J. Vet. Malaysia (2009) 21(2): 17 - 20

ASSESSMENT OF CELL MEDIATED IMMUNE RESPONSE IN SPONTANEOUSLY OCCURRING CANINE MAMMARY GLAND TUMOURS BY LEUCOCYTE MIGRATION INHIBITION TEST (LMIT)

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SUMMARY

In the present study, cell mediated immune response in the mammary gland tumour-bearing bitches was studied by evaluating functional capacity of sensitised lymphocytes using Leucocyte Migration Inhibition Test (LMIT). Cell membrane antigens (allogenic type) of different molecular weights separated from mammary gland tumour tissue were subjected to LMIT against leucocytes separated from twenty tumour-bearing bitches and six apparently healthy dogs used as control group. Statistically, the tumour bearing dogs had significantly lower migration index values in comparison to healthy control dogs. Thus, leucocytes separated from tumour-bearing animals showed positive LMIT response while there was normal migration in the healthy control dogs. It is seen that the functional capacity of T lymphocytes in the mammary gland tumour-bearing bitches played a major role in cellular immunity and was not affected as indicated by migration inhibition index.

Keywords: Canine, Leucocyte Migration Inhibition Test (LMIT), mammary tumour

INTRODUCTION

Cell mediated immune response, which is said to be the more important arm of tumour immune response, has been well characterised in molecular, cellular and clinical studies involving allografts and destruction of various neoplastic cells. To assess the cellular immune response in the tumour affected animals, various methods have been employed which include macrophage migration inhibition assay (Kronman *et al.,* 1969), mixed lymphocyte tumour culture technique, blastogenesis assay (Hess *et al.,* 1975), leucocyte migration inhibition assay (Lindsay *et al.,* 1978), leucocyte adherence inhibition microassay [LAI (Yang *et al.* 1991)], chromium-51 released cytotoxicity assay (Cohen, 1980) and skin delayed type hypersensitivity tests (Dennis *et al.,*1985). Among all those tests, LMIT is known to be simple, specific, time saving and reproducible and hence, it was performed for the assessment of cell mediated immune response in the mammary tumour-bearing bitches.

MATERIALS AND METHODS

Leucocyte migration inhibition test (LMIT) was performed by the method suggested by Bendixen and Soborg (1969) with slight modifications. Cell membrane antigens of different molecular weights prepared by 3M KCL method (McCoy *et al.*, 1975) from mammary gland tumour tissue were subjected to LMIT against leucocytes separated from twenty tumour-bearing bitches and six apparently healthy dogs irrespective of sex.

Viable white blood cell count was estimated with 0.2 % Trypan blue to get 85 – 95 % leucocyte viability. After the incubation period, migration area was measured on graph paper by using camera Lucida.

Migration Index (M.I.) was calculated as follows:

$$\text{M.I.} = \frac{\text{Mean migration from two capillary tubes in presence of antigen}}{\text{Mean migration from two capillary tubes in absence of antigen}}$$

The results were classified as follows:

i. Migration enhancement	M.I. > 1.2
ii. Normal migration	1.2 > M.I. > 0.8
iii. Migration inhibition	M.I. < 0.8

The cut-off limits of ± 20% of the control migration was taken as the contribution of non-specific factor in order to avoid false positive responses.

Data generated in the present study were subjected to statistical analysis as suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

This test was performed with an object to estimate the cellular immune response and the functional capacity of sensitised lymphocytes in the tumour bearing animals. Four allogenic mammary carcinoma antigens were used against the leucocytes separated from mammary tumour-bearing animals. Out of twenty tumour-bearing animals, four animals did not show any leucocyte migration

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inhibition and thus, 80% migration inhibition was noted in the present study.

The leucocytes from all the six healthy dogs free from mammary gland tumours or any other growths, when exposed to carcinoma antigen showed no toxic effect and they migrated easily in the growth medium (Plate A). But when the leucocytes from mammary gland tumour-bearing animals were exposed to allogenic carcinoma antigen, they showed typical migration inhibition indicating sensitisation and therefore liberation of lymphokines especially MIF (Migration Inhibition Factor) by the lymphocytes which were previously exposed to those antigens *in vivo* (Plate B).

The migration indices values of the mammary tumour bearing animals against homologous mammary antigens ranged from 0.2800 to 0.8610 with a mean of 0.5451 ±0.037 while the healthy control animals had indices values ranging from 0.9880 to 0.9090 with a mean of 0.9515 ± 0.012 (Table 1).

Statistically, the tumour bearing dogs had significantly lower migration indices values in comparison to healthy control dogs. Thus, leucocytes separated from tumour bearing animals showed positive LMIT response i.e. migration inhibition (M.I. < 0.8) while there was normal migration (1.2 > M.I. > 0.8) in the case of healthy control dogs.

