Arch Virol (2003) 148: 153–164
DOI 10.1007/s00705-002-0903-6

Archives of Virology
Printed in Austria

# Apoptosis in canine distemper

**L. Moro**[1], **A. de Sousa Martins**[2], **C. de Moraes Alves**[1], **F. G. de Araújo Santos**[1], **J. E. dos Santos Nunes**[1], **R. A. Carneiro**[3], **R. Carvalho**[1], and **A. C. Vasconcelos**[1]

[1]Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[2]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[3]Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Received March 28, 2002; accepted August 7, 2002
Published online October 30, 2002 

**Summary.** Canine distemper is a systemic viral disease characterized by immunosuppression followed by secondary infections. Apoptosis is observed in several immunosuppressive diseases and its occurrence on canine distemper *in vivo* has not been published. In this study, the occurrence of apoptosis was determined in lymphoid tissues of thirteen naturally infected dogs and nine experimentally inoculated puppies. Healthy dogs were used as negative controls. Samples of lymph nodes, thymus, spleen and brain were collected for histopathological purposes. Sections, 5 μm thick, of retropharingeal lymph nodes were stained by HE, Shorr, Methyl Green-Pyronin and TUNEL reaction. Shorr stained sections were further evaluated by morphometry. Canine distemper virus nucleoprotein was detected by immunohistochemistry. Retropharingeal lymph nodes of naturally and experimentally infected dogs had more apoptotic cells per field than controls. In addition, DNA from thymus of infected dogs were more fragmented than controls. Therefore, apoptosis is increased in lymphoid depletion induced by canine distemper virus and consequently play a role in the immunosuppression seen in this disease.

## Introduction

Canine distemper (CD), a contagious systemic disease, causes respiratory, gastrointestinal and nervous system disorders [4]. Despite the widespread use of vaccines, CD remains an important viral disease [17, 16, 27]. *Canine distemper*

*virus* (CDV) is a member of the genus *Morbillivirus* of the family *Paramixoviridae* as well as *Measles virus* and *Rinderpest virus* [21].

CDV infects dogs of all ages, but puppies are more susceptible [4, 10, 14]. The incubation period ranges from one to four weeks or more [3]. Clinical signs vary in range and severity, and some dogs show no symptoms [5]. Some dogs develop neurological signs combined or following systemic or subclinical disease [2, 3]. The most consistent gross lesion is thymic hipotrophy [2]. Lymph nodes may be enlarged or shrunken, depending on the phase of the disease [6]. Lymph nodes and thymus begin to show lymphoid depletion in absence of necrosis on the sixth day post infection [16].

Apoptosis plays a central role in the homeostasis of all multicellular organisms by eliminating dangerous cells such as mutant or viral infected [30]. Apoptotic cells are isolated (anoikyc), shrunken with acidophilic cytoplasm and nuclei show chromatin margination, condensation and fragmentation. Cell fragments into apoptotic bodies [32] and is rapidly sequestered by professional phagocytes or neighboring cells, avoiding inflammatory reaction [19]. Internucleosomal cleavage of DNA into multiples of 180 bp fragments is a marker of apoptotic process as consequence of endonucleases activities [8, 31].

Apoptosis can be triggered by physiological and pathological stimuli. It is prevalent in several immunosuppressive diseases of humans and animals [1, 18, 24]. Ito et al. [12] suggested that apoptosis may play a role in the measles virus (MV) immunosuppressive mechanism. Guo et al. [11] showed apoptosis *in vitro* caused by CDV. Others have addressed the question of apoptosis or necrosis importance in the pathogenesis of oligodendroglial degeneration in distemper [25]. Likewise CD immunosuppression may also involve apoptosis. Additionally, lymphoid depletion in lymph nodes and thymus, in absence of necrosis, has been already reported in CD [16].

Little is known about apoptosis in the pathogenesis of CD *in vivo*. In this regard, this work sets out to investigate apoptosis in lymphoid tissues of natural and experimentally CDV infected dogs. Whether involved as a mechanism of lymphoid depletion, apoptosis may play an important role in the CD immunosuppression.

## Materials and methods

### *Virus*

Snyder Hill strain of CDV, kindly supplied by Dr. Max J. G. Appel (Cornell University, Ithaca, New York, 14853, USA), was used for animal inoculation.

