

# Eosinophilic Granule Cells of Salmonids: A Potential Target for Anti-inflammatory Therapy?

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## INTRODUCTION

Eosinophilic granule cells (EGCs) were first characterised by Roberts and co-workers in studies of the skin of the European plaice, *Pleuronectes platessa* [1]. Work since then has shown that these cells are found in a number of species including salmonids [2,3,4,5], cyprinids [6,7] and anguillids [8]. EGCs are commonly found in the *stratum granulosum* of the intestinal mucosa, in the gill around the central venous sinusoid and in connective tissue of the filament, in the connective tissue surrounding the spinal cord and meninges of the brain, and in the epidermis of the skin. Cells resembling EGCs have also been identified in the microvasculature of the gill [9]. A related cell type, the periodic acid-Schiff granular leucocyte is often observed in blood smears of cyprinids [6]. EGC-like cells have also been identified in the skin of carp (*Cyprinus carpio* L.) [7].

Morphologically EGCs appear as large ovoid-shaped cells with a large, eccentric nucleus and characteristic large, membrane-bounded granules in the cytoplasm [3]. The granules stain positively with acid dyes such as eosin due to the presence of basic proteins, hence their name [1,2,3,4]. In addition EGC granules also stain positively with Alcian blue and periodic acid-Schiff indicating the presence of sulphated and neutral glycosaminoglycans [3,4].

Because of their location in tissues and histochemical staining characteristics (Table I), the suggestion has been made that EGCs are analogous to mammalian mast cells [10,11]. The ability of these cells to degranulate to substances which induce degranulation of mast cells lends further credibility to their analogy. Further similarities have been demonstrated experimentally and shall be discussed in this review.

Table I. Comparison of some of the characteristics of mammalian mucosal and connective tissue mast cells (MC) and eosinophilic granule cells (EGC). + = a positive reaction, - = no reaction, N.D. = not determined.

Comparison	Mucosal MC	Connective tissue MC	EGC	Reference
Size of granules ( $\mu\text{m}$ )	<0.2	0.2-0.4	0.2-1.5	26
Histamine content (pg/cell)	0.1-2.0	1.0-30.0	N.D.	26
O-phthaldehyde	N.D.	+	-	4, 26
Chondroitin sulphates	95%	minor	N.D.	26
Heparin	5%	major	N.D.	26
Alcian Blue	+	-	+	4, 26
Luna's MC stain	+	+	+	4
<u>Degranulation by:</u>				
IgE	+	+	- <sup>a</sup>	26
Calcium ionophore A23187	+	+	+ <sup>b</sup>	27
Compound 48/80	-	+	+	16, 26
Bee venom peptide	-	+	N.D.	26
Substance P	+	+	+	11, 28
<i>A. salmonicida</i> and <i>V. anguillarum</i> ECPs	N.D.	N.D.	+	15, 17, 18

<sup>a</sup> Fish do not possess IgE.

<sup>b</sup> Powell unpublished observations.

## RESPONSE TO DISEASES

### Parasitic Infections

EGCs have been demonstrated to infiltrate the intestinal mucosa in response to parasitic infections of the gastrointestinal tract of salmonids [12]. EGCs have also been observed in the fibrotic cyst of an encapsulated microsporidian parasite in the intestine of the non-salmonid Sergeant Major fish (*Abudefduf saxatilis* L.) [13]. Parasitic infections of the skin of brown trout (*Salmo trutta* L.) showed an increase in the concentration of acidophilic granular cells matching the description of EGCs. The number of granular cells in the epidermis also increased in the absence of parasites in response to continuous irritation by formalin baths [14].

