Use of the Dorfman-Warnke classification (modified by Burke) in veterinary pathology in the evaluation of the lymphadenitis induced through canine parvovirus in guinea-pigs (*Cavia porcellus*)

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Utilização da classificação de Dorfman-Warnke (modificada por Burke) em patologia veterinária na avaliação da linfadenite induzida por parvovírus canino em cobaias (*Cavia porcellus*)

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SUMMARY

In human pathology the adequate system used to analyze and interprete reactive lymphadenopathies is based on the morphological characterization of the various areas within the lymphoid node. In veterinary pathology there is no standardization of histopathological evaluation of lymph nodes. Therefore, in this study, we applied the Dorfman-Warnke (modified by Burke) human classification of reactive lymphadenopathies to evaluate virus-induced lymphadenitis in guinea-pigs. Canine parvovirus was inoculated into the footpads of guinea-pigs and the response of popliteal lymph nodes was histologically studied. The footpads were excised after different periods of time, processed, and stained with HE, Mallory, Gordon & Sweets, Giemsa and by immunohistochemical methods. A significant increase in the weight of ipsilateral lymph nodes to the inoculation site ($p<0.05$) was observed. The follicular response in the lymph nodes was characterized by the reaction of the germinative center, which showed partial loss of delimitation in the mantle zone. Increased numbers of blast cells and hypertrophy of post-capillary venules were evidenced in the paracortical area of the lymph nodes. There was a discrete hyperplasia of the medullary cords and a general enlargement of the reticular net associated with those alterations. The reactional response observed was similar to viral lymphadenitis identified in humans, justifying the attempts to systematize this classification for veterinary use just like it is currently done in human medicine.

UNITERMS: Canine parvovirus; Lymphadenitis; Animal pathology.

INTRODUCTION

Lymph nodes, especially the superficial ones, are the most relevant lymphoid structures – as far as a disease is concerned - that react to the presence of pathogens. Often they are hyperplasic, as a consequence of inflammatory, neoplasic or immunologic stimuli[23]. They can easily be submitted to biopsy or to needle aspiration for diagnostic purposes, and previous knowledge of their normal morphology is essential. The histological aspects of the lymph nodes has been studied in several species, showing wide variability[1,5,7,9]. However, there is a common structure related to the existence of sites populated by different cell types that perform specific functions, the follicular, paracortical, medullary and sinusoidal regions. The interaction between lymphoid tissue and etiological agents leads to modification of the tissue microenvironment generating reactional patterns[2]. This regional specificity in cellularity and function supports a rational classification of lymphadenopathies. The most popular of the systematic classifications of reactive patterns in human lymphadenopathies was elaborated by Dorfman; Warnke[5] and later modified by Burke[2].

Follicle reaction follows a number of stimuli, though lacking specificity and being routinely described in domestic animals[3,14,24]. Paracortical changes are reported in delayed hypersensitivity, viral infections, in drainage of tumor cells and autoimmune diseases[5,21,23]. The medullary cords are sites of plasma cell reaction that usually expand into other lymph node territories. Finally, the sinuses - routes to lymphatic circulation - react to infectious, immunologic or genetic stimuli with histiocytosis[12]. Yet, the morphologic appearance of the hyperplasias varies with preceding experience with the etiologic agent, with individual age and with immunologic capability.

Routine morphologic evaluation of these patterns for correlation with a possible etiology is currently restricted to human medicine, while similar studies are rare in veterinary medicine. In order to contribute to the knowledge of reactional patterns of lymph nodes in animals, particularly against viruses, in the present experimental study we inoculated guinea-pigs with canine parvovirus, and interpreted the findings using the classification of Dorfman; Warnke[5], modified by Burke[2].

MATERIAL AND METHOD

Animals: Twenty-five male and female guinea-pigs (*Cavia porcellus*) weighing 300 to 500 g from the Butantan Institute (São Paulo, Brazil) were used. The animals were kept in cages and received water and balanced ration *ad libitum*. The animals were split into five groups of five animals, the first group being hind leg, a negative control group, and in the other ones the animals were inoculated subcutaneously, into the right footpad with 0.2 ml. of Parvoguard*. The animals of each group were killed and necropsied on days 0, 3, 5, 7 and 10 after inoculation.

