Cellular immune response of gilthead sea bream (*Sparus aurata* L.) to *Enteromyxum leei* (Myxozoa)

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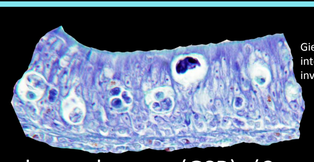
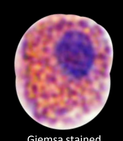
INTRODUCTION



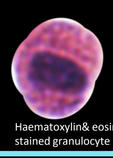
Giemsa stained intestinal section invaded by *E. leei*.

Enteromyxum leei disporoblast in intestinal fresh smear

Enteromyxosis in gilthead sea bream (GSB) (*Sparus aurata*) consists of severe chronic catarrhal enteritis causing a cachectic syndrome and death. The etiologic agent is the intestinal parasite *Enteromyxum leei*, which penetrates and proliferates in the paracellular space between enterocytes. The parasite provokes epithelial desquamation triggering an intense inflammatory response locally.



Giemsa stained granulocyte



Haematoxylin& eosin stained granulocyte

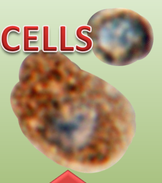
At mucosal and systemic sites, cellular effectors participate in the immune response against pathogens. In GSB, acidophilic granulocytes (AGs), are the predominant cell type recruited during infections and present an eosinophilic, Giemsa magenta-red staining pattern. AGs bear the G7 surface epitope and lack histamine (HIS) granules (=$G7^+HIS^-$). GSB mast cells, though also eosinophilic and Giemsa magenta-red, are $G7^-HIS^+$. Besides, IgM^+ plasma cells and B cells and lymphohaematopoietic melanomacrophages also participate in the immune response of GSB. The involvement of such leukocytes was studied during the acute inflammatory process triggered by enteromyxosis.

MATERIALS & METHODS

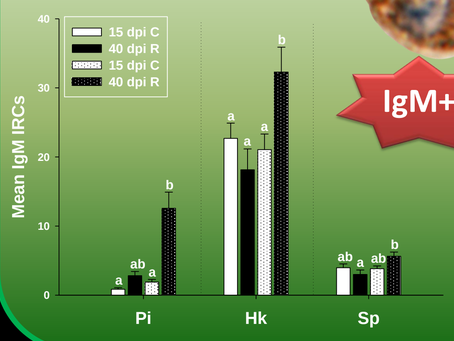
Antibody	Type (origin)	Dilution	Source
GSB G7	Mab (mouse)	1:100	UM[1]
GSB IgM	Pab (rabbit)	1:60,000	IATS [2]
histamine	Pab (rabbit)	1:100	Sigma

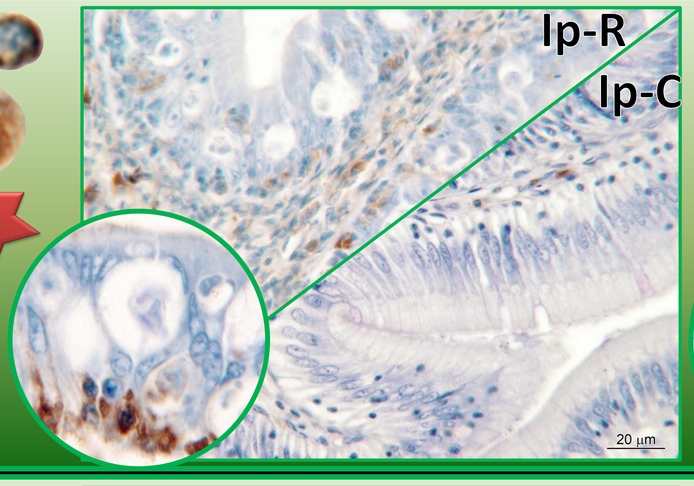
GSB were experimentally infected with *E. leei* by anal intubation. Tissue sections of Bouin fixed and paraffin embedded portions of anterior intestine (Ai), posterior intestine (Pi), head kidney (Hk), spleen (Sp) and thymus (Th) were taken from control (C) and recipient (R) fish at 15 and 40 days post inoculum (dpi). For immunohistochemistry, a monoclonal antibody (Mab) against the G7 epitope on GSB acidophilic granulocytes, a polyclonal antibody (Pab) against histamine stored in mast cell granules and a Pab against GSB IgM labeling plasma cells and B cells were applied. Immunoreactive cells (IRCs) were quantified in all tissues and in splenic sections, the surface of melanomacrophage centres (MMCs) was quantified.