### *Animal groups and samples*

Naturally infected dogs – Forty-two dogs, 3 months to adult, with clinical CD were necropsied. Samples of retropharingeal and tracheobronchial lymph nodes, spleen, thymus, cerebrum and *cerebellum* were *collected*. Experimentally infected dogs – Fifteen mongrel puppies, from Centro de Controle de Zoonoses (Belo Horizonte, MG, Brazil), were dewormed two days before inoculation. Dogs were fed with commercial food three times a day and water *ad libitum*. The experiments were performed in four steps: [i] Four puppies from two different

litters (50 and 100 days old) were split in two groups, each one containing one puppy from each litter: (a) two controls not infected; (b) two inoculated with 0.25 ml of virus inoculum (Snyder Hill strain 5/71) via intra peritoneal and intra nasal routes. Dogs were housed in separate isolation units and clinically evaluated daily up to the tenth day of post inoculation. The dogs were sacrificed ten days post inoculation and necropsied. Thymus, retropharingeal and tracheobronchial lymph nodes, and spleen specimens were collected and processed for light microscopy. [ii] Six puppies, 52 days old, from the same litter were split into 2 groups: two puppies were kept as controls and four were inoculated. [iii] Five puppies, 33 days old, from the same litter were split into two groups: two puppies were kept as controls and three inoculated. Blood was collected on days 0, 3, 6 and 10 days post infection for hematological analysis.

### *Histology and morphometry*

Samples were fixed in 10% buffered formalin, processed and embedded in paraffin. Five μm thick sections were stained by HE and Shorr. Retropharingeal lymph nodes sections stained by Shorr were used for morphometry. Total and apoptotic cells were registered (40 fields/slide) in a digital image analyzer; the apoptotic index was calculated. Morphometry was performed with a specific software (Kontron KS 300 V 2.0).

**TUNEL reaction**: the *in situ* genome fragmentation was detected using the Oncogene–TdT-FragEL–DNA fragmentation detection kit (Cat # QIA33). The reaction was carried out as described by the manufacturer. Briefly, slides were incubated with 20 μg/ml of proteinase K (SIGMA) and endogenous peroxidase was quenched with 3% $H_2O_2$ in methanol. TdT and deoxynucleotides were applied and slides placed in humid atmosphere at 37 °C for 1.5 h. The reaction was stopped by blocking buffer, the slides were treated with peroxidase streptavidin conjungate plus DAB and counter stained with methyl green.

**Methyl Green Pyronin stain (MGP)**: sections from retropharingeal lymph node were deparaffinized, hydrated with distilled water and MGP covered for 2 min. Sections were blot and air dried for 5 min, differentiated with distilled water, dehydrated and mounted.

**Immunohistochemistry**: the anti-CDV nucleoprotein monoclonal antibody (MoAb) was kindly provided by Dr. Alex Walender (Canada). Slides were deparaffinized, predigested with proteinase K and reduced with sodium borohydride. Peroxidase was quenched with hydrogen peroxide. Tissues were stained with MoAb, treated with biotinylated horse anti-mouse IgG, incubated in vectastain ABC reagent and DAB peroxidase reagent applied (Vector laboratories). Sections were counter stained with hematoxylin.

### *DNA electrophoresis*

Approximately 0.2 g of thymus tissue was ground in 0.5 ml of TTE lysis buffer (10 mM Tris; 0.25% Triton x-100; 1 mM EDTA), spun at 500 × ***g***/10 min and the DNA extracted from the supernatant by standard procedures [22]. DNA pattern was analyzed in 1.5% agarose gel standard electrophoresis.

### *Statistical analysis*

A cut-off value of the minimal representative number of microscopic fields was established to quantify apoptosis in canine lymph node, by the standard deviation (SD) instability analysis described elsewhere [23]. A Shorr stained section of tracheobronchial lymph node from a CDV infected dog was used. Apoptotic indices from 300 microscopic fields in the same slide were registered, using image digital analyzer (Kontron KS 300 V 2.0). Ten samples of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 random fields were set as experimental units and had their descriptive statistics calculated. SD for each sample size was plotted against its size to

visualize the trend. SD values decreased as the number of microscopic fields increased. When the oscillations of the SD between consecutive samples were smaller than 5%, a cut-off was established, since the number of fields in that sample was considered representative enough to dispense larger sampling. Hereby, such stabilization of SD occurred at a cut-off of 40 fields per slide. Descriptive statistics and analysis of variance were applied to all morphometric parameters.

## Results

### *Natural infection*

Myoclonus was the most prevalent clinical sign among naturally infected dogs. Others included abdomen pustules, opisthotonos, seizures, pedal movements, bilateral purulent conjunctivitis, apathy, ataxia, chewing, salivation, crying and pad hyperkeratosis. Gross lesions of the lymphoid system varied on necropsy. Lymph nodes were diminished in some animals and enlarged in others. Puppies thymuses were diminished about 5–6 times. One showed only thymus remnants. The histopathological lesions considered for CD diagnosis were: demyelination of the cerebellum and optic tracts; inclusion bodies (Sinegaglia-Lentz) and syncytia formation. Definitive diagnosis was obtained by immunohistochemical detection of viral antigen. The lymph node cortexes were atrophic and depleted. The apoptotic indices of the retropharingeal lymph nodes of 13 CDV and 6 control dogs were plotted in Fig. 1. Statistical analysis showed significant differences ($P < 0.05$) between control and natural infected dogs, considering the X $\pm$ SD of: [i] total number of cells (control $= 218.3 \pm 51.9$; CDV infected $= 196 \pm 53.3$);

