



# Mucosal Mast Cells in Intestinal Tumors of APC<sup>Min</sup> Mice Reveal a TH2 Microenvironment

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## INTRODUCTION

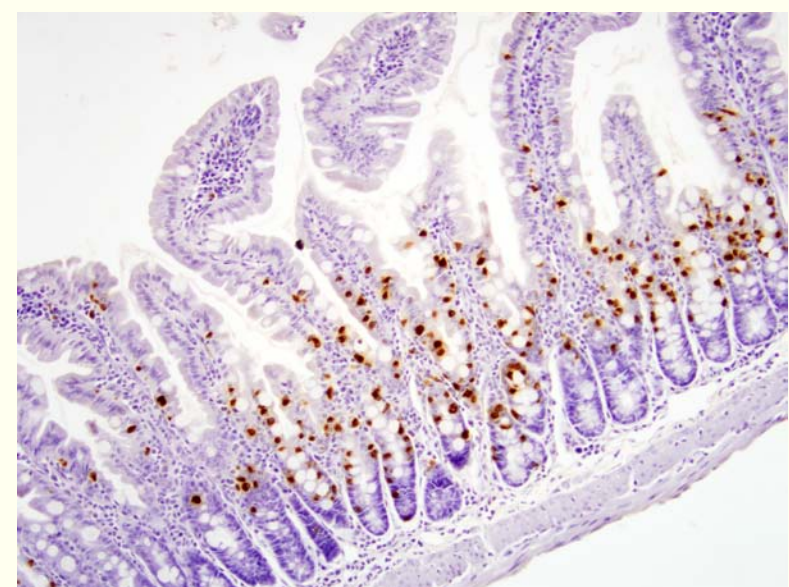
The microenvironment within tumors is critical to neoplasia. Non-neoplastic cells provide nutrients and growth factors that sustain tumor growth and promote progression of the neoplastic process. The complexity of these interactions is very difficult to study *in vitro* or in clinical settings. We have therefore been using a mouse model of intestinal neoplasia to better understand the neoplastic microenvironment in this organ system.

### APC<sup>MIN</sup> Mouse (Min)

- Substitution mutation in murine *Apc* gene truncates protein at 850 amino acids (wt = 2843 aa)
- Fully penetrant autosomal dominant trait
- Homozygous embryonic lethal
- Heterozygous mice spontaneously develop multiple intestinal adenomas predominantly in the small intestine following spontaneous loss of the wild-type *Apc* allele (LOH)
- Loss of *Apc* product is only known mutation driving tumorigenesis
- Modifier genes include *Mom-1* (AKR strain) and *Mom-2*

## Mucosal Mast Cells, TH2 Responses, & Nematode Infections

Min mouse tumors frequently contain a unique cell type not present in the adjacent non-neoplastic mucosa. We've identified these cells as **mucosal mast cells (MMC)** based on immunohistochemical (IHC), histochemical and ultrastructural (EM) characteristics. In H&E stained sections these MMC resemble "globular leukocytes".