Antigen: Attenuated canine parvovirus was used (Parvoguard).

Histopathological analysis: At the end of each experimental period the animals were sacrificed, the skin from the hind legs excised and the popliteal lymph nodes, ipsilateral and contralateral to the site of innoculation were excised – the latter being used as a positive control. The nodes were fixed in 10% formalin, dissected and weighed on an analytical scale (Sartorius-Werke Göttingen) 48 hours post-fixation. The lymph nodes were then processed histologically and sections were stained by the Giemsa, H.E., Gordon & Sweets and Mallory methods.

Immunohistochemical analysis: Sections obtained from the samples embedded in paraffin were submitted to immunohistochemistry using monoclonal antibodies to mouse anti-human T lymphocyte[20] diluted 1:100, and human anti-lymphocyte B monoclonal antibodies, CD45R[25] diluted 1:50. In both cases, amplifications were done with anti-immunoglobulin antibody from biotinylated mice (VECTOR BA2001), using the avidin-biotin-peroxidase complex (VECTOR PK4000) for visualization[11], with 1:1000 dilution in both cases.

Statistical Analysis: The Student t-test was used to analyze the weights, with the level of significance set at $p<0.05$.

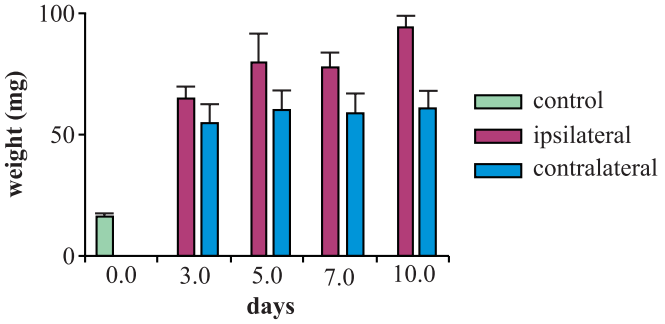

Figure 1

Weight of the lymph nodes in control groups and in ipsilateral and contralateral groups of guinea pigs inoculated subcutaneously with parvovirus into right footpad ($x \pm \sigma$).

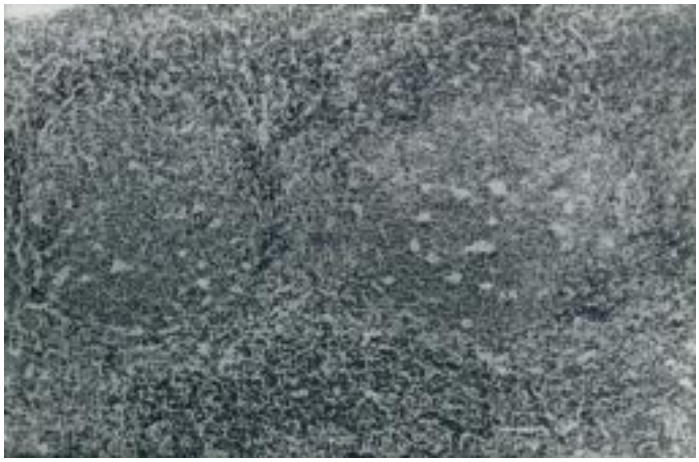

Figure 2

Photomicrograph of the ipsilateral popliteal lymph node ten days after injection of attenuated canine parvovirus into footpad showing in cortex follicular hyperplasia with a starry sky pattern and partial loss of the mantle zone, H.E., 165 x.

RESULTS

Weighing

The lymph nodes ipsilateral to the site of inoculation presented a significant and progressive weight increase (t-test, $p<0.05$) compared to the contralateral lymph nodes. These kept the same weight throughout the experiment, though presenting visible differences in relation to the same lymph nodes from negative control animals (Fig. 1).

Microscopic evaluation

Ipsilateral lymph nodes

Follicles

Three days postinoculation we observed the predominance of primary follicles in the cortical region, with scarce germinal centers of small dimensions. These progressively increased in number and volume, presenting high mitotic activity in the polarized germinal centers and masking of the mantle zone. Ten days after the stimuli we detected follicular hyperplasia with an attenuated or disrupted mantle zone, with a starry sky pattern and an apparent overlap of the follicular and paracortical sites (Fig. 2).

