Immunological aspects of mammary tumors in dogs and cats: a survey including own studies and pertinent literature

V.P.M.G. Rutten[a], W. Misdorp[b,d,e], A. Gauthier[c], M. Estrada[c], J.P. Mialot[c], A.L. Parodi[c], G.R. Rutteman[d] and K. Weyer[e]

[a]*Department of Infectious Diseases and Immunology, Section Immunology, Faculty of Veterinary Medicine, University of Utrecht, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands*
[b]*Department of Pathology, Faculty of Veterinary Medicine, University of Utrecht, The Netherlands*
[c]*École Nationale Veterinaire, D'Alfort, France*
[d]*Department of Companion Animals, Faculty of Veterinary Medicine, University of Utrecht, The Netherlands*
[e]*The Netherlands Cancer Institute, Amsterdam, The Netherlands*

(Accepted 11 April 1990)

ABSTRACT

Rutten, V.P.M.G., Misdorp, W., Gauthier, A., Estrada, M., Mialot, J.P., Parodi, A.L., Rutteman, G.R. and Weyer, K., 1990. Immunological aspects of mammary tumors in dogs and cats: a survey including own studies and pertinent literature. *Vet. Immunol. Immunopathol.*, 26: 211–225.

Naturally occurring cancer in companion animals parallels cancer in man more closely than does experimentally induced cancer in inbred laboratory animals. In dogs and cats, as in man, a role for immune responses is indicated in the development of tumors. A survey is presented based on the literature and our own studies concerning the immunological and immunotherapeutic aspects of canine and feline mammary neoplasia. In dogs bearing mammary neoplasms, circulating immune complexes appear to play a negative role in the generation of effective antitumor immune responses. The functional role of peripheral blood lymphocytes and tumor-infiltrating lymphocytes in dogs and cats with mammary tumors is not yet fully established. No tumor antigen responsible for humoral or cellular responses has yet been identified. Extracorporeal perfusion of serum of dogs with mammary tumors and subcutaneous administration of mitomycin- and neuraminidase-treated autologous tumor cells are associated with improved prognosis. The opposite was true for i.v. treatment with BCG or *Corynebacterium parvum* vaccine in our study, in contrast to a previous report. A number of other treatment modalities in cats and dogs with mammary carcinomas failed to induce tumor regression. Canine and feline mammary carcinomas are good candidates for modern immunotherapeutic approaches.

INTRODUCTION

In animals, as in man, immune responses may interfere with the development of tumors, as is indicated by the occurrence of spontaneous regression and the presence of lymphoid infiltrates (MacEwen, 1986). Naturally occur-

ring cancer in domestic animals parallels cancer in man more closely than does experimentally induced cancer in inbred laboratory animals. Additionally, many cancers in dogs and cats, as in man, are systemic – resulting from metastasis – at the time of first presentation (Misdorp, 1987). Studies of tumor-related immunological reactivity in these animals are therefore more likely to yield results relevant to the disease in man (WHO, 1973; Onions and Jarrett, 1987).

Immunotherapy may provide an effective method for cancer treatment, either alone or in conjunction with surgery, chemotherapy or other approaches leading to tumor reduction. Despite its theoretical efficacy and long history of clinical application, immunotherapy in companion animals is still investigational (Theilen and Hills, 1982; MacEwen, 1986; Theilen and Madewell, 1987). The success of immunotherapy of tumors depends on the possibility of inducing, increasing or unblocking immunological reactivity against the tumor. One important feature is the presence on tumor cells of tumor-associated antigens, and possibly structures – as yet unknown – that are capable of eliciting rejection by activation of immunocompetent cells, or can act as targets for monoclonal antibodies, lymphokine-activated killer activity or tumor-infiltrating lymphocytes. Some virus-induced tumors of domestic animals (feline lymphosarcoma, feline fibrosarcoma) have been shown to possess novel antigens, most likely viral components expressed on the cell membrane, and are highly immunogenic because of these (Sharanjit et al., 1983). The antigenicity of non-virus-induced tumors is less clear (Onions and Jarrett, 1987).

In this review a survey is presented of tumor immunological and immunotherapeutic studies (Phases I, II and III), based on the literature and some of our own unpublished studies. Special attention is paid to mammary neoplasms in dogs and cats.

