ORIGINAL CONTRIBUTION

# Eosinophils in Cancer:
## *Mechanisms and machinery for cytotoxicity*

*Darren James Costain, BSc(Hon), MSc '98*

*Department of Pathology, Dalhousie University, Halifax, Nova Scotia*

Cancer is one of the leading causes of death in North America. For this reason, research into novel therapies to combat tumour growth is an area of intense investigation. Traditional treatment modalities for cancer patients, such as radiation and chemotherapy, have enjoyed only moderate success, partially because these treatments non-specifically target dividing cells and consequently are highly toxic to the patient, and also because some cancers are refractory to such measures. Recently, efforts have been focused towards enhancing the patient's immune response to the tumour. These "immunotherapy" strategies direct the specific recognition of neoplastic tissues, which confers protection from remaining or recurring tumour cells. Most cancer immunotherapy protocols presently under study are aimed towards enhancing type 1 T helper (Th1) immunity. Eosinophilia, traditionally associated with type 2 (Th2) immune responses, has been described in certain tumours and during cancer immunotherapy. Interestingly, correlations have been drawn between good prognosis for recovery and localized eosinophilia in the area of primary tumour. To date, these findings are controversial, as no *in vivo* evidence has demonstrated a direct role for eosinophils in mediating tumour damage. This review will first describe various proinflammatory and cytotoxic molecules produced by eosinophils, and suggest possible mechanisms of inducing anti-cancer immunity. Secondly, evidence suggesting the capacity of eosinophils to kill tumour cells will be provided. Although molecules involved in recruiting and activating eosinophils at the site of tumour growth are largely unknown, candidate molecules will be discussed. Furthermore, recent findings in our laboratory will be described which support the concept that eosinophil-activating cancer immunotherapy merits further investigation.

## INTRODUCTION

The eosinophil acquired its name in 1879 because of its affinity for the acidic dye eosin (1). Soon after their initial characterization, eosinophils were recognized in association with cutaneous disorders, parasite infection, asthma, allergy, and some cancers (2-4). Even to date, the majority of the literature describing the *in vivo* role of eosinophils pertains to parasitic infection or allergic asthma (reviewed in 5 & 6). Eosinophils are non-dividing granulocytes derived from myeloid precursors in the bone marrow. Eosinophils predominantly reside in tissues, particularly near mucosal areas, and typically make up less than 4% of the circulating leukocyte population. Eosinophils are normally only abundant in tissues during parasite infection or inflammatory disorders. For example, eosinophils are the major effector cells in mediating tissue damage in pathological conditions such as inflammatory bowel disease (reviewed in 7), cutaneous disorders, and late phase lung epithelial damage in asthma (reviewed in 6). Because parasite infection is a minor concern in most of North America, most recent eosinophil studies have focused on inhibiting eosinophil activity. Furthermore, Th1 activity normally downregulates type 2 immune responses, including eosinophilia. In that most anti-cancer immunotherapy is directed towards Th1 induction, a competing arm of anticancer immunity may be negated under Th1-promoting therapies.

In the past two decades, a greater ap-

*Address correspondence to:*

Darren J. Costain, Cancer Immunotherapy Research Initiative, Room 10A-D, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia, B3H 4H7

preciation for the pluripotent function of eosinophils has been attained. Although different environments may produce distinct functional activity in the eosinophil, it is neccesary to introduce some basic eosinophil-associated molecules before a case can be made for the eosinophil's role in anti-cancer immunity.

## EOSINOPHIL INFLAMMATORY WEAPONRY- *ARE THEY "DRESSED TO KILL"?*

Eosinophils produce various inflammatory mediators and cytotoxic molecules which are capable of indirectly (by recruiting/activating other cell populations) or directly inducing cell damage. Although some are more well characterized than others, possible roles in tumour cell damage will be discussed.