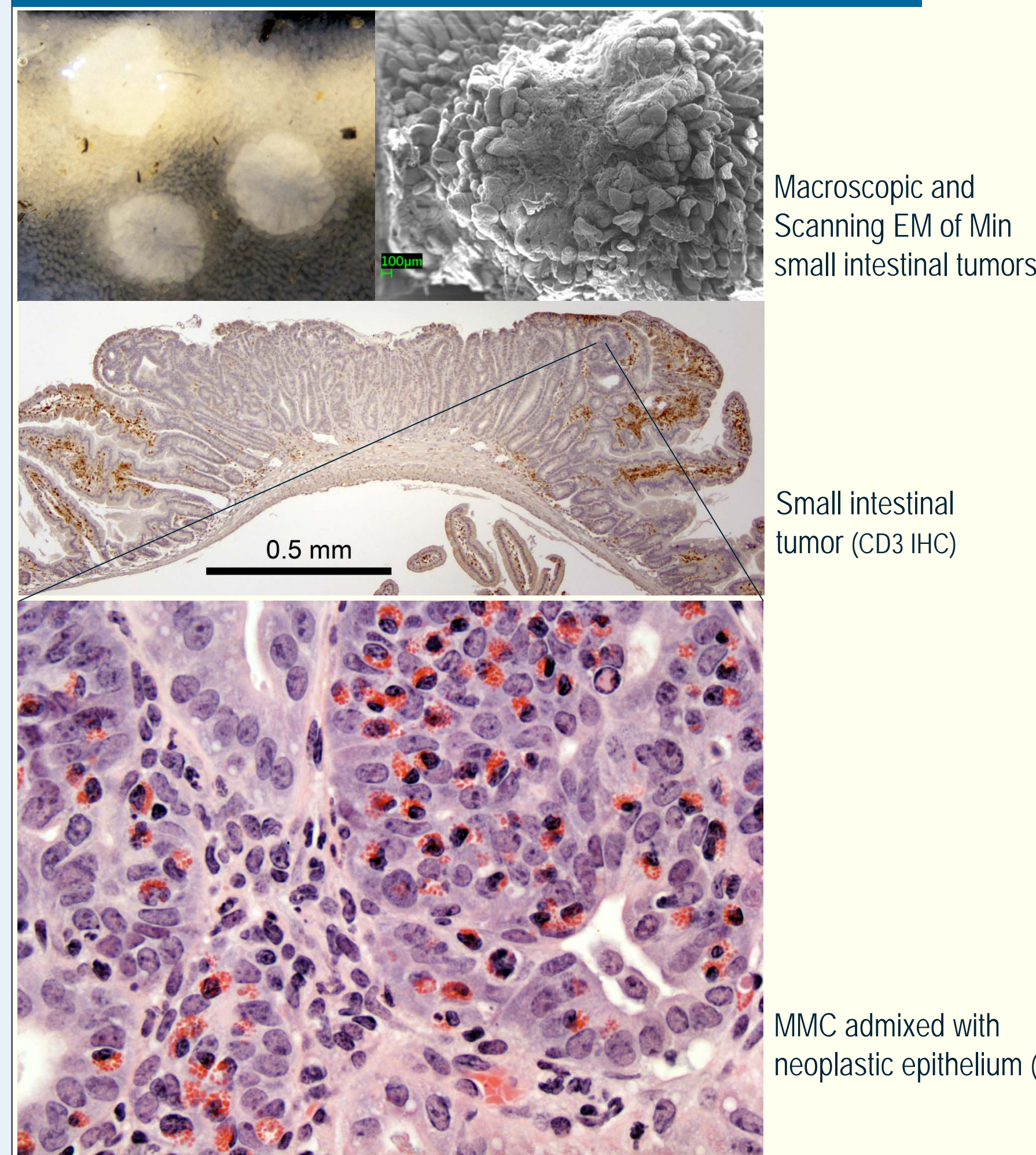
Influx of MMC responding to *Nippostrongylus brasiliensis* in a mouse (mast cell protease-1 IHC)

MMC only appear in the context of TH2 immune responses and have been most extensively studied in intestinal nematode infections where they have been associated with expulsion of parasites. Key factors in this response include:

- TGF- $\beta$ 1
- IL-4 and/or IL-13
- IL-9
- IL-10
- $\alpha$ V $\beta$ 6

Expression of some TH2 cytokines is in fact upregulated in Min mouse tumors compared to the adjacent normal intestine