TUMOR-RELATED IMMUNE REACTIVITY

Dogs

Humoral immune responses. The most pronounced feature of immune reactivity in dogs affected by mammary neoplasia as well as a number of other neoplastic diseases is the occurrence of large amounts of circulating immune complexes (CIC) (Hannant et al., 1978; Gordon et al., 1980; Terman et al., 1980; Terman, 1981; Balint et al., 1982; DeBoer et al., 1982; Holohan et al., 1982; Bowles et al., 1984). Similarly, the presence of CIC in humans with breast carcinomas and other tumors has also been reported (Theofilopoulos et al., 1977).

In 1978, Hannant et al. isolated antigen from tumor cell membranes and CIC from sera of dogs with spontaneous mammary carcinoma. Antigen activ-

ity was attributed to proteins of 150 000 and 65 000 daltons. The exact nature of the antigen moiety of the complexes has not yet been identified (Balint et al., 1982; DeBoer et al., 1982). Balint et al. (1982) reported the presence of IgA together with IgG or IgM in CIC in five of eight dogs bearing mammary adenocarcinomas. They postulated the existence of antiglobulin immune complexes, without explaining the predominance of IgA. Holohan et al. (1982) found that up to 50% of the CIC in various animals was tumor-associated. Terman et al. (1980) showed an increase in circulating tumor-associated antibodies shortly after extracorporeal perfusion of serum over a *Staphylococcus aureus* column, caused either by dissociation of CIC and the subsequent release of antibodies from the column, or by rapid production of antibodies. The latter findings support the observation of Holohan (1982) that tumor antigen is incorporated into a considerable proportion of the complexes.

CIC tend to favor the progression of neoplasms by their immunosuppressive and cytotoxicity-blocking effects (Theofilopoulos et al., 1977; Gordon et al., 1980; DeBoer et al., 1982; Holohan et al., 1982). This is also illustrated by the fact that in responders to *S. aureus* perfusion, removal of complexes seemed to be more efficient than in non-responders (Holohan et al., 1982). Persisting elevated concentrations of CIC after mastectomy indicated higher risks of metastasis (Gordon et al., 1980; DeBoer et al., 1982; Holohan et al., 1982; Bowles et al., 1984).

Terman (1981) claimed an important role for antibodies in antitumor responses. Increased deposits of IgG, IgM and complement in necrotic areas of the tumor, and decreased IgG and C1q concentrations were noticed after extracorporeal protein A treatment of serum of animals with several types of tumors. Using a number of variants of this therapy, Bowles et al. (1984) showed that it is not the removal of CIC per se that is important, but the change in CIC content of the serum. Gordon et al. (1983) and Klausner et al. (1985) even postulated that non-protein A bacterial products are responsible for tumor regression.

Cellular immune responses. Holohan et al. (1982) stated that removal of CIC is neither a sufficient nor a necessary condition for induction of tumor regression. They suggested a role for the cellular immune response based on the appearance of an impressive peritumorous cellular infiltrate following protein A therapy. In a morphological study on the biological behavior of canine mammary tumors (Gilbertson et al., 1983) two types of lymphoid cellular infiltrate were recognized. Diffuse lymphoid infiltration of the tumor was found to consist mostly of plasma cells, and was found especially in and around undifferentiated, invasive carcinomas. Perivenous infiltrates were predominantly composed of small lymphocytes, and were more frequent near lesions considered to be precancerous. The survival time of dogs with lymphoid cel-

lular reactions near the mammary tumor was longer than that of dogs lacking such reactions (MacEwen, 1986). Although immunoglobulin deposits were also observed in metastases and in untreated tumors (Holohan et al., 1982), the possibility of an initial role of the antibody response cannot be ruled out. High B-cell activity was noticed in regional lymph nodes after BCG therapy of canine mammary tumors (Parodi et al., 1983). This treatment, in combination with surgery, was not beneficial, and the relevance of B-cell activation has to be questioned. In 1974 Fidler et al. showed in vitro cytotoxicity of lymphocytes against autologous cells of canine mammary neoplasms and a number of other tumors. This cytotoxicity could be blocked by autologous serum, which in some cases stimulated in vitro tumor growth. A similar mechanism possibly contributes to growth of the tumors in vivo. Ulvund (1975) showed in vitro reactivity of lymphocytes against autologous and allogeneic canine mammary carcinoma cells, using the leucocyte migration technique. Using a chromium-release assay, Betton and Gorman (1978) demonstrated cell-mediated antitumor cytotoxicity by peripheral blood lymphocytes from tumor bearers, as well as by lymphocytes from healthy dogs, against autologous or allogeneic tumor cells from various histologic types of tumors. The same authors (Gorman and Betton, 1978) confirmed their finding of T-cell reactivity in a direct leucocyte migration inhibition test. In these experiments they proved that cross-reactivity of lymphocytes with allogeneic tumor cells of the same type does not exist per se. The explanation for the cross-reactivity observed in their first study (Betton and Gorman, 1978) is the possible presence of other types of cells in the effector population. In the observations of Ulvund (1975), the non-specificity of the response measured could be due to low effector:target cell ratios.