### Leukotrienes

During activation of several types of leukocytes, including eosinophils, arachadonic acid metabolism can occur via the cyclooxygenase or the lipoxygenase pathways. The latter pathway can yield four different leukotrienes (LT; known as $LTB_4$, $LTC_4$, $LTD_4$, and $LTE_4$) which induce smooth muscle contraction/bronchoconstriction, mucus production, and increased vascular permeability, some of the manifestations of the acute asthmatic response. Increased production of $LTC_4$, the predominant LT produced by eosinophils (8-10), is a measure of increased eosinophil activity (11-12). Also, IgE- or IgG-induced degranulation by eosinophils enhances their production of $LTC_4$ (13). Consistent with this observation is that increased levels of $LTC_4$ are produced by eosinophils from asthmatic patients (14-16) and *in vitro* activated human eosinophils (9, 17), implicating a role for activated eosinophils in asthma.

LTs have also been shown to be stimulatory for other cells. For example, $LTB_4$ has been shown to enhance hydrogen peroxide, interleukin (IL)-1, and tumour necrosis factor-$\alpha$ (TNF-$\alpha$) production by macrophages (18). In addition, inhibitors of the 5' lipoxygenase pathway reduce nitric oxide production, TNF-$\alpha$ secretion, and tumour cytotoxic activity of thioglycollate-elicited mouse peritoneal macrophages (19), demonstrating a requirement for LT for these effector functions. $LTB_4$ has also been described for its chemotactic activity for human monocytes and guinea pig eosinophils (20-21), cytotoxicity enhancing activity for human neutrophils against complement opsonized schistosomula of *Schistosoma mansoni* (22), and *in vitro* guinea pig eosinophil antibody-dependent cell-mediated cytotoxicity (ADCC)(23). Furthermore, inhibitors of the lipoxygenase pathway were shown to reduce rat natural killer cell (NK) activity in a $^{51}Cr$-release cytotoxicity assay of tumouricidal function (24). The addition of either $LTB_4$ or $LTC_4$ was able to reverse the inhibitory effect of lipoxygenase pathway blockade in this study, demonstrating the specificity of tumouricidal activation to these LTs. Consistent with this observation, human NK cytolytic activity against the NK-sensitive human tumour cell line K562 was significantly enhanced by addition of exogenous $LTB_4$ (25), and lipoxygenase pathway inhibitors could reduce human NK and Lymphokine Activated Killer (LAK) cytotoxicity towards K562 cells in a manner reversible by addition of exogenous $LTB_4$ (26). It is conceivable, therefore, that eosinophils accumulating around areas of tumour growth may not only enhance localized inflammation, but also increase anti-tumour activity of leukocyte populations infiltrating into the tumour mass through the release of LTs.

### Major Basic Protein

The eosinophil major basic protein (MBP) is the major component of eosinophil granules, accounting for more than half of the granular protein (27) and approximately 25% of the total cellular protein (28). This suggests an important biological role for MBP in eosinophil function. Although enzymatic functions of MBP have not been described, MBP is highly cationic and basic in nature, and is thought to induce cellular damage through membrane disruption and cell lysis by interacting with anionic lipid membranes (29).

MBP has been shown to be cytotoxic to *S. mansoni* (30), *Trichinella spiralis* newborn larvae (31), splenocytes, monocytes, epidermal and tracheal epithelial cells (32), murine ascites tumour cells (30), and *Staphylococcus aureus* and *Escherichia coli* (33). In support of *in vivo* MBP toxicity, correlations have been noted between deposited MBP and tissue damage in lymph nodes of Hodgkin's disease patients (34), patients with bronchial asthma (35, reviewed in 4), and patients undergoing episodic kidney or liver allograft rejection (36-37).

In addition to the cytotoxic properties of MBP, the protein has also been shown to induce human eosinophil degranulation, $LTC_4$ and IL-8 production (38), basophil and mast cell histamine release (39-40), neutrophil surface protein expression (41) and degranulation (42), release of platelet inflammatory mediators (43), and alternative complement pathway activation (44). Tumour-associated release of MBP by eosinophils could thereby not only directly, but also indirectly enhance localized recognition and destruction of tumour cells through recruitment and activation of other inflammatory cells.

