normal oral mucosa. These findings prompted the present authors to consider thalidomide as a treatment, as it is widely used in human conditions where a Th1 response is preferable to a Th2 response, such as human immunodeficiency virus (HIV) infection, *Mycobacterium tuberculosis* infection and tumours (Verbon and others 2000, Dredge and others 2002). In contrast, corticosteroids, which are often used to treat FGS, suppress both Th1- and Th2-type responses (Moreira and others 1997, Rowland and others 1998). Additionally, thalidomide has been reported to reduce some proinflammatory cytokine responses (tumour necrosis factor-α, interleukin [IL] 6 and IL-10) (Moreira and others 1997, Rowland and others 1998) and has been successfully used in the treatment of oral aphthous stomatitis in humans (Weinstein and others 1999).

Lactoferrin was also considered as a therapeutic component as it has been reported to be beneficial in cases of FGS (Sato and others 1996). Lactoferrin aids the phagocytic activity of polymorphonuclear leucocytes and binds iron, rendering it unavailable to bacteria that require iron to replicate (Hasegawa and others 1994). Lactoferrin has also been reported to have antiviral activity (Hasegawa and others 1994, Harmsen and others 1995, Marchetti and others 1996, Superti and others 1997, Swart and others 1998), although not specifically against FCV.

CASE HISTORY

Presentation

An entire male domestic shorthaired cat was presented to the referring veterinary sur-

Please circle which descriptions most appropriately describe the cat's mouth at present:

Pain on vet opening cat's mouth
(1) no evasive reaction
(2) slight evasive reaction
(3) obvious evasive reaction
(4) threatening and bite action

Salivation
(1) none
(2) slight
(3) moderate
(4) marked

Appetite
(1) normal
(2) 1/2 to 1/3 of usual food intake
(3) 1/5 or <1/5 of usual food intake
(4) no appetite

Oral inflammation
(1) normal
(2) reddened
(3) severe congestion
(4) ulcerative inflammation and proliferation of granulation tissue in fauces

Haemorrhage from lesions
(1) none
(2) some bleeding when swabbed
(3) spontaneous haemorrhage

Halitosis
(1) normal cat breath
(2) present
(3) dreadful

Submandibular lymph nodes
(1) normal
(2) one or other enlarged – please specify which
(3) both raised

Cat's weight today:

FIG 1. Feline chronic gingivostomatitis – veterinary surgeon's questionnaire

geon in January 1998 as an ex-stray of unknown age, with gingivitis. The cat was castrated, and vaccinated with attenuated FCV, feline herpesvirus, feline panleuco-penia virus and inactivated feline leukaemia virus (FeLV) (Katavac Eclipse; Fort Dodge Animal Health). The cat's gingivitis was treated with antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs). The cat was presented again in August 1998 with signs of upper respiratory tract disease following a stay in a boarding cattery. In March 1999, the cat was presented again with gingivitis and received antibiotic and NSAID treatment. The following month all of the cat's teeth, except the canine and incisor teeth, were removed. The cat continued to be treated for chronic gingivitis and stomatitis with clindamycin (Antirobe; Pharmacia & Upjohn), amoxycillin (Clamoxyl; Pfizer), spiramycin and metronidazole (Stomorgyl; Merial), marbofloxacin (Marbocyl; Vetoquinol), prednisolone, carprofen (Rimadyl; Pfizer) and ketoprofen (Ketofen; Merial) until referral to the University of Glasgow Veterinary School in July 1999.

The cat was examined by one of the authors (D. D. A.) on seven occasions. Results of the physical examination were recorded on a form based on that of Sato and others (1996) (Fig 1), so that a clinical score could be devised. The results are shown in Table 1. On presentation, the cat had a clinical score of 16, which was high; a normal score would have been 7 (see Fig 1). The worst possible score would be 25 without weight loss (see Table 1) and 27 with weight loss; however, the cat was not weighed at first presentation. In addition, the mouth lesions were recorded on a mouth map.

A questionnaire was completed at each examination, recording what the cat ate and drank, and establishing that it was occasionally exposed to cigarette smoke, was not known to hunt, and that the owner was not applying any dental care products. The animal was from a single cat household and was free-ranging.