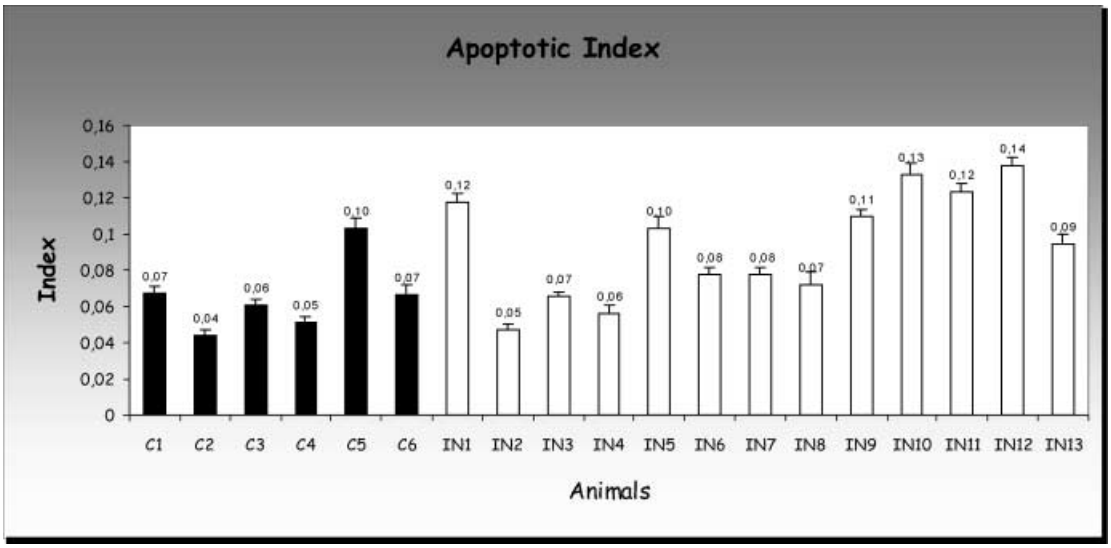



**Fig. 1.** Average of the apoptotic index (number of apoptotic cells/total number of cells) per 40 fields studied per retropharingeal lymph node of CDV naturally infected animals. Standard deviations (SD) are shown as bars. Control (*C*) and natural infection (*IN*) X $\pm$ SD were $0.06 \pm 0.03$ and $0.09 \pm 0.04$ respectively

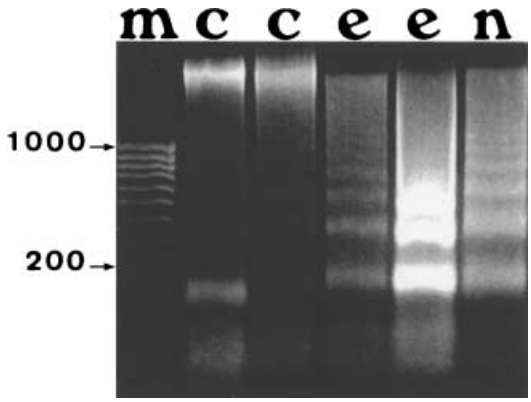



**Fig. 2.** Typical agarose gel electrophoresis of DNA extracted from thymus of control (*c*), experimentally (*e*) and naturally (*n*) CDV infected puppies. Electrophoresis was performed in a 1.5% agarose gel, 1 × TE, 1 h at 120 volts. DNA bands were stained with ethidium bromide (0.5 μg/ml). *m* = marker (gene ruler – 100 bp DNA ladder, MBI); Qualitative differences of high molecular DNA and ladder pattern of degradation are observed between controls and infected animals

[ii] number of apoptotic cells (control = 13.2 ± 5.1; CDV infected = 17.4 ± 7.7) and [iii] apoptotic indices (control = 0.06 ± 0.03; CDV infected = 0.09 ± 0.04) (Fig. 1).

The classical DNA "ladder pattern" was observed by agarose gel electrophoresis of the thymus DNA from infected and control animals (Fig. 2). Controls showed more integral high molecular DNA than infected dogs. The DNA ladder was more intense in the infected animals.

### *Experimental infection*

Puppies had similar clinical signs varying in intensity between those in the same litter and among different litters. Signs were apathy, anorexia, bilateral ocular and nasal discharge (serous to mucopurulent), cough, diarrhea and vomiting, dehydration and progressive loss of weight. Two puppies died before the end of the experiment. The relative counts of lymphocytes and platelets varied. Five dogs showed lymphopenia after six days post infection. Leukopenia was present in one dog (Table 1). Apoptotic cells in blood smear were more observable in inoculated animals. The main gross lesion was hipotrophy of lymphoid organs, more evident in thymus which was diminished 5–6 times. Sometimes only remnants were present. Retropharingeal lymph nodes showed lymphoid depletion and no cortex-medulla demarcation (Fig. 3A). Apoptotic cells, shrunken and surrounded by an halo with condensed and fragmented nuclei, were observed either isolated or in groups (Fig. 3C, D). Apoptosis was confirmed by TUNEL reaction (Fig. 3F). Several syncytia formation, sometimes presenting apoptotic nuclei, were also labelled (Fig. 3G, H). Additionally, MGP also evidenced apoptotic cells (Fig. 3D, G).