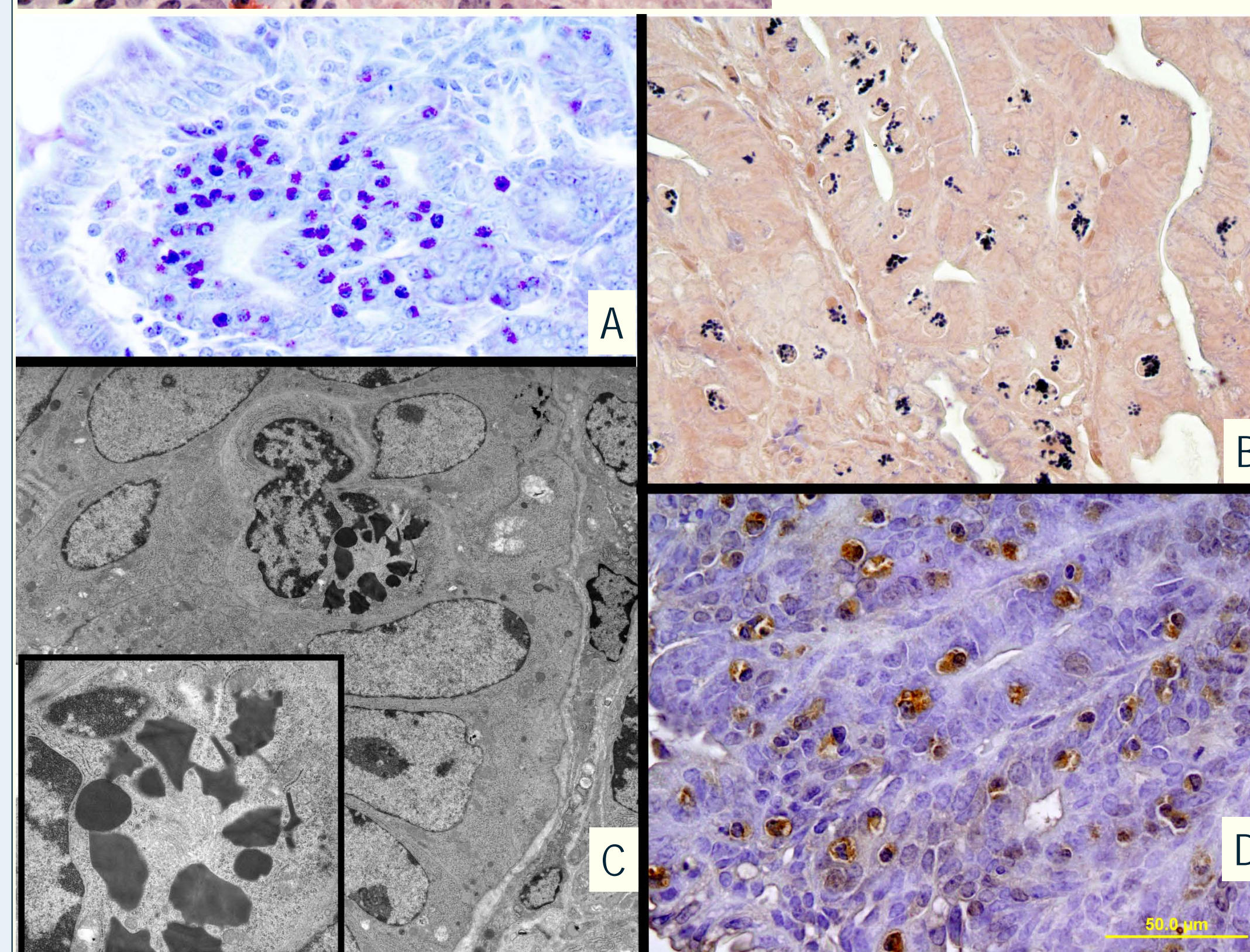
## RESULTS



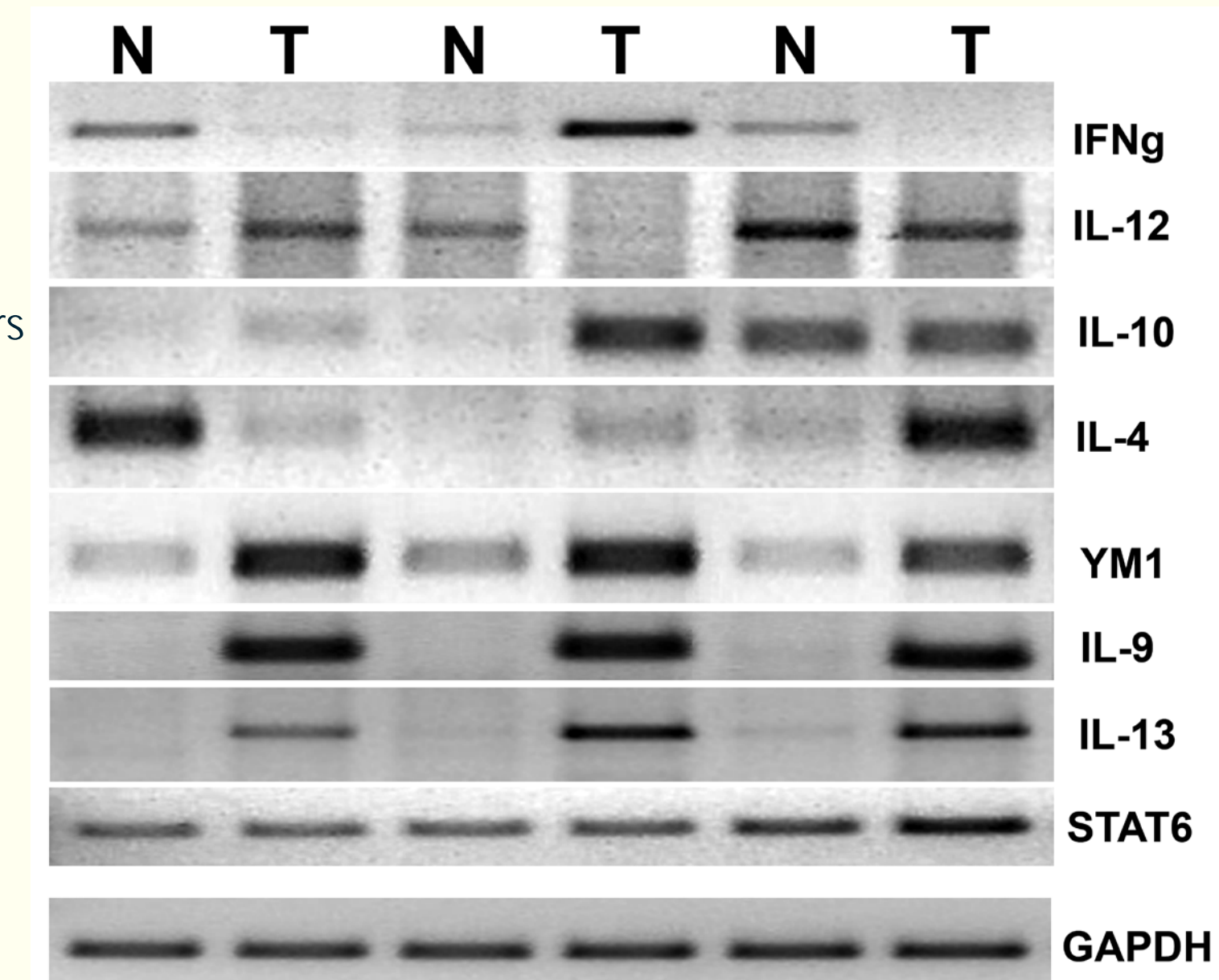
Macroscopic and Scanning EM of Min small intestinal tumors