At the beginning and end of the study, the cat was FeLV negative (Jarrett and Ganière 1996) and negative by immunofluorescence for feline immunodeficiency virus (Pedersen and others 1987) and

Table 1. Clinical score based on the questionnaire shown in Fig 1. A lower clinical score indicates improvement

Date	Lymph node enlargement	Halitosis	Pain score	Salivation	Appetite	Inflammation	Haemorrhage	Weight score*	Weight (kg)	Total score
14/7/99	2	2	2·5	1	2·5	4	2	ND	ND	16
3/8/99	3	1	1	1	1	2	1	–1	4·1	9
20/10/99	2 (left only)	1	2	1	1	3	2	–1	4·82	11
16/2/00	2	1	2	1	1	3	1	+2	4·26	13
13/6/00	2	2	1	1	1	2	1	–1	5·18	9
27/9/00	1	1	1	1	1	1·5	1	+2	4·3	9·5
2/5/01	2	1	1	1	1	1	1	+2	3·9	10

*Weight loss = +2, Weight gain = –1, Same weight = 0
ND = Not done

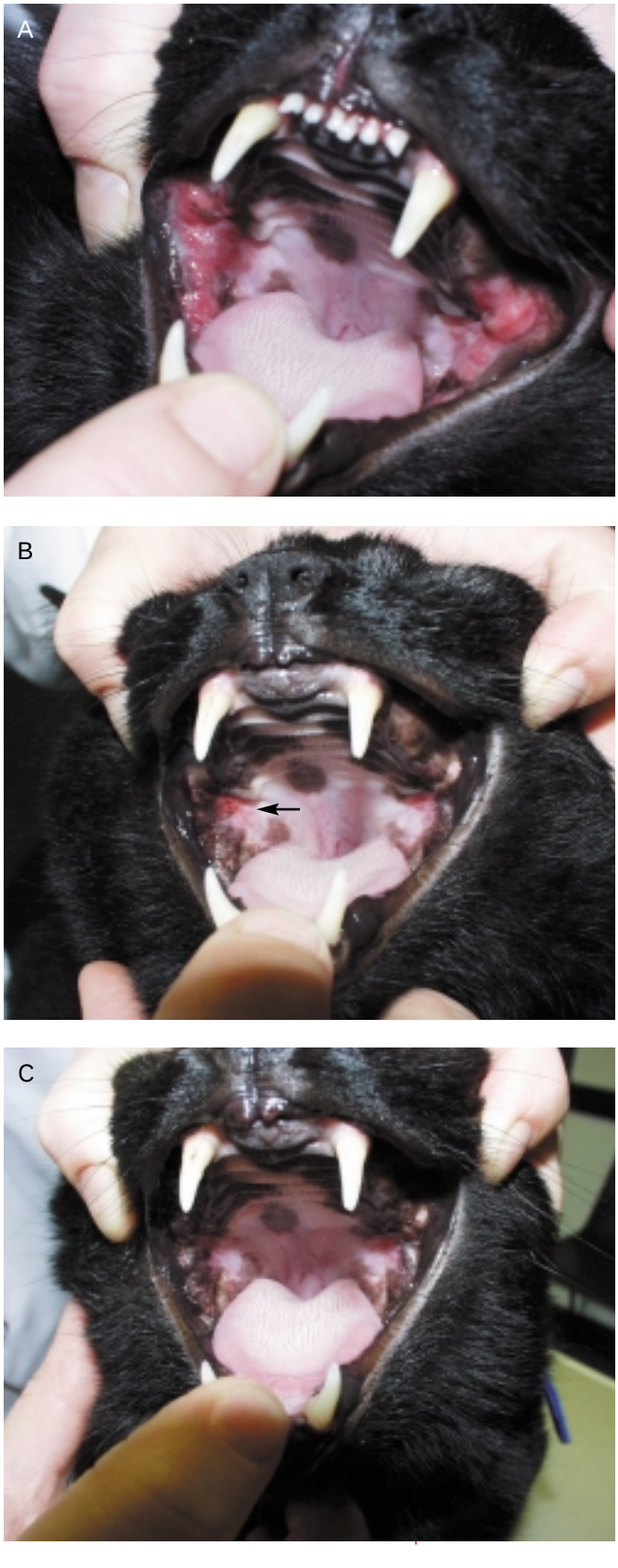

FIG 2. Chronic gingivostomatitis in a cat being treated with thalidomide and lactoferrin. (A) Appearance of the lesions in February 2000. (B) In September 2000, after 14 months of treatment, there remained one small area of inflammation (arrow), so treatment was continued. (C) By May 2001, complete resolution had been achieved. Clinical recovery correlated with cessation of feline calicivirus shedding

corona virus antibodies (Addie and Jarrett 1992). The lesions are shown at three time points in Fig 2. At each clinical examination an oropharyngeal swab was collected into viral transport medium for calicivirus isolation. Bacterial culture was also performed at each examination. Two swabs were rubbed into the lesions and used for bacteriology. A smear was made of one swab and Gram stained for examination for the presence of spirochaetes. The second swab was put into bacterial transport medium (Transwab; Medical Wire & Equipment Company, Corsham, Wiltshire) for both aerobic and anaerobic culture. Swabs were transported by hand to the bacteriology laboratory and examined immediately. Swabs were inoculated onto 7 per cent sheep blood agar and MacConkey agar for aerobic incubation, onto 'chocolate' agar for incubation in 10 per cent carbon dioxide, and onto 7 per cent horse blood agar for incubation in an anaerobic work station (Don Whitley Mark III). Organisms considered to be of clinical significance were identified using the appropriate analytical profile index (Biomerieux). The results of these examinations are presented in Table 2.

Treatment