In conclusion, CIC play a clear negative role in antitumor responses to mammary neoplasms in dogs. Their partial removal leads to the production (redistribution) of tumor-associated antibodies. These antibodies may be responsible for the initial antitumor activity, which is improved by the activity of T-cells already present but suppressed, as shown by in vitro experiments with lymphocytes from untreated animals.

Cats

Very little is known about immune reactivity in cats with mammary carcinoma. To our knowledge the only relevant study on this subject was reported by one of the present authors (Weyer, 1980).

Cell-mediated cytotoxicity was measured in microcytotoxicity assays. Peripheral white blood cells from cats with mammary carcinoma, taken at least 14 days after mastectomy ($n=9$), and from healthy cats ($n=7$), were incubated for 48 h at 37°C with autologous or allogeneic feline ($n=7$) or canine ($n=3$) mammary carcinoma cells. The number of tumor cells remaining was

compared with the number grown without lymphocyte interference. Lymphocytes from three cats were tested with autologous cells, whereas all other tests were performed with allogeneic cells. Lymphocytes from four of seven healthy cats and from three of nine tumor-bearing cats caused weak cytotoxicity (23–31% in specific ^{51}Cr release assays) against feline mammary carcinoma cells at least once. Cytotoxicity against canine mammary carcinoma cells was seen only with lymphocytes from one of five control cats and one of seven tumor-bearing cats. Thus, specific cell-mediated cytotoxicity could not be demonstrated. The low incidence of cytotoxicity may have been caused by insensitivity of cultured tumor cells, by the absence, or presence in low numbers, of specific lymphocytes, or by functional blocking of these effector cells (effector:target cell ratio in the tests was 400:1). Most likely, however, is that natural killer cells rather than tumor-specific T lymphocytes were responsible for the cytotoxicity. In some cases cocultivation of tumor cells with lymphocytes caused increased growth rates rather than cytotoxic effects.

In a clinicopathological study (Weyer and Hart, 1983) it was found that the prognosis in cats with mammary carcinoma worsened with increasing lymphoid infiltration. The amount of lymphoid infiltrate was statistically associated with tumor necrosis, which is even more characteristic of a poor prognosis.

IMMUNOTHERAPY

Studies on normal animals

Toxicity studies (phase I). Intravenous application of Glaxo BCG resulted in the formation of multiple small granulomas without intact bacilli in lungs and liver of normal (and osteosarcoma-bearing) dogs (Owen and Bostock, 1974). Mild pyrexia was the only sign attributable to toxicity. In view of the possible beneficial effects of local immunotherapy in bladder cancer, the potential reactivity to BCG in that organ was studied. Installation of 7×10^7 particles of Tyze BCG into the urinary bladder was not followed by marked clinical or histological changes, either in dogs sensitized to BCG by repeated intradermal injections or in naive dogs. Injection of the same number of particles into the vesicular wall caused distinct but transient, inflammatory reactions in all sensitized dogs and some of the non-sensitized dogs (Bloomberg et al., 1982). Intravesical installation together with intradermal application of RIVM BCG proved to be a safe procedure in dogs. Small granulomas were found in the suburothelial tissue only (Van der Meijden et al., 1986).

Corynebacterium parvum vaccine did not give rise to signs of general cytotoxicity when administered in various dilutions, whether i.v., i.m. or s.c. Only local inflammatory reactions occurred in dogs receiving undiluted *C. parvum* vaccine (10^9 units), either s.c. or i.m. Histological changes comprising gran-

ulomas in lungs and liver after i.v. injection were similar, but less marked than after i.v. BCG administration (Owen et al., 1980).