### Eosinophil cationic protein

Like MBP, eosinophil cationic protein (ECP) is highly cationic in nature. ECP forms transmembrane pores structurally similar to both perforin and C9 (45), which polymerize in cell membrane to induce cytolysis. ECP has been described for its ability to exert cytolytic activity on red blood cells, chicken embryo myotubules, and P388, CTLL-A11, and J774 cell lines (45). In addition, ECP demonstrates ribonuclease (RNase) activity (46). Ribonuclease activity is apparently not required for cytotoxic activity, as shown using *S. aureus* targets (47). ECP is at least as potent as MBP on a molar basis for schistosomula cytotoxicity (48-49), and has also been shown to be toxic for *T. spiralis* (50) and tracheal epithelial cells (51). High levels of ECP detected in sputum (52-54), lung (55-56), and blood (55,57) in asthmatic patients have implicated the involvement of ECP in *in vivo* airway damage. In addition, ECP deposition correlates with the rejection process of transplanted livers in humans (58). These studies suggest that ECP production by eosinophils at the site of tumour

growth could contribute to tumour cell destruction. However, this possibility has yet to be addressed in the literature.

**Eosinophil-derived neurotoxin**

Eosinophil-derived neurotoxin (EDN) demonstrates approximately 50-100 times more potent RNase activity than ECP (46, 59). EDN is only weakly cytotoxic for parasites and mammalian cells (60, 50-51), but is noted for its neurotoxicity when injected into CNS of experimental animals (referred to as the Gordon phenomenon). Sorrentino (61) has shown that although RNase activity of EDN is required, it is not sufficient for induction of the Gordon phenomenon, suggesting another undefined biological activity of the enzyme. It is of interest to note that onconase, a RNase which belongs to the same RNase A superfamily as EDN, is also capable of causing the Gordon phenomenon (62). Onconase, obtained from oocytes and early embryos of the frog *Rana pipiens* (71), is noted for its anti-tumour properties both *in vitro* (63) and *in vivo* (64-65), and is currently undergoing phase III clinical trials for cancer treatment (66). Preliminary studies in which chimeric molecules of EDN and onconase were produced using recombinant technology revealed that a chimera with enzymatic activity and antigenic identity more characteristic of EDN was more cytotoxic to tumour cell lines than recombinant onconase (67). Furthermore, onconase RNase activity correlates with the protein's ability to induce the Gordon phenomenon, and EDN is orders of magnitude more enzymatically active than onconase (62). Wu and colleagues (68) have suggested that RNase activity is required for onconase tumouricidal activity (68). In this report, alkylated onconase with dramatically reduced RNase activity was more than 100 times less efficient in preventing protein translation in glioma cells. Onconase-induced cytotoxicity has been proposed to involve disruption of 28S and 18S ribosomal enzymes (68) or tRNA degradation (69). It is tempting to speculate that EDN may have anti-tumour activity similar to onconase. Although no study has yet addressed this possibility, EDN may require additional factors to acquire this cytotoxic property, as has been demonstrated with other members of the RNase A superfamily (70). Presently, a direct role for EDN-mediated tumour cell cytotoxicity has not been presented. Given its similarity to onconase, however, it is suggested that EDN merits further attention.