From July 1999, one 50 mg capsule of thalidomide (Sauramide; Penn Pharmaceuticals, Tredegar) was given daily by mouth in the evening. In accordance with advice from the Veterinary Medicines Directorate, the owner was informed that thalidomide is not licensed for use in cats. Lactoferrin powder (200 mg) was sprinkled directly onto the lesions each day and the cat's mouth was held closed for a few minutes to stop it expelling it. Treatment was not given during December 1999 and January 2000, when the owner was unable to attend the clinic, and the cat's condition deteriorated. Significant improvement was noted 11 months after the start of treatment and a gradual withdrawal of treatment was effected, with thalidomide being given every other day and lactoferrin for four days in seven; all treatment had ceased

Table 2. Results of viral isolation and bacteriological investigation

Date	FCV isolation	Bacteriology
14/7/99	FCV	Sparse culture of *Moraxella* species
3/8/99	FCV	*Pasteurella multocida*
20/10/99	FCV	*Pasteurella multocida*, Gram+ve rods, Gram–ve coccobacilli
16/2/99	FCV	*Pasteurella multocida*
13/6/00	No virus isolated	*Pasteurella multocida*, streptococci, ß-haemolytic streptococci
27/9/00	No virus isolated	Sparse growth of *Pasteurella multocida*, staphylococci
2/5/01	No virus isolated	*Pasteurella multocida*

by 15 months after its instigation. No antibiotics were given at any time after referral, and the cat's oral flora were carefully monitored.

Clinical progress

Initially, the cat's condition gradually improved, as seen from the decrease in inflammation and total clinical scores, and increase in bodyweight recorded in Table 1. However, at examination in February 2000 (Fig 2A), it was found that the cat had relapsed after the owner discontinued treatment for two months, and this was manifest as a drop in weight and increased discomfort on opening the mouth. In June 2000, the incisor teeth had become loose and subsequently had to be removed, but otherwise a marked clinical improvement was noted. The extent of the lesions in the fauces had reduced considerably and they did not bleed when swabbed. The owner reported that the cat's appetite had improved and it had gained weight. By May 2001, the cat's mouth had completely recovered (Fig 2C).

Viral and bacterial monitoring

The results of virus isolation are presented in Table 2. The inability to isolate FCV coincided with the marked clinical improvement noted in June 2000 (Table 2). RNA sequence analysis on the FCV isolates obtained in July 1999 and October 1999 showed that the cat was infected with the same strain at these two time points (data not shown).