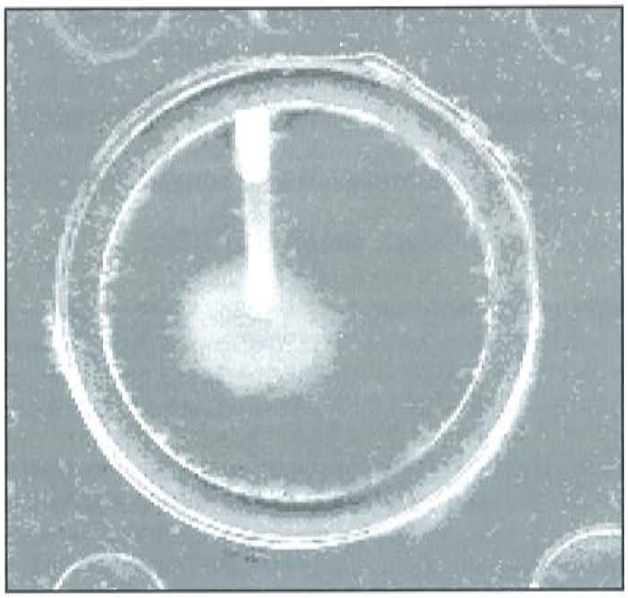
In the present study, all the antigens, used against the leucocytes of tumour-bearing animals were allogenic antigens. It is well known that compared to allogenic antigens, autochthonous antigens exhibit a higher inhibitory effect on migration of leucocytes (Ulvand, 1975; Nehete, 1995). However, with alloantigens too, the migration inhibition was distinctly noted as compared to non-tumour bearing animals (Shingatgeri, 1983). Ulvand (1975) reported that the average migration inhibition of the leucocytes, separated from mammary tumour-bearing dogs was 61% in case of homologous antigen whereas in case of autochthonous antigen, it varied from 72.2 – 92.3%, indicating strong antigenicity in comparison to homologous antigens.

In the present study, 80% migration inhibition was noted in the tumour bearing animals. Similar observations regarding percentage of migration inhibition was recorded by Ulvand (1975).

Table 1: LMIT in tumour bearing and healthy control dogs

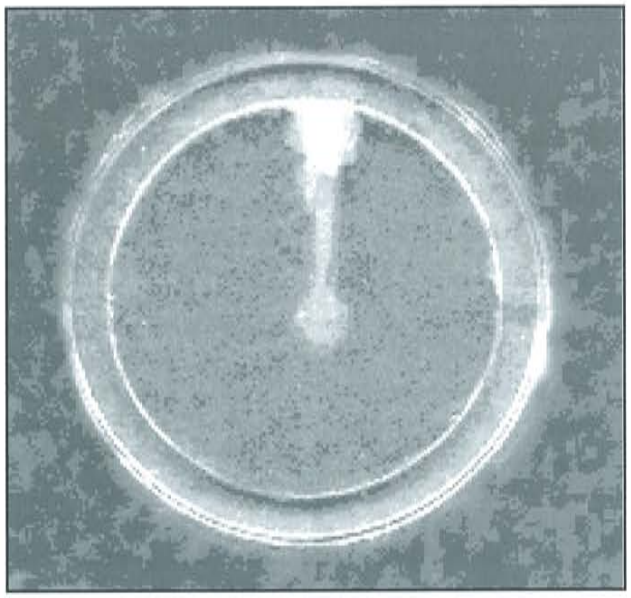
Source of leucocytes	Source of antigen	Leucocyte Viability (%)	Migration index(MI)
CMT-1	CMT-1	85	0.5112
CMT-2	CMT-1	88	0.4522
CMT-3	CMT-2	89	0.4421
CMT-4	CMT-1	85	0.5200
CMT-5	CMT-2	92	0.5526
CMT-6	CMT-2	93	0.8110
CMT-7	CMT-1	92	0.3850
CMT-8	CMT-2	92	0.6019
CMT-9	CMT-1	87	0.8610
CMT-10	CMT-2	85	0.6051
CMT-11	CMT-8	94	0.5370
CMT-12	CMT-1	91	0.2800
CMT-13	CMT-10	94	0.4109
CMT-14	CMT-10	89	0.3951
CMT-15	CMT-8	85	0.4256
CMT-16	CMT-1	88	0.8091
CMT-17	CMT-8	86	0.6610
CMT-18	CMT-1	90	0.3957
CMT-19	CMT-2	88	0.8021
CMT-20	CMT-8	89	0.4436
Cont-1	CMT-1	90	0.9520
Cont-2	CMT-10	92	0.9690
Cont-3	CMT-8	86	0.9210
Cont-4	CMT-10	87	0.9700
Cont-5	CMT-8	89	0.9880
Cont-6	CMT-1	96	0.9090
CMT	Mean± S.E.	89.1	0.5451±0.037*
Control	Mean± S.E.	90	0.9515±0.012

(CMT= Canine Mammary Tumour, * Significant at 5% level)



A

Plate A - Normal migration of leucocytes in the absence of antigen



B

Plate B - Migration inhibition of leucocytes in the presence of antigen

Generally, antigens from other tumours did not react against the leucocytes separated from a specific type of tumour (Chattopadhyay *et al.* 1983). In the present study, there was extensive immunological cross-reactivity among the mammary neoplasms of different histological features as 80% of tumour-bearing animals showed positive LMIT response against mammary carcinoma antigens from homologous mammary tumours. Similar observations were recorded by Rieche *et al.* (1976) who found that 89.66% of breast cancer patients showed positive LMIT response against homogenate from homologous mammary tumours.

To conclude, the functional capacity of T lymphocytes, that play a major role in cellular immunity, was not affected as indicated by migration inhibition index and separated soluble cell membrane antigens were found to be immunogenic and cross-reactive as they stimulated sensitised lymphocytes from the tumour-bearing animals.

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, Bombay Veterinary College for providing necessary facilities.

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