Distribution studies. These studies were performed in order to find out whether (1) BCG bacilli and associated granulomas were recognizable in the lungs, the first blood filter in the case of distant metastases, (2) viable BCG bacilli – potential infectious agents – were present in the sputum, and (3) severe necrosis would occur after injection into subcutaneous tissue. Cats were injected i.v. or s.c. with varying amounts of live, irradiated or heat-killed RIVM BCG. The presence of bacilli was investigated in microscopic sections (Ziehl–Neelsen stain) and/or by culture of sputum obtained immediately after euthanasia. In the first study acid-fast bacilli were found in microscopic sections of the lungs of cats 1 and 2 weeks after single i.v. injections of 36×10^4, 36×10^5 or 36×10^6 live or irradiated bacilli. They were present in macrophages or occurred freely in lung tissue. In the three cats injected with two of the doses (with a booster at 4 weeks), no bacilli were detectable in the lungs after 13 weeks. Only in the lungs of cats receiving the higher doses were a few granulomas detected. In sputum cultures of two of five cats, findings were in accordance with the histological results. None of the cats had shown signs of toxicity.

In a second experiment eight cats were treated with high doses of BCG (108×10^6 or 36×10^7), either i.v. ($n = 4$) or s.c. ($n = 4$). Bacilli were present in sections of the injection site 4 weeks after a single s.c. injection of the lower dose in one cat and in sections of the lungs of a cat that received the higher dose. In one cat, injected twice (36×10^7, booster at 4 weeks), acid-fast bacilli were found both in subcutis and lungs. Of the four cats treated intravenously, only one (injected twice at the higher dose) showed bacilli in its lungs. The s.c. injections were associated with extensive and necrotizing inflammation, and the i.v. injections with a few scattered pulmonary granulomas.

In a third experiment, four cats received one or two i.v. or s.c. injections of a high dose (108×10^6 or 36×10^7) of heat-killed BCG; acid-fast bacilli were subsequently found in the lungs of three of the cats. In the two animals injected s.c. they were also found in subcutaneous granulomas. The presence of bacilli, and the granulomatous reaction elicited by them in subcutis and lungs, could have therapeutic significance in tumor-bearing animals.

In an other experiment, using dogs, the highest levels of ^{125}I-*C. parvum* vaccine 1 h after i.v. injection were found in the liver (48%), the lungs (19%) and the spleen (4.5%). Radioactivity declined at a higher rate in the lungs than in liver and spleen (Owen et al., 1980).

Activity studies. Alveolar macrophages from three of four normal dogs injected intravenously with *C. parvum* vaccine exhibited increased in vitro cytotoxicity against a number of target cells: canine melanoma, osteosarcoma

and mammary carcinoma (Betton et al., 1979; Owen et al., 1980). Natural killer cell activity increased after i.v. BCG (0.1 mg/kg) administration (Betton et al., 1979). Using lymphocytes from healthy BCG-treated dogs in ^{51}Cr release assays (Betton and Gorman, 1978) or in the leucocyte migration inhibition technique (Gorman and Betton, 1978), non-specific reactivity resulting from treatment was demonstrated.

Studies on tumor-bearing animals

Intratumorous injection of BCG and C. parvum *vaccine before surgery.* A combined study in Paris/Alfort and Amsterdam was performed to investigate this treatment. In Paris/Alfort, dogs with mammary cancer ($n=25$) were treated with a single combined intralesional injection of Pasteur BCG (10^7 live bacteria) and *C. parvum* vaccine (10^9 killed bacteria) 2 weeks before surgery. The survival of these dogs did not differ significantly from that of dogs ($n=23$) treated by surgery and placebo. Similarly, in our parallel study in Amsterdam, survival of dogs ($n=30$) treated with a single intralesional injection of *C. parvum* vaccine (10^9 killed bacteria), followed by surgery was not different from that of dogs ($n=37$) treated by surgery only (Parodi et al., 1983).

Incomplete surgery and C. parvum *vaccine.* The effect of a single injection of *C. parvum* vaccine (10^9 killed bacteria) at the operation site of histologically confirmed, incompletely removed tumors was studied in a prospective randomized trial. Three animal tumor diseases were incorporated in the study: canine haemangiopericytoma, canine mastocytoma and feline mammary carcinoma. In none of the tumor diseases studied did the number of recurrences differ between the vaccine-injected and non-injected controls (Table 1). Neither were there differences between treated and control groups of animals in recurrence-free interval (Misdorp, 1987).