Small intestinal tumor (CD3 IHC)

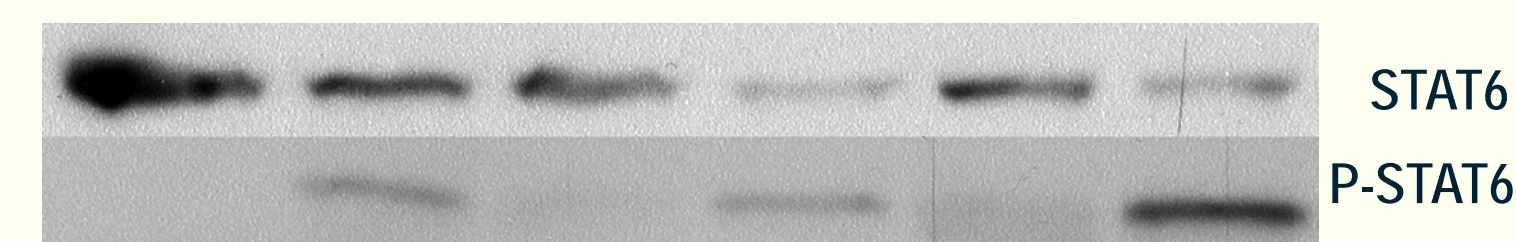
MMC admixed with neoplastic epithelium (H&E)