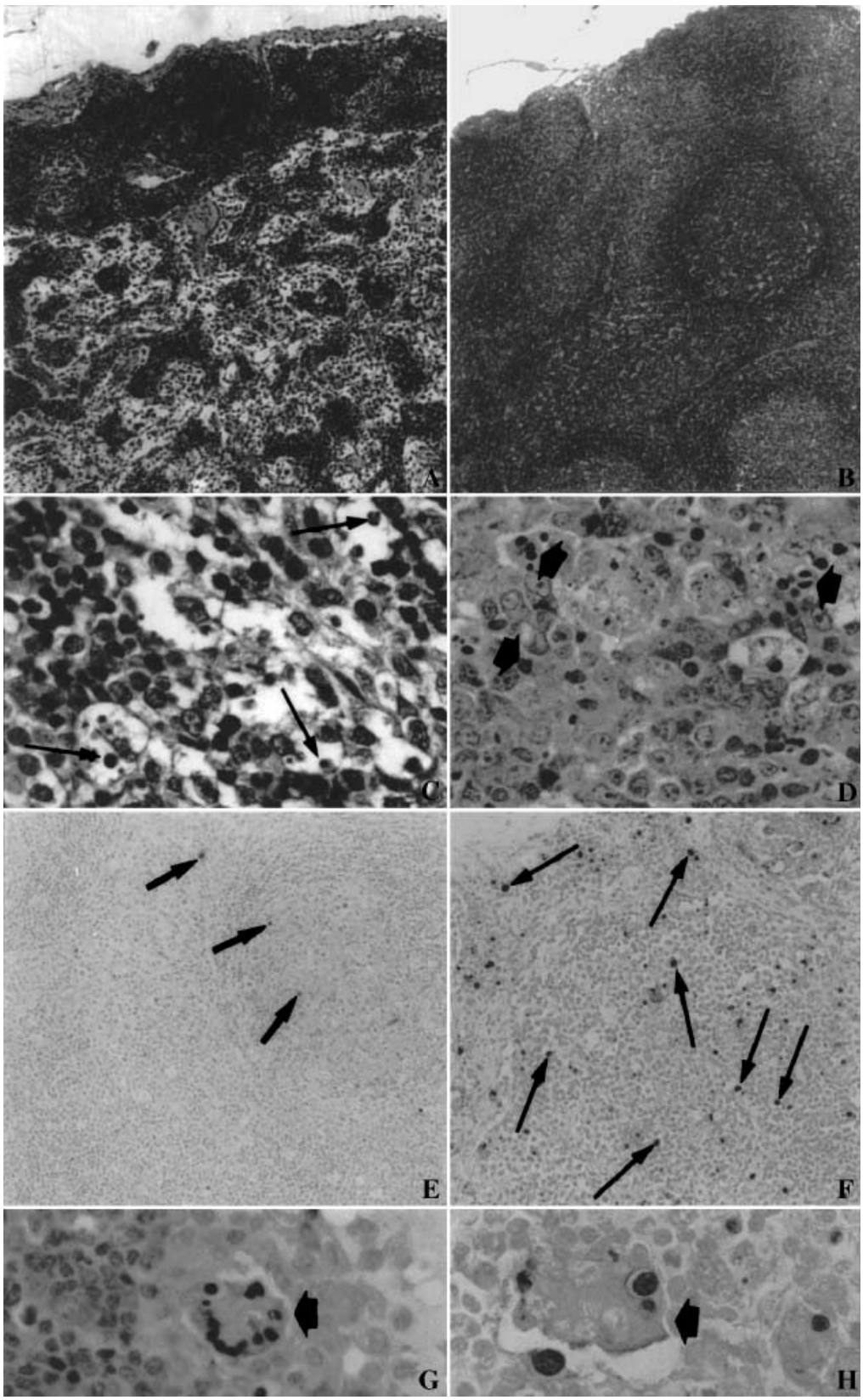
**Table 1.** Hematological results from puppies experimentally inoculated with CDV