Paracortex

In the first experimental period (3 days) there was a diffuse distribution of lymphocytes, predominating blast cells, especially immunoblasts, associated with high mitotic activity and cellular pleomorphism (Fig. 3). The high-endothelial venules exhibited a transitory hyperthrophic response with a large amount of lymphocytes on their walls (Fig. 4). The lymphoid expansion persisted throughout the experimental periods, with a progressive densification of the reticle framework (Fig. 5) and the presence of a discrete polymorphonuclear infiltration. Ten days after inoculation there was a mixed reactive pattern, including follicular and paracortical hyperplasia.

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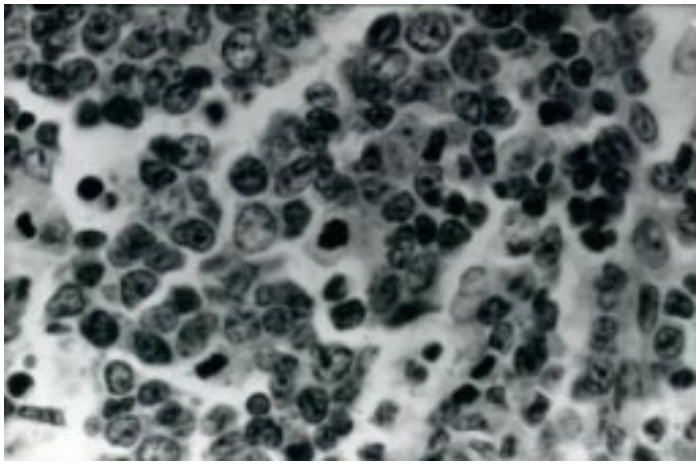

Figure 3

Photomicrograph of the ipsilateral popliteal lymph node, three days after injection of attenuated canine parvovirus into footpad showing the paracortical zone, with a high mitotic index, many immunoblasts and pleomorphic cells. H.E., 660 x.

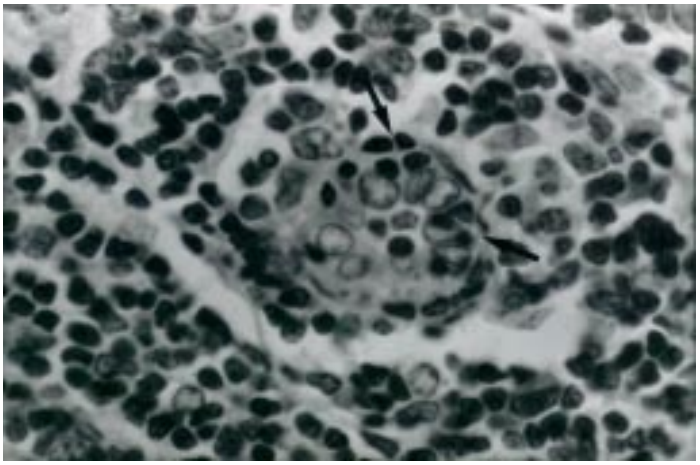

Figure 5

Photomicrograph of the ipsilateral popliteal lymph node, three days after injection of attenuated canine parvovirus into the footpad showing the paracortex with a hypertrophic high endothelial venule with some lymphocytes during transmigration (arrows). H.E., 660 x.