**Eosinophil peroxidase**

Eosinophil peroxidase (EPO) is another highly cationic enzyme present within eosinophil granules. In addition to charge, EPO has a high mannose content (71). These biochemical properties of EPO have led to two hypotheses regarding EPO binding to target cells. The first model proposes the attraction of EPO towards anionic phospholipid in cell membrane (72). Alternatively, mannose receptor ligation may facilitate EPO deposition onto target cells (73). Although EPO alone is cytotoxic to various tumour cell lines, including human K562 and HL-60 cell lines, and murine P815 and FO tumours (74), its combination with $H_2O_2$ and halide dramatically enhances EPO toxicity (75-76). EPO can kill schistosomula (77), bacteria (78-79), respiratory endothelium (51), and mammalian tumour cells (74, 80). EPO also enhances eosinophil degranulation (38) and macrophage production of $H_2O_2$ and the tumouricidal cytokine TNF-$\alpha$ (81). By enhancing macrophage activity and directly mediating cytotoxicity, EPO released in areas of tumour mass could restrict tumour progression. Whether this process occurs *in vivo* remains to be determined.

**CD30 ligand**

CD30 ligand (CD30L), a member of the TNF superfamily (reviewed in 82), has recently been shown to be expressed on human eosinophils (83). The role of CD30L-signalling in tumour pathogenesis has enjoyed considerable attention of late. However, no clear model of CD30 signalling exists which would classify CD30L as beneficial or detrimental to tumour growth. For example, depending on the $CD30^+$ target, CD30-CD30L interactions can either enhance (83-84) or inhibit (84-85) tumour cell proliferation and viability. Of particular interest, however, is that the Hodgkin's disease cell line HDLM-2 demonstrates a different pattern of protein tyrosine phosphorylation than other lymphoma cell lines following CD30L signalling (86). Similarly, the Hodgkin's cell line H-RS appears to receive CD30 signals as an activation signal for cytokine production (87) and proliferation (83). This renders much of the expanding literature on CD30L-mediated signalling in tumours difficult to interpret, since the most recent work has been performed in the above cell lines. Although it would be interesting to further investigate the role of CD30L in eosinophil-mediated damage of different tumours, CD30 signalling remains too poorly characterized at present for a generalized model of its involvement in neoplastic pathology.

**Other inflammatory weaponry**

Eosinophils have been described for their ability to act as antigen presenting cells (APC) for T cells (88-89), suggesting that eosinophils localized to the area of tumour growth might assist in T cell activation towards tumour targets. Eosinophils also express various proinflammatory molecules, including (but not restricted to) IL-1 (88, 90), IL-2 (91), IL-3 (92), IL-4 (93), IL-5 (94-95), IL-6 (96), IL-8 (97), IL-10 (98), interferon-$\gamma$ (IFN-$\gamma$) (98), TNF-$\alpha$ (99), macrophage inflammatory protein 1-$\alpha$ (MIP-1$\alpha$) (99), granulocyte-macrophage colony stimulating factor (GM-CSF)(94), regulated upon activation normal T cell expressed and secreted protein (RANTES)(100) and inducible nitric oxide synthase (iNOS)(101). These inflammatory molecules are well characterized for their immunomodulatory and inflammatory enhancing activity, thus demonstrating the capacity of eosinophils to dramatically influence a developing immune response. More recently, human eosinophils have been shown to express CD95L (FasL)(102-103), while mouse eosinophils have been shown in our laboratory to express mRNA transcripts for FasL, granzyme B, and perforin by RT-PCR (manuscript in preparation). FasL, granzyme B, and perforin are classical components of cytotoxic T lymphocyte (CTL) and NK cell cytolytic machinery involved in the destruction of tumour and virally-infected cells. These recent observations

suggest that eosinophils may be capable of inducing apoptosis in tumour cells in a CTL- or NK-like manner.