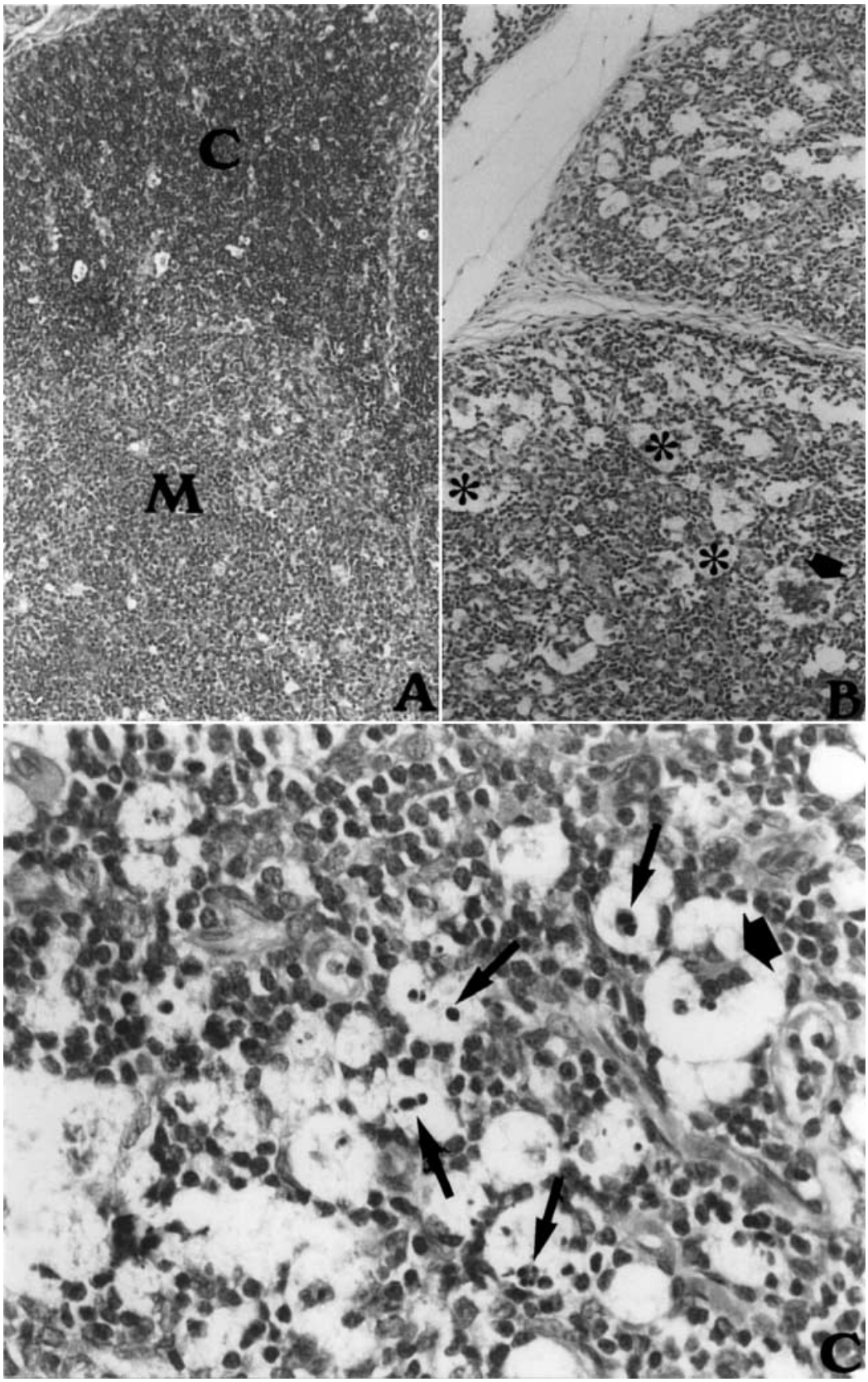
| Animal/step | Main results/collection | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | day 0 pi | | day 3 pi | | day 5 pi | | day 10 pi | |
| | Li (%) | Pla | Li (%) | Pla | Li (%) | Pla | Li (%) | Pla |
| C1/1 | 50 | 34200 | 26 | 23300 | 72 | 454000 | 28 | 618800 |
| C1/2 | 20 | 205000 | 53 | 352000 | 32 | 480000 | 35 | 514800 |
| I1/1 | 49 | 205000 | 55 | 14100 | 53 | 450000 | 8 | 108000 |
| I1/2 | 31 | 179400 | 58 | 452000 | 9 | 453000 | 3 | 330400 |
| C2/1 | 83 | 277300 | NA | NA | NA | NA | ✞ | NA |
| C2/2 | 38 | 220000 | NA | NA | NA | NA | ✞ | NA |
| I2/1 | 27 | NA | NA | NA | NA | NA | 18 | 343000 |
| I2/2 | 39 | NA | NA | NA | NA | NA | ✞ | NA |
| I2/3 | 29 | 359450 | NA | NA | NA | NA | 25 | 180000 |
| I2/4 | 25 | 264960 | NA | NA | NA | NA | 14 | 250000 |
| C3/1 | 27 | 288000 | NA | NA | 45 | 430000 | 16 | 474000 |
| C3/2 | 18 | 501120 | NA | NA | 21 | 430000 | 43 | 473000 |
| I3/1 | 14 | 331200 | NA | NA | 12 | 404000 | ✞ | NA |
| I3/2 | 28 | 593750 | NA | NA | 8 | 480000 | 14 | 412000 |
| I3/3 | 20 | 244860 | NA | NA | 18 | 296000 | 5 | 398000 |