Intramammary injection of BCG cell-wall vaccine. Single intramammary injections (4 mg/ml) of BCG cell-wall vaccine induced high titers of interferon

TABLE 1

Local recurrence rate after incomplete surgery

	Local Cpv[a]	Control[b]
Canine haemangiopericytoma	9/22	8/20
Canine mastocytoma	7/26	7/17
Feline mammary Carcinoma	14/17	5/8

[a]Animals treated with *Corynebacterium parvum* vaccine after surgery.
[b]Surgery only.

in the circulation of eight of ten healthy adult beagle bitches. Titers were maximal 3 weeks after injection. A single injection in histologically confirmed canine mammary cancers caused high titers (maximal after 4 weeks) of interferon in seven of ten dogs. Effects of the therapy on tumor size or survival time were not reported (Winters and Harris, 1982).

Intravenous injection of BCG or C. parvum *vaccine after surgery.* Dogs with mammary cancer in a Cambridge trial received repeated (eight) i.v. injections of Glaxo BCG (0.1 mg/kg, live bacteria) after mastectomy. The survival time increased significantly, being 100 weeks compared with 24 weeks in control animals. According to the authors, the longer survival was due to elimination, or at least postponement, of distant metastasis (Bostock and Gorman, 1978). The WHO Comparative Oncology Group proposed to repeat this study on a larger scale, and to add *C. parvum* vaccine as a third treatment. The double-blind prospective study involved 130 bitches with histologically confirmed mammary cancer (95 in Paris/Alfort and 35 in Amsterdam and Utrecht). Tumor material was removed by chain resection from dogs without detectable distant metastasis. After tumor removal, dogs with a single malignant tumor were entered in the study.

In the first treatment group, dogs received seven i.v. injections of live Pasteur BCG at 2-week intervals, starting 7–11 days after mastectomy. The initial dose of 1 mg/kg had to be lowered to 0.5 mg/kg in Paris/Alfort because of signs of toxicity, which included anaphylactic shock in five dogs, in spite of the use of the antihistaminic drug Piriton. In the Amsterdam series, BCG Pasteur administration had to be stopped in two of nine dogs because of severe toxicity (high fever, apathia, anorexia or aggression).

In the group treated with *C. parvum* vaccine (0.25 mg/kg; Burroughs Wellcome) the treatment scheme was similar to that for BCG. Side effects were less serious than in the BCG-treated dogs. One dog in Paris/Alfort developed

TABLE 2

Survival of dogs after mastectomy followed by i.v. immunotherapy with BCG or *Corynebacterium parvum* vaccine (Cpv)

	Survival rate[a]			Survival time (weeks)
	1 year	2 years	3 years	
BCG, Paris/Alfort	55%	40%	30%	50
Amsterdam	43%	30%	10%	75
Cpv, Paris/Alfort	65%	50%	50%	65
Amsterdam	75%	50%	17%	93
Control, Paris/Alfort	80%	65%	40%	67
Amsterdam	65%	35%	20%	78

[a]Percentage of treated animals alive after 1 year etc.

a transient malaise, and in two bitches in the Amsterdam series mild local inflammation was noticed at the injection site. The control group in Paris/ Alfort received seven i.v. injections of isotonic physiological saline after i.m. administration of Piriton, in addition to mastectomy. The control group in Amsterdam was treated surgically only. The results of the experimental treatment in terms of 1-, 2- and 3-year survival and average survival time were not significantly different from those in the control groups (Table 2). Clinical and post-mortem studies (Amsterdam) showed that death was attributable to the tumor disease in more than 50% of the dogs that died or were euthanized (BCG, 4/7; *C. parvum*, 6/12; control, 10/14).

Levamisole treatment per os. Levamisole is believed to influence immune responsiveness in a positive way. Continuous administration of levamisole per os (5 mg/kg, 3 days a week) as an adjuvant to surgery was found to be ineffective in altering the recurrence rate of feline malignant mammary tumors, and did not increase the survival time of treated cats compared with that of control groups (MacEwen et al., 1984). The same treatment regimen did not improve survival time, the cancer-free interval or recurrence rate in dogs after radical or simple mastectomy for mammary cancer (MacEwen et al., 1985).