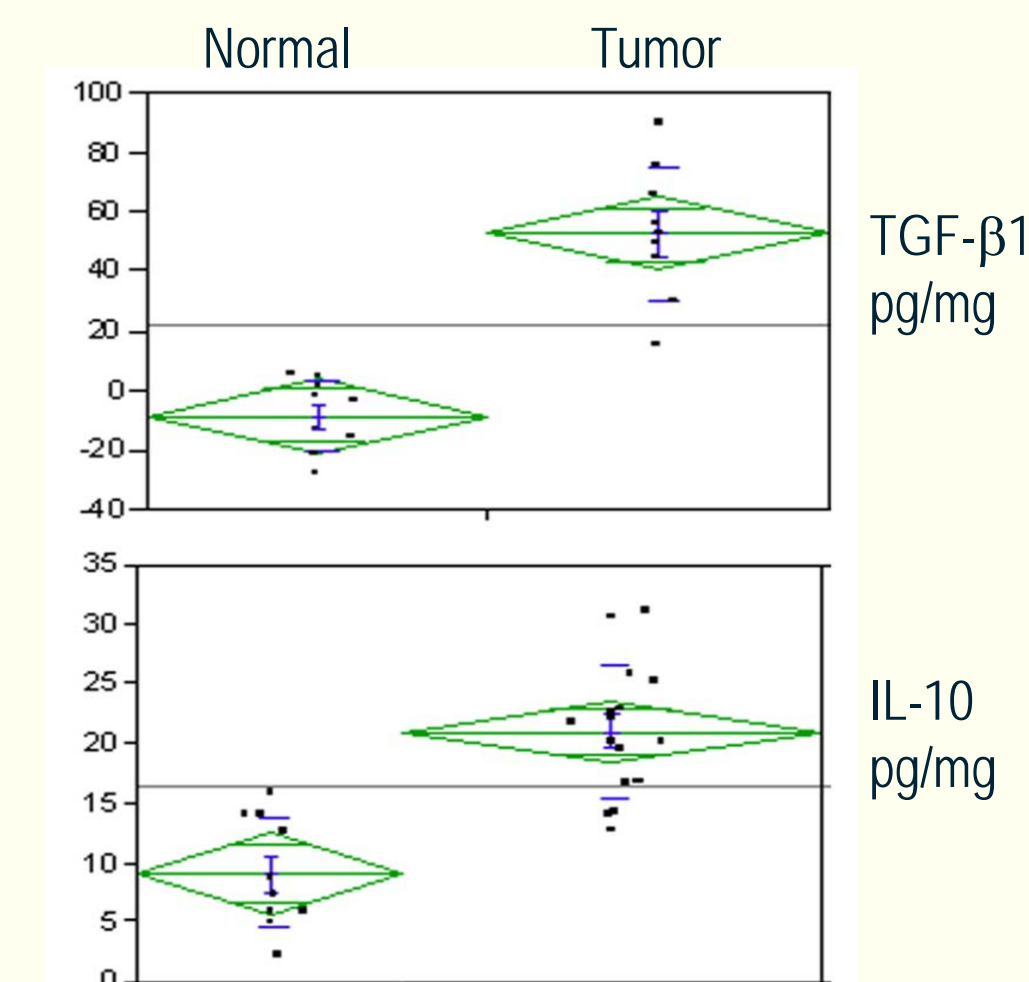
## RT-PCR



## WESTERN



Tumors and adjacent normal intestinal samples were cultured overnight *in vitro* and supernatants evaluated by ELISA (pg cytokine/mg tissue).



- A – Toluidine Blue (Carnoy's fix; no metachromasia with formalin fixation)
- B – PTAH stained section
- C – Transmission EM
- D – Mast cell protease-1 IHC (specific for murine MMC)

## Relation to Tumorigenesis and Treatment Effects

MMC may be found in small intestinal tumors from:

- Min mice
- Min x AKR mice carrying the dominant modifier allele *Mom-1*
- *Apc*1638 mice, including invasive tumors
- Unrelated mouse strains (spontaneous carcinoma)
- Not all small intestinal tumors in Min mice contain MMC and they are not present in Min colonic polyps.
- MMC infiltrates do not appear to be related to affects of select pharmaceutical and dietary factors on tumorigenesis.
- PGE2-receptor agonist treatment may increase the number of intratumoral MMC, but this is not associated with a significant impact on tumor growth.

Diet	MMC count	Tumor Load		
			Mean	Stnd Error
Arachidonic Acid	1.5	0.4	48	
Eicosapentaenoic Acid	2.3	2.1	22 **	
Drug	Untreated	4.5	0.6	46
	Sulindac	6.1	1.8	22 **
	dimethyl-PGE2	11.2 **	2.5	38
Genotype	<i>Mom1</i> (-/-)	4.5	0.6	99
	<i>Mom1</i> (+/-)	5.3	0.6	39 **

\*\* p < 0.05

## CONCLUSIONS

### Mucosal mast cells:

- Specifically accumulate within the neoplastic epithelial compartment of small intestinal tumors of mice, in particular those that develop with LOH in *Apc*.
- Do not appear to enhance or retard tumor growth.
- Reflect a TH2 cytokine microenvironment within these tumors characterized by increased production of IL-9, IL-10, IL-13, and YM1.

STAT6 phosphorylation is increased in these tumors, attributed to signaling via IL-13.

It remains to be determined if this TH2 signaling pathway is important in mucosal mast cell infiltration in tumors, contributes to Min tumorigenesis, or is a target of various treatments or environmental factors that affect tumorigenesis in this model.

## Select References

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