*Li* Lymphocytes; *Pla* platelets; *pi* post inoculation; ✞ = dead before the end of the experiment; *NA* not available

Severe lymphoid depletion was observed in spleen, associated to increased apoptosis in lymphoid follicles. No demarcation cortex to medulla along with decreased cellularity and cystic formation were observed in thymus (Fig. 4). Syncytia and apoptosis were frequent (Fig. 4B, C). Morphometric analysis showed statistical differences ($P < 0.05$) between control and natural infected dogs, considering the $X \pm SD$ of: [i] total number of cells (control $= 225.7 \pm 37.03$; CDV infected $= 187.6 \pm 41.4$); [ii] number of apoptotic cells (control $= 12.2 \pm 4.7$; CDV infected $= 28.2 \pm 16.6$) and [iii] apoptotic indices (control $= 0.05 + 0.02$; CDV infected $= 0.16 \pm 0.1$) (Fig. 5). Agarose gel electrophoresis of thymus DNA showed typical genome fragmentation of apoptosis. Controls had more integral high molecular DNA than infected dogs, while DNA ladder was more intense in CDV-inoculated animals (Fig. 2).

**Fig. 3.** Retropharingeal lymph node of: **A** puppy inoculated with CDV with a reduced cortical and absence of follicles (HE, 60X); **B** control puppy with evident lymphoid follicles (HE, 60X); **C** presence of apoptotic cells (arrows, HE, 600X); **D** apoptotic cells evidenced by MGP (arrows, 600X); **E** control puppy with few apoptotic cells labeled by TUNEL (60X); **F** CDV-inoculated puppy with apoptotic cells labeled by TUNEL (arrows, 600X); **G** syncytium (arrow) containing apoptotic nuclei (MGP, 600X) and **H** labeled by TUNEL (arrow, 1400X)


A
B
C
D
E
F
G
H


C
M
A
B
C

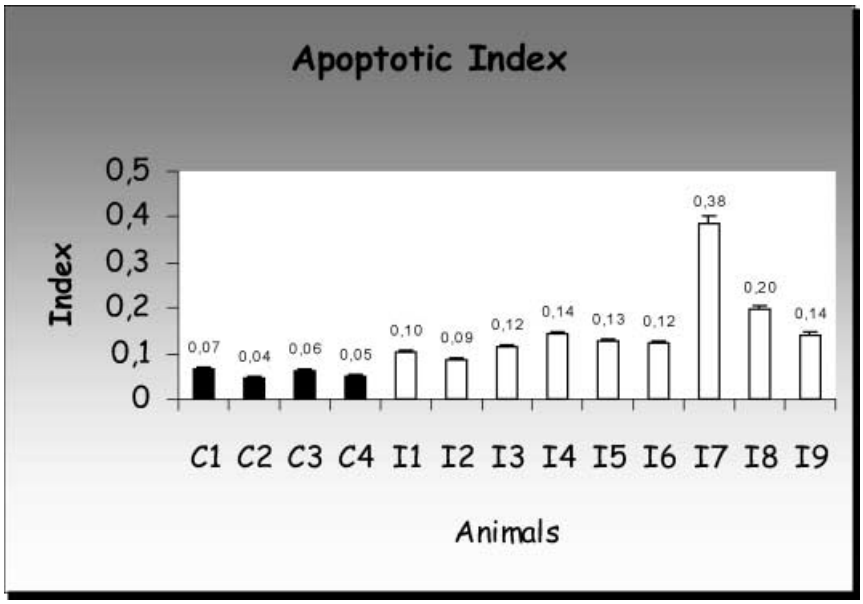



**Fig. 5.** Average of the apoptotic index (number of apoptotic cells/total number of cells) per 40 fields studied per retropharingeal lymph node of CDV experimentally infected animals. Standard deviations (SD) are shown as bars. Control (*C*) and experimental infection (*I*) X ± SD were 0.05 ± 0.02 and 0.16 ± 0.1 respectively

## Discussion

The clinical signs manifested by natural and experimentally infected dogs were similar to those reported for canine distemper [2, 4, 27, 28]. A great variation in clinical signs among litters and within puppies from same litter was also present in accordance to others observation [5]. Hematological results varied as much as clinical signs. Lymphopenia was the most important alteration as described before [15] reflecting immunosuppression [28]. The most severe lymphopenia occurred in two puppies, 60 and 100 days old, at the time of inoculation. It is well known that puppies are more susceptible when passive antibodies are fading away [4, 10].

Gross lesions observed in lymphoid tissues were compatible to those already described [2, 16]. Thymus hipotrophy varied in severity though present in all natural and experimentally infected puppies. These results are in agreement with other findings [2, 16, 20].