Subcutaneous injection of mitomycin and neuraminidase-treated autologous tumor cells. One tumor was excised from dogs with multiple mammary tumors, and a single cell suspension was made (Sedlacek et al., 1977). Tumor cells were treated with mitomycin and *Vibrio cholerae* neuraminidase (VCN). Cells were injected s.c. on the day of surgery and the day thereafter. Regression of the residual tumor was apparently dose-related. In 13 of 15 dogs receiving 2×10^7 cells, regression to less than 10% of the original size was observed, followed by progressive tumor growth in three of them. Treatment with 10^8 cells resulted in progression, including metastasis. Injection of lower doses of cells (10^6) caused regression in most cases, followed by rapid recurrence. In a control group of dogs receiving 2×10^7 cells treated with mitomycin only, most of the residual tumors progressed (8/12). The residual tumor was in most cases of a type similar to that of the extirpated tumor. Another group of dogs was treated with 16–20 intradermal injections of different combinations of mitomycin-treated autologous tumor cells (from 0.4×10^8 to 44×10^8 cells) and VCN (0.65–65 international milliunits), according to a chessboard vaccination scheme. Complete tumor regression was observed in eight of ten animals (Sedlacek et al., 1979), and recurrence occurred in two cases. Survival rate and time increased after chessboard vaccination (Sedlacek et al., 1979, 1986).

Absorption of sera of tumor patients to Staphylococcus aureus *(protein A).* Extracorporeal perfusion of serum from a human colon carcinoma patient over *S. aureus* resulted in considerable tumoricidal effect (Bansal et al., 1978).

After this initial observation, absorption of sera to *S. aureus* or protein A became a widespread treatment modality in small animal experimental tumor therapy (Jones et al., 1980; Terman et al., 1980; Terman, 1981; Holohan et al., 1982; Gordon et al., 1983; Bowles et al., 1984; Klausner et al., 1985).

In general, extracorporeal perfusion of serum in cases of canine mammary tumor leads to tumor reduction, but only rarely to 100% cure. Terman et al. (1980) and Terman (1981) observed tumor necrosis in each of twelve breast adenocarcinomas after therapy. In eight of the dogs, healing of large ulcerating areas occurred. Similar regression was observed in dogs with other tumor types. Holohan et al. (1982) reported 0–50% reduction of tumor size in five of ten treated dogs, and found that removal of CIC seemed to be more efficient in responder animals. Results obtained by Klausner et al. (1985) after perfusion of sera over protein A were less favorable; in only one of eleven tumor-bearing dogs was tumor reduction observed after therapy.

Although tumor reduction was initially attributed to removal of immuno-suppressive and cytotoxicity-blocking CIC, some authors have speculated about the possibility that the release of non-protein A bacterial products contributes to tumor reduction (Theofilopoulos et al., 1977; Jones et al., 1980; Terman et al., 1980; Terman, 1981; DeBoer and Madewell, 1982; Holohan et al., 1982; Gordon et al., 1983; Bowles et al., 1984; Klausner et al., 1985). The mechanism of induction of tumor reduction is still not clear.

DISCUSSION

One of the indications for the involvement of immune responses in tumor diseases is spontaneous regression of tumors (MacEwen, 1986). Complete regression is extremely uncommon in canine and feline mammary cancer (Parodi et al., 1983). Progression, on the other hand, characterized by rapid occurrence of metastases, is frequently observed (Misdorp and Hart, 1976; Weyer and Hart, 1983). In addition one case of spontaneous development of mammary adenocarcinoma following prolonged immunosuppression has been reported (Joseph et al., 1970). Histological studies have indicated that the prognosis for dogs is better when lymphoid infiltrates are present in the vicinity of the tumor (MacEwen, 1986). Although the greater part of the infiltrate in canine invasive carcinomas consists of plasma cells, the potential role of T lymphocytes, which are predominant in precancerous lesions, may be more important (Gilbertson et al., 1983). In histological studies during successful protein A therapy, large amounts of peritumorous cellular infiltrates have been seen (Holohan et al., 1982). Plasma cells and the antibodies produced may play an initiating role in immune responses leading to regression (Terman et al., 1980). Parodi et al. (1983) showed that high B-cell activity induced by BCG therapy was not associated with tumor regression. In addition, antibody deposits have been found in metastases and untreated tumors (Holohan et

al., 1982). The formation of CIC, which seem to contribute to unfavorable conditions in many tumor diseases in man and animals, is also associated with poor prognosis in canine and feline mammary carcinoma. Although it has been reported that at least some of the antibodies are directed against tumor-associated antigens (Holohan et al., 1982), CIC seem to be suppressive in antitumor immune responses (Theofilopoulos et al., 1977; Gordon et al., 1980; DeBoer and Madewell, 1982; Holohan et al., 1982). This is supported by the fact that protein A therapy, which changes only the CIC level, can lead to regression of the tumor (Holohan et al., 1982, Bowles et al., 1984). While peritumorous lymphoid infiltrate has a favorable prognostic significance in dogs (MacEwen, 1986) it is difficult to explain why the reverse is true in the cat (Weyer and Hart, 1983). More knowledge needs to be gathered about the functional capacity of the lymphoid infiltrate (MacEwen, 1986).