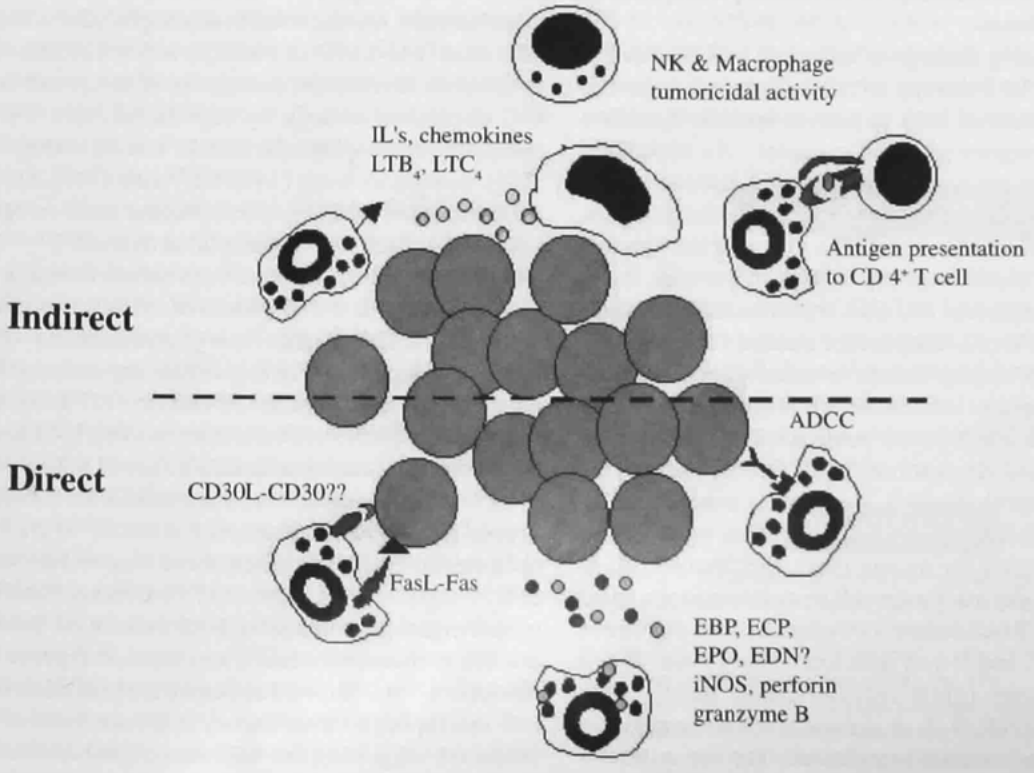
Although the potential for these diverse molecules to operate in an inhibitory fashion to regulate tumour progression has been implicated (Figure 1), few studies have directly investigated the involvement of these products released by eosinophils in reducing tumour burden. Several reports do suggest, however, that eosinophils may be operative in mediating tumour cell damage or restricting neoplastic growth. Some of these studies are described below.

## EOSINOPHILS IN CANCER

Many clinical reports describe localized eosinophilia in association with certain types of tumours. However, the role of eosinophils in tumour pathology remains obscure due to conflicting reports. Some studies have indicated good prognosis for recovery in tumours associated with eosinophilia, including gastric, colonic, cervical, and lung cancers (104-109) and pleural malignancies (110). Furthermore, EPO deposition has been described in association with certain lymphoid malignancies, and has been suggested as a "tumour-associated enzyme" in need of exploitation (111). The prognostic value of tumour-associated eosinophilia was later contested, however, by two studies demonstrating that favourable prognosis with localized eosinophilia failed to reach significance when tumour specimens were separated based on stage (112-113). It is interesting to note, however, that eosinophil number was strongly associated with lower Duke's stage in the former study. This latter observation may in itself suggest that eosinophils negatively influence development of tumour into more a aggressive neoplasm, although more evidence is required to support this hypothesis. One possible explanation of these conflicting reports, suggested by Lowe and colleagues (114), is that tumours with tissue eosinophilia only are indicative of active anti-tumour inflammatory reaction associated with favourable prognosis for survival, while concomitant tumour-associated blood eosinophilia indicates metastatic spreading of the cancer with a decreased likelihood of survival.