A severe lymphoid depletion was observed in retropharingeal lymph nodes of natural and experimentally infected dogs. Iwatsuki et al. [13] related necrosis to lymphoid depletion in lymph node follicles of CDV naturally infected dogs. On

**Fig. 4.** Thymus of **A** a control puppy with evident demarcation between cortex (*c*) and medulla (*M*) (HE, 60X); **B** inoculated puppy with absence of demarcation between cortex and medulla, and presence of cysts (*) and syncytium (arrow) (HE, 60X); **C** presence of apoptosis (smaller arrows) and syncytium (bigger arrow, HE, 600X)

the other hand, Krakowka et al. [16] reported lymphoid depletion in absence of necrosis. In the present study, while depletion occurred in the absence of necrosis, apoptosis was the main process observed. To our knowledge, this is the first work approaching apoptosis as a mechanism involved in the pathogenesis of the canine distemper immunosuppression. In this regard, apoptosis does not induce inflammatory reaction, differently from necrosis [8, 19]. In this study, no inflammatory response in lymphoid organs was detected, despite the lymphoid depletion. Others have reported the involvement of apoptosis in immunosuppressive diseases [1, 18, 24]. Therefore, CD may be a target for further studies *in vitro* and *in vivo* of the apoptotic pathway that leads to immunosuppression.

A characteristic feature of CDV infection is the syncytia formation, which is a diagnostic tool for distemper [26]. In this study, apoptotic nuclei were observed inside several syncytia. Esolen et al. [7] also reported apoptosis in syncytia, as induced by other morbillivirus – the measles virus, *in vitro*. This observation of apoptosis in syncytia is worthy of future research for better understanding of cell–virus interactions.

Shorr stain was primarily used to obtain better visualization of Sinegaglia-Lentz inclusion bodies, since trichromic stains can provide better differentiation as for other cellular components [9]. Surprisingly, Shorr also helped to distinguish apoptotic cells yielding a better contrast of nuclei. Thus, it was used for sections under morphometric analysis. Morphometry showed higher cellularity and less apoptosis in control dogs than in infected animals. Apoptosis is remarkably increased in lymphoid depletion, which suggests that its importance as the main mechanism involved in the cellularity decrease in these immunosuppressive conditions.

Thymus DNA showed higher degree of genomic fragmentation in CD than in controls, suggesting a higher incidence of apoptosis in these tissues, as observed in Fig. 2. Moreover, apoptosis may represent an important mechanism in thymus hipotrophy during distemper evolution. In such a way, several viruses infecting lymphoreticular tissues can cause transient or prolonged immunosuppression [29]. This interaction of viruses and lymphoid system can induce inadequate activation and apoptosis of lymphocytes as in AIDS [1], Gumboro's and Newcastle diseases in chickens [18] and feline immunodeficiency virus infection in cats [24].

In conclusion, the present work is the first approach showing important observations that infection of CDV results in increased apoptotic cell population in lymphoid tissues. The data here obtained by morphometry, DNA electrophoresis, TUNEL and MGP staining support that apoptosis play an important role on lymphoid depletion induced by CDV. A remarkable aspect here is the usage of an animal model system to address the importance of CDV infection in canine distemper pathogenesis. Further research is necessary to elucidate the molecular mechanisms involved in these conditions. The analysis of the CDV RNA expression may contribute to elucidate the role of viral components that participate in the pathogenesis of CD. For the first time apoptotic cells were seen in syncytia and in the blood smear of CDV-infected animals. These observations should be more explored in the future. Blood smear from inoculated dogs reflected what

was occurring in the lymphoid organs. The presence of apoptotic cells in blood smear may be helpful in diagnostic purposes.

## Acknowledgements

We thank: Dr. Max J. G. Appel (Cornell University, Ithaca, New York, 14853, USA), for the Snyder Hill strain of CDV; Dr. Alex Wandeler for monoclonal antibodies (Canadian Food Inspection, Agency's Animal Diseases, Research Institute/Centre for Plant Quarantine Pests, Canada); Dr. Steven Krakowka, Dr. Mike Oglesbee, Candy Glendening and Susan Ringler (Goss Laboratory, Ohio State University, Columbus, Ohio, USA) for their assistance; Dr. Ivan B. M. Sampaio (Departamento de Zootecnia, EV/UFMG, Brazil) for assistance with statistical analysis; Dr. Alfredo Miranda de Góes (Departamento de Bioquímica, ICB/UFMG, Brazil) for suggestions; Dr. Maria L. P. Corrêa, CCZ-MG and Veterinary Hospital of Veterinary School (UFMG, Brazil) for providing animals; CAPES and CNPq for financial support.