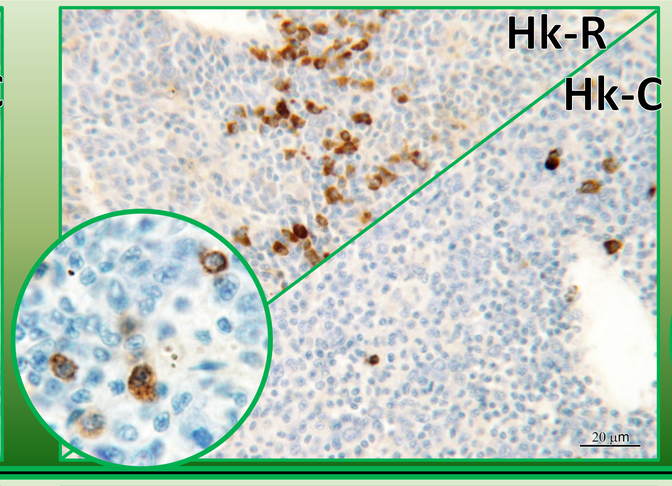
PLASMA CELLS / B CELLS

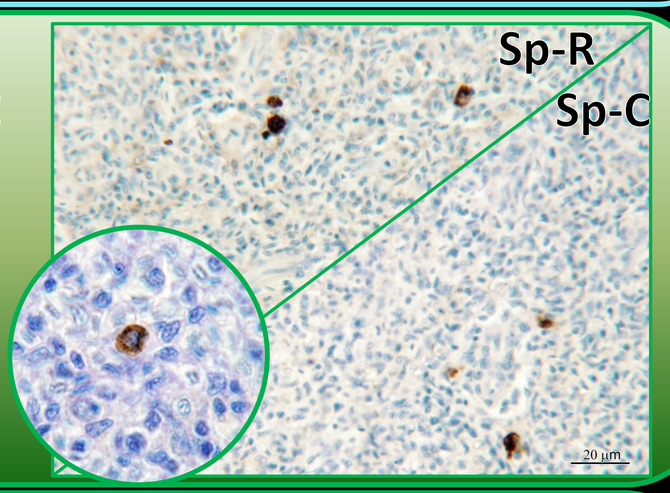


IgM+

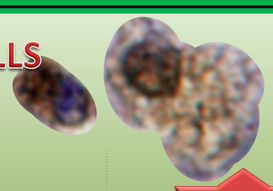




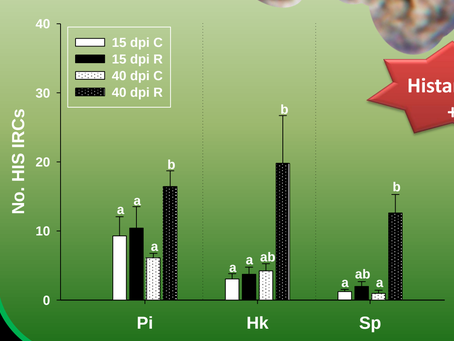


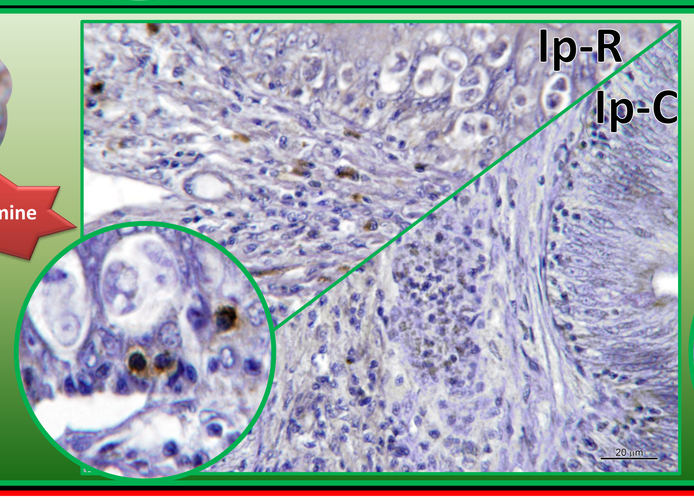


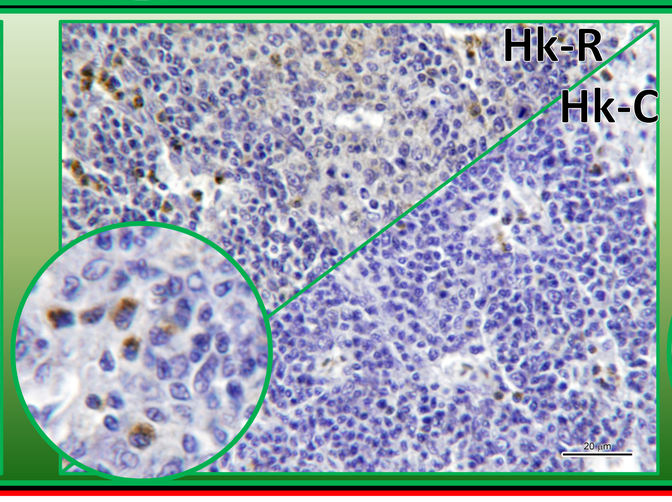
MAST CELLS

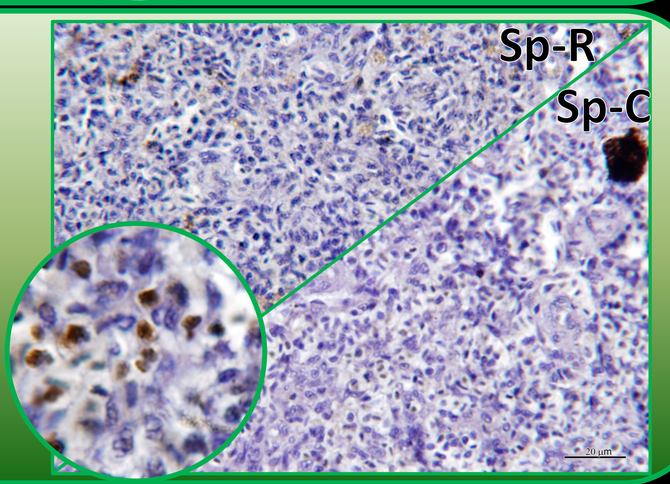


Histamine +



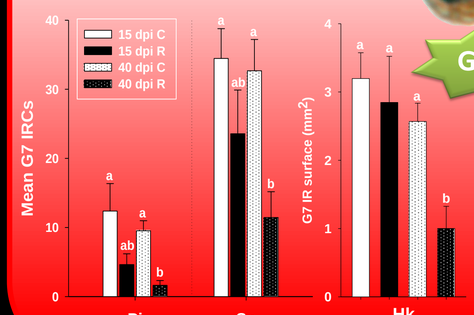


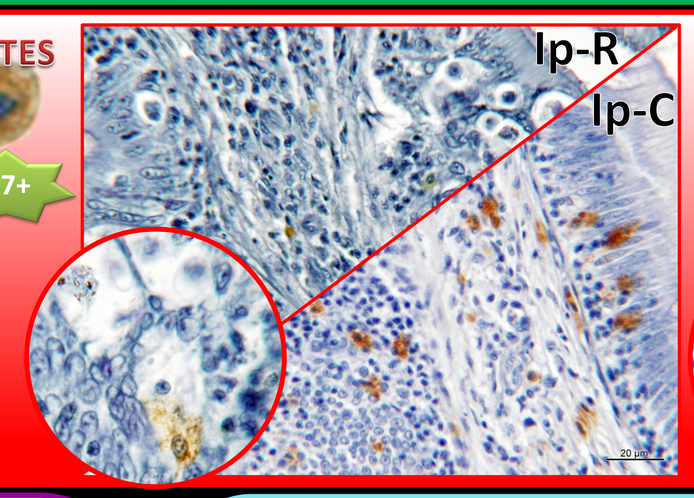


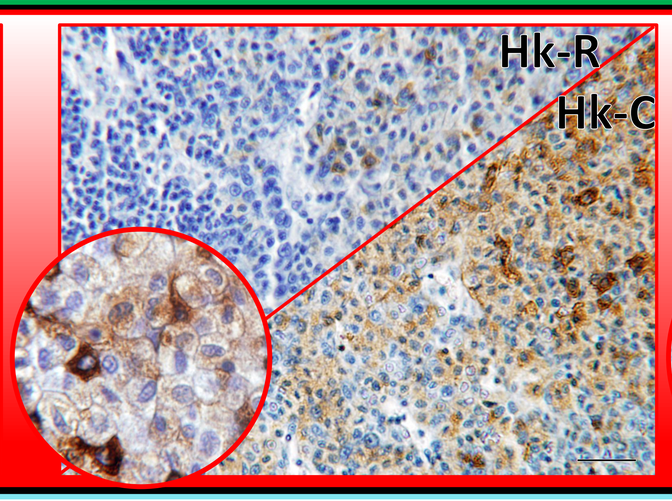


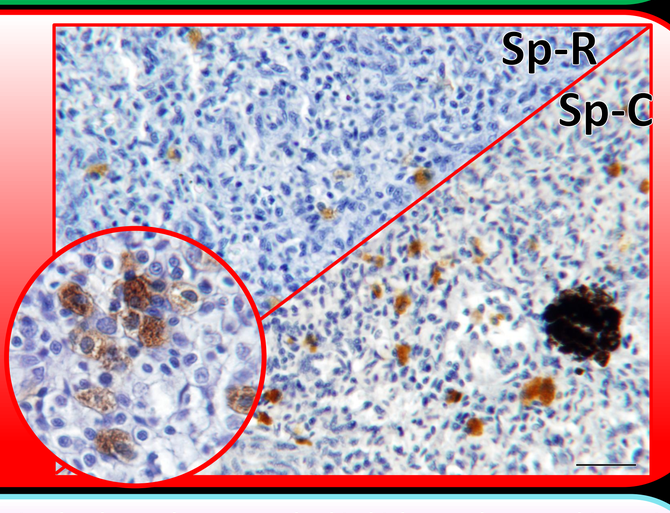
ACIDOPHILIC GRANULOCYTES

G7+

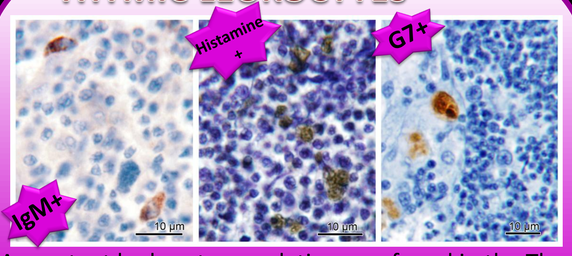






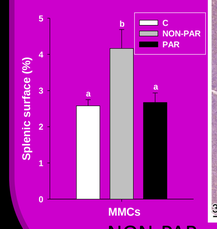


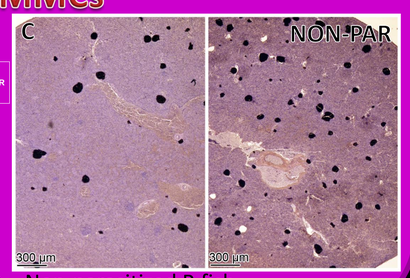
THYMIC LEUKOCYTES



A constant leukocyte population was found in the Th, regardless of the infective status.

SPLENIC MMCs

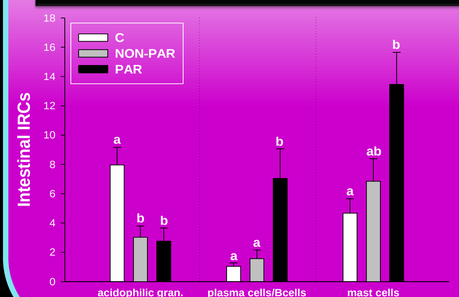




NON-PAR = Non-parasitized R fish