Betton and Gorman (1978), as well as Fidler et al. (1974) demonstrated the cytotoxicity of lymphocytes in in vitro functional studies with lymphocytes of dogs with mammary carcinoma. Moreover, in leucocyte migration inhibition tests Gorman and Betton (1978), in contrast to Ulvund (1975) demonstrated specific reactivity to autologous tumor cells. Lymphocytes of mammary carcinoma-bearing cats could not be shown to be specifically reactive to tumor cells. In fact, the reactivity registered may be only NK activity (Weyer, 1980). Although in vitro studies have given an indication of the molecular weight of tumor antigen in mammary carcinoma (Hannant et al., 1978), the true nature of the antigen in feline and canine mammary carcinoma has still to be determined (Balint et al., 1982; DeBoer et al., 1982).

Of the immunotherapy protocols described so far, only two have had any success. Extracorporeal absorption of serum to protein A resulted in tumoricidal activity in various tumor diseases, including canine mammary carcinoma and feline lymphosarcoma (Jones et al., 1980). The mechanism of antitumor reactivity may include the following (partial) removal of CIC, redistribution of tumor-associated antibodies, activation of complement and activation of cytotoxic T lymphocytes (Jones et al., 1980; Terman et al., 1980; Terman, 1981; Holohan et al., 1982; Gordon et al., 1983; Bowles et al., 1984; Klausner et al., 1985). The other successful biological treatment of canine mammary carcinoma is immunization by means of s.c. injection of mitomycin and VCN-treated autologous tumor cells (Sedlacek et al., 1977) or a combination of mitomycin-treated autologous tumor cells and VCN (Sedlacek et al., 1979, 1986). The success of this treatment, which is clearly dose-dependent, is assumed to be based on the unmasking of tumor-associated antigens. It seems worthwhile to perform these experiments in dogs and cats with other locally advanced primary and recurrent tumor diseases. Distribution, toxicity and activity studies of BCG and *C. parvum* vaccine in normal cats and dogs yielded administration schedules, routes and doses allowing safe administration of vaccines (Owen and Bostock, 1974; Owen et al., 1980; Weyer, 1980;

Bloomberg et al., 1982; Van der Meijden et al., 1986). Treatment of canine and feline mammary tumors using immunomodulators like BCG, *C. parvum* vaccine and levamisole, administered either intratumorously or s.c., eventually in combination with surgery, was not successful in our own and some other studies (Winters and Harris, 1982; Parodi et al., 1983; MacEwen et al., 1984, 1985). The results of our study in Paris/Alfort and Amsterdam (Gauthier, 1985), involving the intravenous injection of BCG or *C. parvum* vaccine after surgery, did not confirm those of the promising Cambridge study (Bostock and Gorman, 1978). Differences in the experimental set-up regarding the types and doses of BCG used, may have been the cause of our disappointing results. In some dogs with mammary cancer examined post mortem (Amsterdam), it was found that Pasteur BCG-induced granulomas were not necessarily located at the site of micrometastases. This is an important observation if BCG–tumor cell contact is indeed a prerequisite for successful treatment of malignant tumors (Winters and Harris, 1982). In case of early metastasizing carcinomas, as most feline and many canine mammary cancers are (Misdorp and Hart, 1976; Weyer and Hart, 1983) lung metastases have to be eradicated as early as possible. In this context, mammary cancers in dogs and in cats, as well as other spontaneous tumors in small and large animals (Klein et al., 1986a, 1986b), are good models for new therapeutic tools such as autochthonous tumor cell vaccines (Peters et al., 1979; Jeglum et al., 1986), monoclonal antibodies, lymphokine-activated killer cells, tumor-infiltrating lymphocytes, interleukin-2 alone or in combination with one of the former, or other tumor-reducing modalities (Rosenberg, 1988). In bovine ocular squamous cell carcinoma, low-dose local interleukin-2 therapy has yielded promising results (Rutten et al., 1989). Immunotherapy and studies of natural and induced immunological phenomena in such tumor model systems deserve continued attention.

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