## References

1. Ameisen JC, Capron A (1991) Cell dysfunction and depletion in AIDS: the programmed cell death cell hypothesis. Immun Today 12: 102–105
2. Appel MJG (1969) Pathogenesis of canine distemper. Am J Vet Res 30: 1167–1182
3. Appel MJG (1987) Virus infections of vertebrates; vol 1, Virus infections of carnivores. Elsevier, Amsterdam, pp 133–159
4. Appel MJG, Summers BA (1995) Pathogenicity of morbilliviruses for terrestrial carnivores. Vet Microbiol 44: 187–191
5. Cornwell HJC, Campbell RSF, Vantsis JT, Penny W (1965) Studies in experimental canine distemper. I. Clinico-pathological findings. J Comp Pathol 75: 3–17
6. Dungworth DL (1993) The respiratory system. 4th edn. In: Jubb KVF, Kennedy PC, Palmer N (eds) Pathology of domestic animals, vol 2. Academic Press, San Diego, pp 539–699
7. Esolen LM, Park SW, Hardwick JM, Griffin DE (1995) Apoptosis as a cause of death in measles virus-infected cells. J Virol 69: 3955–3958
8. Gerschenson LE, Rotello RJ (1992) Apoptosis: a different type of cell death. Faseb J 6: 2450–2455
9. Gorham JR (1960) Canine distemper (la maladie de Carrè). Adv Vet Sci 6: 287–351
10. Greene CE, Appel MJ (1990) Canine distemper. In: Greene GE (ed) Infectious diseases of the dog and cat. WB Saunders, Philadelphia, pp 226–241
11. Guo A, Lu CP (2000) Canine distemper virus causes apoptosis of Vero cells. J Vet Med B 47: 183–190
12. Ito M, Watanabe M, Ihara T, Kamiya H, Sakurai M (1997) Measles virus induces apoptotic cell death in lymphocytes activated with phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore. Clin Exp Immunol 108: 266–271
13. Iwatsuki K, Okita M, Ochikubo F, Gemma T, Shin Y-S, Miyashita N, Mikami T, Kai C (1995) Immunohistochemical analysis of the lymphoid organs of dogs naturally infected with canine distemper virus. J Comp Path 113: 185–190
14. Krakowka S, Koestner A (1976) Age-related susceptibility to infection with canine distemper in gnotobiotic dogs. J Infect Dis 134: 629–632
15. Krakowka S, Cockerell G, Koestner A (1975) Effects of canine distemper virus infection on lymphoid function *in vitro* and *in vivo*. Infect Immun 11: 1069–1078
16. Krakowka S, Higgins RJ, Koestner A (1980) Canine distemper virus: review of structural and functional modulations in lymphoid tissues. Am J Vet Res 41: 284–292

17. Krakowka S, Axthelm MK, Johnson GC (1985) Canine distemper virus. In: Olsen RG, Krakowka S, Blakeslee JR, Comparative pathobiology of viral diseases, vol 2. CRC, Boca Raton, pp 137–164
18. Lam KM, Vasconcelos AC (1994) Newcastle disease virus-induced apoptosis in chicken peripheral blood lymphocytes. Vet Immun Immunopathol 44: 45–56
19. Leist M, Nicotera P (1998) Apoptosis, excitotoxicity and neuropathology. Exp Cell Res 239: 183–201
20. Mccullough B, Krakowka S, Koestner A (1974) Experimental canine distemper virus-induced lymphoid depletion. Am J Path 74: 155–170
21. Norrby E, Kövamees J, Blixenkrone-Möller M, Sharma B, Örvell C (1992) Humanized animal viruses with special reference to the primate adaptation of morbillivirus. Vet Microbiol 33: 275–286
22. Sambrook J, Fritsch DF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, New York
23. Sampaio IBM (1998) Estatística aplicada à experimentação animal Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia Belo Horizonte
24. Sarli G, Della Salda L, Zaccaro L, Bendinelli M, Piedimonte G, Marcato PS (1998) Apoptotic fraction in lymphoid tissue of FIV-infected SPF cats. Vet Immun Immunopath 64: 33–34
25. Schobesberger M, Zurbriggen A, Summerfield A, Vandevelde M, Griot C (1999) Oligodendroglial degeneration in distemper: apoptosis or necrosis? Acta Neuropathol 97: 279-287
26. Summers BA, Appel MJG (1985) Syncytia formation: an aid in the diagnosis of canine distemper encephalomylitis. J Comp Path 95: 425–435
27. Summers BA, Cummings JF, Lahunta A (1995) Veterinary neuropathology. Mosby, Saint Louis, pp 95–188
28. Tipold A, Jaggy A, Zurbriggen A, Vandevelde M (1996) Neurological signs in canine distemper encephalomyelitis – a clinical study. Eur J Comp Anim Pract 6: 33–38
29. Trautwein G (1992) Immune mechanisms in the pathogenesis of viral diseases: a review. Vet Microbiol 33: 19–34
30. Verhagen AM, Voux DL (1999) Molecular mechanisms of apoptosis. Res Problems Cell Differ 23: 10–24
31. Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284: 555–556
32. Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68: 251–305

Author's address: Dr. Luciana Moro, Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Campus, Pampulha, Belo Horizonte, MG, 31 270-901, Brazil; e-mail: moro@icb.ufmg.br