### Bacterial Infections

Experimentally, it has been possible to stimulate EGCs to degranulate in a manner similar to that shown for mammalian mast cells in response to bacterial pathogen extracellular products. Intraperitoneal injection of *Aeromonas salmonicida* extracellular products induced a detectable release of biogenic amine which could be correlated with an anaphylactic-like exocytosis of the cytoplasmic granules from intestinal EGCs of rainbow trout (*Oncorhynchus mykiss*) [15,16]. More recent evidence suggests that exotoxin-induced anaphylactic-like exocytosis is probably a function of the concentration of exotoxin used. At lower concentrations of exotoxin to those used by Vallejo & Ellis [16] the granules become highly vesiculated with the presence of many cytoplasmic vesicles. This morphology is consistent with a controlled processing and release of the granule contents [17]. A similar response is seen following injection of extracellular products of *Vibrio anguillarum* [17]. Exposure of trout to *V. anguillarum* and its extracellular products also induces diapedesis of EGCs into the vasculature and infiltration into organs usually devoid of such cells such as the kidney, liver and spleen [18]. It is not known if exposure to *A. salmonicida* extracellular products also induces diapedesis of EGCs.

### Pharmacological Manipulation

To date, pharmacological studies of EGCs have been carried out either *in vivo* or *in vitro* on intact pieces of tissue. It has been shown that mast cell secretagogues used *in vivo*, such as compound 48/80 (1 µg/g body weight of fish) and concanavalin A (5 µg/g body weight of fish), will induce degranulation similar to that shown with *A. salmonicida* exotoxins in Atlantic salmon [15,16]. In rainbow trout it has been demonstrated that EGC degranulation upon exposure to compound 48/80 *in vivo* and *in vitro* appears to be dose-dependant [19]. At low dosages (0.05 µg/g and 0.5 µg/g body weight of fish *in vivo*) a multivesiculate degranulation occurs. Degranulation induced by compound 48/80 (10 µg/mL) *in vitro* is similar to the anaphylactic-type of exocytosis usually associated with mast cell degranulation [19]. Calcium ionophore A23187 also induces EGC anaphylactic-like exocytotic degranulation (Powell et al. unpublished), suggesting that degranulation is mediated by an influx of extracellular calcium into EGCs. Details of the mechanism of degranulation are unknown at this time.

Another similarity between salmonid EGCs and mammalian mast cells is evidence of a possible functional link between intestinal EGCs and non-myelinated nerves [11]. It has been shown that EGCs are frequently observed in close association with non-myelinated axons in the trout intestine [11]. It has also been demonstrated that injection of substance P, a putative neurotransmitter of the peptidergic nervous system of trout, will induce multivesiculate degranulation similar to that seen with low doses of compound 48/80 [11]. A similar response is seen after injection of the neurotoxin and substance P releaser, capsaicin [11]. This form of granule breakdown and release is not lysosomally mediated [20]. A similar link is suggested between mammalian mucosal and connective tissue mast cells [21, 22]. Mast cells are known to degranulate *in vivo* in response to such agents as substance P [23] and capsaicin [24]. Interaction between EGCs and peripheral nerves suggests an association between the nervous system and immunological function.

It is interesting to note that experimentally induced EGC degranulation responses, whether anaphylactic or multivesiculate in nature, appear to occur very rapidly within a matter of hours [11, 17, 19]. Rapid cellular responses of this nature suggest that EGCs may be important in the mediation and initiation of inflammatory responses. Mammalian mast cells have been shown to release a mixture of inflammatory mediators including histamine, serotonin, leukotrienes, prostaglandins and platelet-activating factor, as well as proteoglycans such as heparin and chondroitin sulphates [25]. Although the contents of the EGC granule remain a mystery at present, they appear to be intimately involved in the non-specific defense processes of the host.

### **THERAPEUTIC IMPLICATIONS**

The similarities between mast cells and EGCs strongly suggests that EGCs may be analogous to mammalian mast cells. Further confirmatory characterization of the EGC granule is required to determine the nature of the biogenic amine and mediators within the granules. By monitoring the number and morphological state of EGCs, early pathological changes caused by infectious or, possibly, polluting agents can be detected and preventative measures taken. The involvement of EGCs in apparently inflammatory reactions in responses to diseases suggests a potential for the development of anti-inflammatory therapies targeted at EGCs.

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