The results of bacterial culture are presented in Table 2. On initial presentation, the bacterial flora present was very sparse. On subsequent occasions, profuse cultures of *Pasteurella multocida* were recovered (with the exception of the examination performed on 27 September 2000). Anaerobic bacteria were not recovered from these samples at any time.

DISCUSSION

To the authors' knowledge, this is the first time that FCV has been monitored for the duration of a case of natural FGS, and that FCV shedding has been recorded to cease when clinical signs resolved. This finding contrasts with other studies performed by one of the authors (D. D. A.) in which FCV was isolated from 12 further cats which continued to show signs of FGS on 46 of 48 occasions, over a mean follow-up period of 4·75 months (range one to 13 months) (unpublished observations).

In the present case, treatment may have led to elimination of the virus by stimulating an effective antiviral immune response. Alternatively, reduction of the lesion may have removed cells required for viral growth. Although this finding does not prove that FCV is the cause of this condition, it must be added to the weight of evidence that suggests that FCV is involved.

Since FCV vaccination has been shown to prolong long-term shedding of FCV in experimental infections (Dawson and others 1991), and this cat was clearly prone to being a long-term FCV carrier, the authors advised the referring veterinary surgeon to use only killed FCV vaccines in this case when booster vaccinations were due.

The sparse culture of bacteria on presentation was undoubtedly the result of the thorough antimicrobial treatment given by the referring veterinary surgeon. *P multocida* was isolated on every subsequent examination. Mallonee and others (1988) reported a correlation between the presence of *P multocida* and gingival scores: the healthier the gums, the more *P multocida* were present. A clear association between *P multocida* and the extent of lesions was not revealed in this case. The absence of anaerobes from the lesions is of interest. Spirochaetes are an indicator of an anaerobic flora and were not recovered from the sites sampled in this animal. The failure to culture anaerobes may be associated with the areas sampled, as the teeth and gingival crevices of this animal were not involved and were not sampled.

As resolution of the clinical signs took so long, the authors cannot claim to have cured the condition using thalidomide and lactoferrin: human aphthous ulcers show an improvement within a week of commencement of thalidomide treatment (Weinstein and others 1999) and cats treated with lactoferrin were reported to have shown an improvement in two weeks (Sato and others 1996). However, the cat's appetite did improve soon after the beginning of treatment and the lesions ceased to bleed when swabbed. Veterinary surgeons in practice treating a further two cases of FGS in collaboration with the authors have noted a similar clinical improvement using thalidomide, although neither cat recovered completely, even after months of treatment, and FCV was isolated from both cats every time they were sampled, on 21 occasions. Some cases of FGS spontaneously regress, and that may have happened in this case.

The immunomodulatory properties of thalidomide are not fully understood, and have never been examined in the cat. The literature contains conflicting reports of the drug's immunomodulatory effects: McHugh and others (1995) reported that it induced Th2 and inhibited Th1 cytokine production in peripheral blood mononuclear cells in vitro; however, a single dose of thalidomide given to human volunteers enhanced their Th1-type immune response (Verbon and others 2000). Thalidomide is used widely in humans where a shift towards the Th1 response is protective, while a Th2 response is deleterious; for example, in

HIV or *Mycobacterium* species infection (Verbon and others 2000).

The safety of thalidomide in the cat is well documented, the only side effect being mild sedation in some cases (Frederickson and others 1977, Kaitin 1985). As in other species, thalidomide is teratogenic (Khera 1975) and so should not be prescribed to pregnant or possibly even entire female cats. For FGS, a disease that is notoriously refractory to conventional treatments, thalidomide therapy warrants further investigation.

There are several additional factors that may have influenced the outcome in the present case. Notably, the owner chose to change the cat's main diet (to Classic cat food; Butcher's Pet Care) just before the first major improvements were seen in June 2000. This change of diet may have been significant on three counts. First, in humans, the occurrence of gingivostomatitis has been associated with the consumption of certain food additives (Wray and others 2000). Therefore, the authors cannot be sure that this case did not resolve because some noxious component had been removed from the diet. This warrants further investigation. Secondly, deficiencies of certain micronutrients have been implicated in some human stomatitis cases (Wray and others 1975), and thus the new diet might have supplied some nutrient that the cat had previously lacked. Thirdly, Classic food contains the antioxidant vitamins A, D_3 and E, all of which have been claimed to improve immune function (Harbige 1996), while vitamin A plays a vital role in mucosal health (Harbige 1996).