While the role of the eosinophil remains unclear in cancer patients, recent findings suggest that cytokine manipulation of the immune system may enhance both eosinophil activity and tumour regression. Work initiated by Rosenberg and colleagues identified a mechanism whereby tumour-reactive T cells could be expanded *in vitro* by the exogenous addition of IL-2 (115-116). Adoptive immunotherapy strategies in which these LAK cells were injected alone or in combination with systemic IL-2 treatment severely impaired tumour growth and metastasis *in vivo* (reviewed in 117). Based on these studies, various clinical trials using IL-2 therapy were initiated. Several of these studies reported both eosinophilia

***Figure 1:*** *Direct and indirect mechanisms of eosinophil-mediated reduction in tumor growth*



and enhanced eosinophil activation. Although eosinophils bear the IL-2 receptor (118-119), IL-2-induced eosinophilia appears to be mediated by endogenous production of the eosinophil reactive cytokine IL-5 (120-124).

Of particular interest was the observation that eosinophils isolated from IL-2-treated human cancer patients demonstrated enhanced cytotoxic activity against different tumour targets both in the presence and absence of tumour-specific antibody (125). This indicated that eosinophils might be operative in slowing tumour progression *in vivo* during IL-2 therapy. Furthermore, eosinophils isolated following IL-2 therapy showed characteristics of hypodense (HD) eosinophils (120, 122, 125-126). HD eosinophils are activated eosinophils well characterized for their enhanced cytotoxic potential. This suggests that normodense-hypodense transition may be instrumental in eosinophil acquisition of tumouricidal activity.

In an effort to further characterize which effector cells are required for IL-2 enhanced destruction of tumours, IL-2-transfected human tumour cells were injected into T cell deficient nude mice and monitored for growth and cellular infiltrate (127). This study demonstrated that T cells were not required for the IL-2-induced anti-tumour response, and that substantial macrophage infiltration, followed by neutrophils, mast cells, and eosinophils, correlated with destruction of the tumour. Cooperation between eosinophils and macrophages leading to enhanced cytotoxic activity is well documented (81, 128-130). Combined with the observation that mouse macrophages (131) and eosinophils (132-133) alone do little in terms of inhibition of tumour growth in some cytokine-transfected tumour cell lines, it is possible that successful eradication of tumour cells depends on active participation of both cell populations.

The promising findings of enhanced anti-tumour cytotoxicity during IL-2 therapy stimulated interest in genetically modifying tumour cells to secrete specific cytokines localized to the tumour microenvironment. An expanding literature describes the varying capacity of cytokines to produce a tumour-specific inflammatory response, including localized eosinophilia (132, 134-135). Of particular interest was a study by Leder's group in which various IL-4-transfected mouse tumour cell lines were reported to be rapidly rejected in a T cell-independent manner (136). Histological analysis of tumour lesions revealed substantial eosinophil and macrophage infiltration, while lymphocytes were notably absent. In a subsequent publication, the same group reported that neutralizing antibody to IL-5 could partially restore the tumourigenicity of IL-4-secreting tumours (137). Consistent with this finding was a significant reduction in eosinophils infiltrating the tumour mass. Furthermore, IL-4-transfected J558L plasmacytoma or B16 melanoma cells failed to grow in *nu/nu* (T cell deficient), *bg/bg* (NK cell deficient), *bg/nu/xid* (NK, T, and B cell deficient), *scid* (T and B cell deficient), or $w/w^v$ (mast cell deficient) mice. This demonstratred that NK, B, T, or mast cells are not involved in the IL-4-mediated tumour regression. On the contrary, monoclonal anti-granulocyte antibody RB6-8C5, which obliterates eosinophils and neutrophils from mice, restored growth of IL-4-transfected tumours, implicating either granulocyte in the rejection process. Histological analysis failed to reveal a substantial neutrophil accumulation, while eosinophils were the predominant inflammatory recruit. Furthermore, although macrophages were also found to infiltrate the tumour mass, this occurred later than eosinophil accumulation, and tumour destruction correlated with times of aggressive eosinophil influx. Inasmuch as macrophage accumulation persisted during treatment with RB6-8C5, it appeared as if macrophages alone were not sufficient for tumour rejection. Although a cooperative role for macrophages and eosinophils in tumour cytotoxicity cannot be ruled out by this study, overwhelming circumstantial evidence points towards the eosinophil as being the principle effector cell in the observed anti-tumour response.