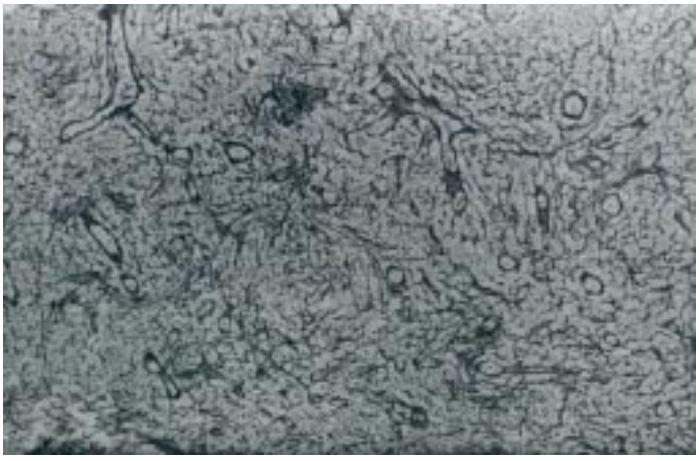

Figure 4

Photomicrograph of the ipsilateral popliteal lymph node ten days after injection of attenuated canine parvovirus into the footpad showing the densification of the reticular framework of the reactive lymph node. Gordon & Sweets, 165 x.

Medulla

A discrete polymorphonuclear infiltrate, arranged in strings was observed at all experimental times, initially without any local lymphoid expansion. This was detected five days after the stimuli, with thickening of the medullary cords. The sinuses were preserved without any histiocytic reaction throughout the experimental period.

Contralateral lymph nodes

A predominance of primary follicles in the contralateral lymph nodes was observed during the experiment, with the presence of sporadic, small secondary follicles. An inflammatory infiltrate of polymorphonuclear cells could be seen during the experiment, especially in the paracortical and sinusoidal regions. The paracortex and medullary compartments were unaltered.

Immunohistochemistry

Immunostaining showed, in the ipsilateral popliteal lymph nodes during the last experimental time, a diffuse distribution of T cells in paracortical hyperplasia, with sporadic nodular aggregates and a secondary expansion of B cells during the germinative center reaction.

DISCUSSION

According to the Dorfman; Warnke's classification[5], later modified by Burke[2], the observed reaction was of the mixed type, characterized by precocious paracortical expansion of the lymph node that drained the injured site, and followed by progressive follicular expansion between the 5th and 10th response day. Medullary cords and lymphatic sinuses played a secondary role. Mehrotra[18], reported a similar reaction picture in rabbits with lymphadenitis induced by the vaccinia virus.

The used stains were those routinely described for histopathological analysis of lymph nodes[15], complemented with immunohistochemical evaluation. They allowed the visualization of the local architecture, as well as the morphofunctional characterization of the lymphocyte population.

Probably the increase in the weights of the stimulated lymph nodes was related to the local replicative activity and to a decreasing exit of the lymphocytes from that structure. These findings were reproduced in mice, by anti-interferon globulin simultaneously injected with Newcastle disease virus, resulting in the inhibition of the expected lymphadenopathy characteristic of this infectious picture[8]. The mechanism implicated in this apparent local sequestration of lymphocytes seems likely to be related to the interferon-induced alterations in the surface proteins necessary for the binding with high-endothelial venules[10]. Since most of the cells parasitized by viruses do produce large amounts of interferon, this mechanism may be correlated with the occurrence of lymphadenopathies and lymphopenia resulting from viral infections and often clinically reported[26].

Hypertrophy of high-endothelial venules during early stages of the reaction was a characteristic of the observed paracortical response, probably due to local liberation of cytokines produced by activated lymphocytes and macrophages[16], together with the effect of cell to cell interactions and lymphocyte transmigration[6].

Although cellular immunity is most important in viral infections, the expansion of the observed follicular and cordonal medullary compartments is the structural expression of the sprouting of humoral immunity, also present in viral infections[4,17]. Follicular hyperplasia reached extreme levels with partial loss of delimitation of the mantle zone, a scarcely researched event that can also be observed in other viral infections[4,22].

With the evolution of the lymphoid response comes the progressive reticulum proliferation and fiber condensation at perifollicular sites, simultaneously with the reaction of the germinal center. The occurrrence of cellular pleomorphism in lymphocytes populating proliferative compartments is worth reporting. The explanation for this resides in the different processes to which lymphocytes are submitted in those territories, particularly somatic mutation[17], a potential generator of atypical cells.

The utilization of lymph nodes ipsilateral to the site of inoculation as control was pertinent, since there was a significant difference between the weights of these and the contralateral ones, as also observed in a previous similar experiment[10]. It is worth to stress the large difference in weights between control non-inoculated lymph nodes and contralateral controls, with the presence of polymorphonuclear cells in the latter, indicating the existence of an inflammatory reaction. In some animals were also detected contralateral controls follicles presenting a discrete proliferative response, probably due to the ocurrence of viremia after inoculation of the attenuated parvovirus[19].