PAR = parasitized R fish

RESULTS & DISCUSSION



The relatively fast progression of the *E. leei* infection by the anal route resulted in high prevalence and intensity of infection as well as an early inflammatory response. Our results suggest that the cellular response of GSB to enteromyxosis induces proliferation of plasma cells/B cells and mast cells in lymphohaematopoietic organs and recruitment into intestines. This occurs likely *via* blood circulation since blood vessels of the examined R organs contained IRCs for the applied antibodies. At the Ai the mucosal inflammatory response was weaker and occurred later than in the Ip, the main target site of the parasite. Interestingly, the observed mast cells presented diverse morphologies and staining patterns, worth to be further studied. Acidophilic granulocytes, by contrast, presented an opposite pattern of response to the infection with an overall depletion in R fish compared to C fish. The distribution pattern of all three leukocyte types in the Th did not vary along the trial, being the IgM-IRC population the largest. However, the studied leukocyte types in Th do apparently not participate in the acute response. The significant increase of splenic surface covered by MMCs in NON-PAR fish (*vs* PAR and C) was related to a greater MMC number rather than to their size increase. An early proliferation of MMCs, related to antigen retention and processing, may indicate the onset of an adaptive immune response conferring protection against parasite invasion. The limited protection of GSB against *E. leei* may be explained in part by the acidophilic granulocyte depletion and an immunosuppressive effect of the parasite cannot be discarded.

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