Because IL-4 has pleiotropic effects on the immune system, the above evidence demonstrating eosinophil recruitment to site of tumour and subsequent destruction of the tumour failed to describe whether IL-4 acts directly or indirectly to induce eosinophil cytotoxic activity. It has been reported that IL-4 fails to enhance sIgA-induced eosinophil degranulation (138), while 16h incubation with human rIL-4 reduced IgG-induced degranulation by up to 65%, and suppressed antibody dependent cell-mediated cytotoxicity of *S. mansoni* schistosomula by up to 39% (139), suggesting that IL-4 might, if anything, directly downregulate eosinophil activity. Furthermore, mRNA transcripts for CD16 (FcγRIII) and CDw32 (FcγRII) are downregulated following 24h incubation with IL-4 (140). However, IL-4 has been demonstrated to upregulate mRNA, but not protein, expression of the high affinity IgE receptor Fc RI in eosinophils (141). Indirectly, IL-4 acts to upregulate VCAM-1 expression on human endothelial cells, which enhances eosinophil adhesion and transmigration (142-144). In addition, indirect effects of IL-4 on eosinophils involve the production of the potent eosinophil C-C chemokine eotaxin by endothelial cells (145), which stimulates eosinophil adhesion to human endothelial cells (146), respiratory burst (147), $Ca^{2+}$ flux (148), oxygen radical production, Mac-1 expression, and actin reorganization (149), all indicative of eosinophil activation.

Furthermore, IL-4 has been shown to bias developing Th0 cells towards a Th2 pattern of cytokine secretion (150-154). IL-5, a Th2 cytokine, is well characterized for its activity on eosinophils, including enhancing eosinophil granule release (155-156), survival in culture (157-159), mobilization of eosinophils from bone marrow (160-161), and chemotaxis/homing of eosinophils into inflamed tissues (160, 162-163). These observations, combined with the finding by Tepper's group that neutralizing antibody to IL-5 partially abolishes eosinophil recruitment and restores tumourigenicity of IL-4-transfected tumours (137), encouraged another group to investigate the potential of IL-5-transfected tumours to induce an eosinophil-mediated anti-tumour response (133). In this report, it was shown that despite the prominent eosinophil and macrophage inflammatory response, tumour fate was unaltered, suggesting that additional signals that are induced by IL-4, but not IL-5, are required for eosinophil-mediated tumour cell destruction. Although one recent report suggests

that eotaxin might activate eosinophil tumouricidal activity (145), little evidence further defines the signals involved. Transfection of tumour cells with eotaxin also does not confer protection from tumor growth in animal studies (Jack Gauldie, personal communication). Of particular interest was the finding that liposome-encapsulated glucose oxidase, a $H_2O_2$-generating compound, eradicated 46% of IL-5-transfected tumours (164). This suggests that EPO from eosinophils and locally produced $H_2O_2$ might cooperate to damage tumours under some conditions. Because eosinophil-stimulated macrophages can be a source of $H_2O_2$ (81), and eosinophils and macrophages are both abundant during localized production of IL-4 by tumour cells, it is interesting to speculate that macrophage-eosinophil interplay induces tumour cell cytotoxicity through the production of these molecules.

The factor(s) required for eosinophil infiltration into the site of primary tumour remains to be elucidated. Although IL-5 and eotaxin might be likely candidates, an investigation of the expression of these factors in areas of tumour growth in individuals demonstrating tumour-associated eosinophilia has not been performed. At present, the best information available in terms of eosinophil attracting molecules at the site of tumour growth comes from the mouse models of immunotherapy. The relevance of these studies to actual *in vivo* conditions of tumour growth in humans remains unclear at present.