In this study, the use of peripheral lymph nodes was due to their restricted drainage area when compared with central lymph nodes - frequent stimulation targets[15] - resulting in a more selective response. In short, in the presence of the same antigen, the reaction of lymph nodes to the canine parvovirus injected into the guinea-pigs foot pads was quite similar to the reaction identified in dogs[19], rabbits and even in human beings, and in other types of viral diseases[4,18]. This suggests the validity of using the Dorfman-Warnke modified by Burke for classifying the lymphoid responses in animals as it is done for humans, thus permitting their correlation with an accurate diagnosis.

RESUMO

Em patologia humana, a análise e interpretação das linfadenopatias reacionais baseia-se na caracterização morfológica das várias regiões do linfonodo. Em patologia veterinária não há essa padronização. No presente estudo, utilizamos as bases da classificação de Dorfman-Warnke (modificada por Burke), utilizada na área médica humana para avaliarmos uma linfadenite viral experimental em cobaias. Parvovírus canino foi inoculado em coxim plantar de cobaias e a resposta dos linfonodos poplíteos avaliada. No fim de cada período experimental, linfonodos foram excisados, pesados e processados histologicamente em cortes corados pelos métodos de Giemsa, Mallory, Gordon & Sweets e Hematoxilina-Eosina e por meio de imunoistoquímica com anticorpos pan-T e pan-B. Foi observado aumento de peso significante ($p<0,05$) dos linfonodos ipsilaterais ao sítio de inoculação em comparação com os contralaterais. A resposta folicular nos primeiros linfonodos foi caracterizada por reação do centro germinativo, apresentando perda parcial da delimitação da zona do manto. Em região paracortical aumentou o número de células blásticas, ocorrendo hipertrofia de vênulas pós-capilares. Associou-se discreta hiperplasia de cordões medulares e densificação do estroma reticular. A reação observada apresenta semelhanças com a identificada em humanos frente a estímulo viral, sugerindo a adequação do uso dessa classificação também em medicina veterinária.

UNITERMOS: Parvovírus canino; Linfadenite; Patologia animal.

REFERENCES

1- BROWN, E.M.; DELLMANN, H-D.; NICANDER, L. Lymphatic organs. *In*: DELLMANN, H-D.; BROWN, E.M. (eds.). **Textbook of veterinary histology**. 3.ed. Philadelphia : Lea & Febiger, 1987. p.164-84.

2- BURKE, J.S. Reactive lymphadenopathies. **Seminars in Diagnostic Pathology**, v.5, p.312-6, 1988.

3- CALLANAN, J.J.; THOMPSON, H.; TOTH, S.R.; O'NEIL, B.; LAWRENCE, C.E.; WILLET, B.; JARRET, O. Clinical and pathological findings in feline immunodeficiency virus experimental infection. **Veterinary Immunology and Immunopathology**, v.35, p.3-13, 1992.

4- DIEBOLD, J.; LE TORNEAU, A. Diagnostic histopatologique des lymphadenites virales. **Revue Française des Laboratoires**, v.154, p.97-108, 1986.

5- DORFMAN, R.F.; WARNKE, R. Lymphadenopathy simulating the malignant lymphomas. **Human Pathology**, v.5, p.519-50, 1974.

6- DUIJVESTIJN, A.M.; REP, M.; BUTCHER, E.C.; HENDRIKS, H.R.; KRAAL, G. Regulation of functional and morphological aspects of high endothelium in mouse. *In*: FOSSUM, S.; ROSTALD, B. (eds.). **Histophysiology of the immune system**. New York : Plenum Press, 1987. p.491-7.

7- GILLMAN, J.; GILLMAN, T. The pathogenesis of experimentally produced lymphomata in rats (including Hodgkin's-like sarcoma). **Cancer**, v.5, p.792-846, 1952.