The owner was devoted to the cat and took great pains to ensure that it received its treatment daily. Fortunately, the cat was gentle and, although the lesions were painful, this cat did allow treatment to be applied. Many cats with FGS are in too much discomfort to allow such treatment and this is a factor that needs to be considered before pursuing this option.

Acknowledgements

The authors are grateful to the veterinary surgeons participating in this study and especially to Gerard Henry for referring the case and for provision of excellent clinical records prior to referral. They thank Oswald Jarrett for advice in preparation of the manuscript; Richard Irvine for photography; Matthew Golder, Michael McDonald and Joyce Simpson for technical assistance; Maria Williams and Janet McGrane for administrative help; and Andrew Gordon, Drew McConnell and Graeme McCombe for assistance with computing. The lactoferrin powder was kindly supplied by Mr Ko Shiino, of Morinaga Foods, Germany. The work was funded by Companion Animal Diagnostics, University of Glasgow.

References

Addie, D. D. & Jarrett, O. (1992) A study of naturally occurring feline coronavirus infection in kittens. *Veterinary Record* **130**, 133-137

Dawson, S., Smyth, N. R., Bennett, M., Gaskell, R. M., McCracken, C. M., Brown, A. & Gaskell, C. J. (1991) Effect of primary-stage feline immunodeficiency virus infection on subsequent feline calicivirus vaccination and challenge in cats. *AIDS* **5**, 747-750

Dredge, K., Marriott, J. B., Todryk, S. M., Muller, G. W., Chen, R., Stirling, D. I. & Dalgleish, A. G. (2002) Protective antitumor immunity induced by a costimulatory thalidomide analog in conjunction with whole tumor cell vaccination is mediated by increased Th1-type immunity. *Journal of Immunology* **168**, 4914-4919

Frederickson, R. C., Slater, I. H., Dusenberry, W. E., Hewes, C. R., Jones, G. T. & Moore, R. A. (1977) A comparison of thalidomide and pentobarbital – new methods for identifying novel hypnotic drugs. *Journal of Pharmacology and Experimental Therapeutics* **203**, 240-251

Harbige, L. S. (1996) Nutrition and immunity with emphasis on infection and autoimmune disease. *Nutrition and Health* **10**, 285-312

Harley, R., Helps, C. R., Harbour, D. A., Gruffydd-Jones, T. J. & Day, M. J. (1999) Cytokine mRNA expression in lesions in cats with chronic gingivostomatitis. *Clinical and Diagnostic Laboratory Immunology* **6**, 471-478

Harmsen, M. C., Swart, P. J., de Bethune, M-P., Pauwels, R., de Clercq, E., The, T. H. & Meijer, D. K. F. (1995) Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vivo. *Journal of Infectious Diseases* **172**, 380-388

Hasegawa, K., Motsuchi, W., Tanaki, S. & Dosako, S. (1994) Inhibition with lactoferrin of in vitro infection with human herpes virus. *Japanese Journal of Medical Science and Biology* **47**, 73-85

Jarrett, O. & Ganiere, J-P. (1996) Comparative studies of the efficacy of a recombinant feline leukaemia virus vaccine. *Veterinary Record* **138**, 7-11

Kaitin, K. I. (1985) Effects of thalidomide and pentobarbital on neuronal activity in the preoptic area during sleep and wakefulness in the cat. *Psychopharmacology* **85**, 47-50

Khera, K. S. (1975) Fetal cardiovascular and other defects induced by thalidomide in cats. *Teratology* **11**, 65-69

Knowles, J. O., McArdle, F., Dawson, S., Carter, S. D., Gaskell, C. J. & Gaskell, R. M. (1991) Studies on the role of feline calicivirus in chronic stomatitis in cats. *Veterinary Microbiology* **27**, 205-219