In addition to the evidence in the literature, it has been shown in our laboratory that rat eosinophils prevent $G_0$-S phase transition in rapidly dividing colon carcinoma cells *in vitro* (manuscript in preparation). In addition, infection of rats with the helminth parasite *Nippostrongylus brasiliensis*, a powerful inducer of Th2 activity, including IL-4 production (165), significantly depresses tumour growth of subcutaneously injected mammary carcinoma cells. Consistent with this reduction in growth rate, histologic sections revealed an extensive eosinophilic infiltrate in *N. brasiliensis*-infected animals (manuscript in preparation), implicating eosinophils in the tumour inhibitory response.

These findings, together with those by Tepper and colleagues, prompted us to further investigate tumouricidal capacity of eosinophils *in vitro*. Presently, we have shown that eosinophils isolated from mice infected intraperitoneally with the tapeworm *Mesocestoides corti*, can kill syngeneic A20 B cell lymphoma cells in 18h JAM test, an assay of DNA fragmentation indicative of cellular apoptosis (166). Furthermore, hypodense eosinophils are substantially more effective in their tumouricidal capacity than their normodense counterparts, and macrophages isolated from the same animals increase eosinophil tumouricidal activity in an additive manner (manuscript in preparation). In an attempt to characterize the mechanisms involved in eosinophil-mediated tumour cell cytotoxicity, we have shown that eosinophils do not induce substantial cytolysis of A20 targets in $^{51}Cr$-release cytotoxicity assays, suggesting that apoptosis rather than cytolysis is the principle means by which eosinophils damage tumour cells. This was a surprising finding, as we anticipated that eosinophil granule proteins would be more likely to disrupt target cell membrane integrity than induce apoptosis. We therefore elected to investigate mRNA expression in eosinophils for classical CTL/NK proteins characterized for their ability to induce apoptosis in tumour targets. We have recently shown that eosinophils from *M. corti*-infected animals transcribe messages for perforin, granzyme B, and, to a lesser extent, FasL. Furthermore, we have also shown in preliminary studies that a competitive substrate for granzyme B decreases tumouricidal activity of hypodense eosinophils, a novel finding which should help to shed some light on the mechanisms involved in eosinophil mediated tumouricidal activity.

## SUMMARY

Although the eosinophil has classically been described in "unwanted" immune responses in North America, its prevalence in certain diseases and pathologies, including cancer, suggests that it may have a more important function in immune surveillance than has been previously thought. Tremendous circumstantial evidence suggests a role for eosinophils in mediating tumour cell damage. Eosinophils not only possess several proinflammatory and cytotoxic mediators capable of directly and indirectly enhancing anti-tumour immunity, but they also have been observed both clinically and experimentally in association with a reduction in tumour growth. At present, the factors involved in recruiting eosinophils into tumour tissue and signals required for their activation, secretion, and degranulation are largely unknown. Recent evidence in our laboratory demonstrates that mouse eosinophils induce apoptosis, but not cytolysis in syngeneic tumour cells. Induction of tumour damage does not appear to require degranulation, but may involve the secretion of granzyme B and perforin. Although FasL is likely not operative in our system, we have yet to rule out this possibility. Despite the fact that much research is still required, we believe that immunotherapeutic strategies which enhance eosinophil cytotoxic activity may lead to more effective cancer treatments.

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## AUTHOR BIOGRAPHY

Darren Costain recieved his Bachelor of Science with Honours in Microbiology and Immunology from Dalhousie University in 1995. Prior to the initiation of his MSc program, Darren was employed as a research associate in the Transplantation and Immunology Research Laboratory, where he examined the ability of a chitin derivative to prevent post-surgical adhesions. Darren will attend medical school at Dalhousie in the fall, where he hopes to continue his cancer research.