8- GRESSER, I.; GUY-GRAND, D.; MAURY, C.; MAUNORY, M.T. Interferon induces a peripheral lymphadenopathy in mice. **Journal of Immunology**, v.127, p.1569-75, 1981.

9- HALL, J.G. The functional anatomy of lymph nodes. *In*: STANSFELD, A.G. (ed.). **Lymph node biopsy interpretation**. New York : Churchill-Livingstone, 1985. p.1-25.

10- HALL, J.G.; MORRIS, B. The immediate effect of antigens on the cell output of a lymph node. **Journal of Experimental Medicine**, v.120, p.450-4, 1965.

11- HSU, S.; RAINE, L.; FANGER, H. Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase: a comparison between ABC and unlabeled antibody (PAP) procedures. **Journal of Histochemistry and Cytochemistry**, v.29, p.577-80, 1081.

12- KOMP, D.M.; PERRY, M.C. Introduction: the histiocytic syndromes. **Seminars in Oncology**, v.18, p.1-2, 1991.

13- KOWALA, M.C.; SCHOEFL, G.I. The popliteal lymph node of the mouse: internal architecture, vascular distribution and lymphatic supply. **Journal of Anatomy**, v.148, p.25-46, 1986.

14- LADDS, P.W. **A colour atlas of lymph node pathology in cattle**. Townsville : James Cook University, 1986. p.4-6: General reactions of lymph nodes.

15- LATHAM, G.R. Routine laboratory methods. *In*: STANSFELD, A.G. (ed.). **Lymph node biopsy interpretation**. New York : Churchill Livingstone, 1985. p.31-43.

16- MANTOVANI, U.G.; KIRKPATRICK, C.H. The lymphoid system in mycotic and mycobacterial diseases. *In*: GALLIN, J.I.; FAUCI, A.S. **Advances in host defenses mechanisms**. New York : Raven Press, 1983. p.143-77.

17- McLENNAN, I.C.M.; GRAY, D. Antigen-driven selection of virgin and memory B cells. **Immunology Reviews**, v.91, p.61-85, 1986.

18- MEHROTRA, R. Histological and ultrastructural changes in experimentally produced post-vaccinial lymphadenitis in rabbits. **Journal of Pathology**, v.126, p.39-44, 1978.

19- POLLOCK, R.V.H.; PARRISH, C.R. Canine parvovirus. *In*: OLSEN, R.G.; KRAKOWKA, S.; BLAKESLEE Jr., J.R. (eds.). **Comparative pathobiology of viral diseases**. Boca Raton : CRC Press, 1985. V.I. p.45-77.

20- POPPEMA, S.; LAI, R.; VISSER, L. Monoclonal antibody OPD4 is reactive with CD45RO, but differs from UCHL1 by the absence of monocyte reactivity. **American Journal of Pathology**, v.139, p.725-9, 1991.

21- REE, H.; FANGER, H. Paracortical alteration in lymphadenopathic and tumor-draining lymph nodes: histologic study. **Human Pathology**, v.6, p.363-72, 1975.

22- SCHNITZER, B. Reactive lymphoid hyperplasias. *In*: JAFFE, E.S. (ed.). **Surgical pathology of the lymph nodes and related organs**. 2.ed. Philadelphia : W.B. Saunders, 1995. p.98-132.

23- STANSFELD, A.G. (ed.). **Lymph node biopsy interpretation**. New York : Churchill Livingstone, 1985. p.67-84: An introduction to biopsy interpretation.

24- VALLI, V.E.O. The hematopoietic system. *In*: JUBB, K.V.F.; KENNEDY, P.C.; PALMER, N. (eds.). **Pathology of domestic animals**. 3.ed. Orlando : Academic Press, 1985. p.83-236.

25- WEISS, L.M.; ARBER, D.A.; CHANG, K.L. CD45: a review. **Applied Immunohistochemistry**, v.1, p.166-81, 1993.

26- WOODRUFF, J.F.; WOODRUFF, J.J. Virus-induced alterations of lymphoid tissues I. Modification of the recirculating pool of small lymphocytes by Newcastle disease virus. **Cellular Immunology**, v.1, p.333-54, 1970.

Received: 01/03/1996
Accepted: 21/05/1998