Love, D. N., Vekselstein, R. & Collings, S. (1990) The obligate and facultatively anaerobic bacterial flora of the normal feline gingival margin. *Veterinary Microbiology* **22**, 267-275

McHugh, S. M., Rifkin, I. R., Deighton, J., Wilson, A. B., Lachmann, P. J., Lockwood, C. M. & Ewan, P. W. (1995) The immunosuppressive drug thalidomide induces T helper cell type 2 (Th2) and concomitantly inhibits Th1 cytokine production in mitogen- and antigen-stimulated human peripheral blood mononuclear cell cultures. *Clinical and Experimental Immunology* **99**, 160-167

Mallonee, D. H., Harvey, C. E., Venner, M. & Hammond, B. F. (1988) Bacteriology of periodontal disease in the cat. *Archives of Oral Biology* **33**, 677-683

Marchetti, M., Longhi, C., Conte, M. P., Pisani, S., Valenti, P. & Seganti, L. (1996) Lactoferrin inhibits herpes simplex virus type 1 adsorption to Vero cells. *Antiviral Research* **29**, 221-231

Moreira, A. L., Tsenova-Berkova, L., Wang, J., Laochumroonvorapong, P., Freeman, S., Freeman, V. H. & Kaplan, G. (1997) Effect of cytokine modulation by thalidomide on the granulomatous response in murine tuberculosis. *Tuberculosis and Lung Disease* **78**, 47-55

Pedersen, N. C., Ho, E. W., Brown, M. L. & Yamamoto, J. K. (1987) Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* **235**, 790-793

Reubel, G. H., Hoffman, D. E. & Pedersen, N. C. (1992) Acute and chronic faucitis of domestic cats. *Veterinary Clinics of North America: Small Animal Practice* **22,** 1347-1360

Rowland, T. L., McHugh, S. M., Deighton, J., Dearman, R. J., Ewan, P. W. & Kimber, I. (1998) Differential regulation by thalidomide and dexamethasone of cytokine expression in human peripheral blood mononuclear cells. *Immunopharmacology* **40**, 11-20

Sato, R., Inanami, O., Tanaka, Y., Takase, M. & Naito, Y. (1996) Oral administration of bovine lactoferrin for treatment of intractable stomatitis in feline immunodeficiency (FIV)-positive and FIV-negative cats. *American Journal of Veterinary Research* **57**, 1443-1446

Superti, F., Ammendolia, M. G., Valenti, P. & Seganti, L. (1997) Antirotaviral activity of milk proteins: lactoferrin prevents rotavirus infection in the enterocyte-like cell line HT-29. *Medical Microbiology and Immunology* **186**, 83-91

Swart, P. J., Kuipers, E. M., Smit, C., Van der Strate, B. W., Harmsen, M. C. & Meijer, D. K. (1998) Lactoferrin. Antiviral activity of lactoferrin. *Advances in Experimental Medicine and Biology* **443**, 205-213

Verbon, A., Juffermans, N. P., Speelman, P., van Deventer, S. J., ten Berge, I. J., Guchelaar, H. J. & van der Poll, T. (2000) A single oral dose of thalidomide enhances the capacity of lymphocytes to secrete gamma interferon in healthy humans. *Antimicrobial Agents and Chemotherapy* **44**, 2286-2290

Weinstein, T. A., Sciubba, J. J. & Levine, J. (1999) Thalidomide for the treatment of oral aphthous ulcers in Crohn's disease. *Journal of Pediatric Gastroenterology and Nutrition* **28**, 214-216

Williams, C. A. & Aller, M. S. (1992) Gingivitis/stomatitis in cats. *Veterinary Clinics of North America: Small Animal Practice* **22**, 1361-1383

Wray, D., Ferguson, M. M., Mason, D. K., Hutcheon, A. W. & Dagg, J. H. (1975) Recurrent apthae: treatment with vitamin B12, folic acid and iron. *British Medical Journal* **2**, 490-493

Wray, D., Rees, S. R., Gibson, J. & Forsyth, A. (2000) The role of allergy in oral mucosal diseases. *Quarterly Journal of Medicine* **93**, 507-511