

Report of Short Term Scientific Mission (COST-850)

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Background:

Effective control of insect pests by entomopathogenic nematodes (EPN) and their bacterial symbionts require understanding of the immune response of the insect host in order to produce better EPN-bacterial complexes. . Unraveling the molecular components of these events should aid in understanding why certain hosts are resistant to some strains of EPN and not to others, and on the other hand why some EPN-bacterial complexes have narrow host range while others have a broader host range. Information gained from studying the interaction between these insect pathogens and their hosts will have immediate implications in better tailoring the best EPN strain to a given pest, and should have long-term implications for rational intervention with the pest's immune system to facilitate its control.

The immune response of the insect comprises humoral and cellular aspects. The former have been studied extensively in the best available model insect – *Drosophila*. However little is known on the cellular response. The goal of my Short Term Scientific Mission was to learn how to assay the cellular immune response of *Drosophila*. As reported below we successfully applied Prof. Trenczek's experience and tools for these studies to certain strains of *Drosophila* known to be involved in hematopoiesis and immune response that I brought with me and certain strains she has.

1. Preparation of blood cell monolayers for microscopic observation by phase contrast and illumination.

Genotypes used: (Details of genotypes used are given in Appendix 1)

- Wild type (Canton-S, hereafter referred to as CS)
- *l(3)mbn* (larval lethal with “malignant blood cells”)
- *l(1)mbn* (a different larval lethal with “malignant blood cells”)
- Gal4-e33c/UAS-GFP – (Gal4 driver reportedly enforcing expression of GFP in hematopoietic cells) No expression of GFP was detected in the blood cells.
- Gal4-Dlmo/UAS-GFP - (Gal4 driver of the hematopoietic gene *dlmo* studied in Segal's lab; expected to enforce expression of GFP in hematopoietic cells) No expression of GFP was detected in the blood cells.

2. Identification of different blood cells – Plasmatocytes (PL), lamellocytes (LM) and crystal cells (CC). Observation was conducted by phase contrast.

Genotypes used:

- Wild type (CS) - Most of the cell population is composed of plasmatocytes; lamellocytes are seen only at late 3rd instar larvae, and crystal cells are rare.
- *l(3)mbn* - Over abundance of blood cells, mostly plasmatocytes but lamellocytes are present more than in the wild type and they are bigger and flatter; crystal cells are rare. Plasmatocytes seem to spread more and seem to be bigger than the wild type-plasmatocytes.
- *l(1)mbn* - Over abundance of blood cells, mostly plasmatocytes but lamellocytes are present more than in the wild type and they are bigger; crystal cells are rare.

3. Whole mount preparations of larvae for microscope observation and the identification of the hematopoietic organ and other organs. Observation was conducted by differential interference contrast (Nomarski) and illumination.

Genotypes used:

- Wild type (CS)
- *l(3)mbn* - The hematopoietic organ is enlarged, all lobes.
- *l(1)mbn* - The hematopoietic organ is enlarged, all lobes
- Gal4-e33c/UAS-GFP - Expression of GFP was detected at the larvae brain, proventricle, epidermal cells and the hematopoietic organ.
- Gal4-Dlmo/UAS-GFP - Expression of GFP was detected at the larvae brain and salivary glands.
- Gal4-AP/UAS-GFP - Expression of GFP was detected at the larvae brain and hindgut.
- *domino* (a hematopoietic mutant) - The hematopoietic organ is enlarged, and its first lobes are melanized. Some larvae have floating melanized bodies.

4. Immunohistochemistry of blood cell monolayers - was done using the *l(3)mbn* mutant since in this mutant there is over proliferation of blood cells and all types of cells are present, were in the wild type lamellocytes are present only a short time before pupation. Blood cells were stained by specific first antibody (Table 1) and a mouse anti IgG FITC labelled secondary antibody. Observation was conducted by phase contrast and illumination. A list of the antibodies that were tested and found negative is given in the bottom (CC=crystal cells; LM=lamellocytes; PL=plasmatocytes)..

Table 1: Immunohistochemistry of blood cell monolayers

Antibody	<i>I(3)mbn</i> blood cell observation
12F6	CC* marker, stains the crystal, better with triton
4c1	dotty membrane staining for some of the cells, no particular kind
4b8	one side membrane staining on PL* and LM*, LM stronger with triton
X14E7	uneven staining, stronger for LM, some PL and CC also stain.
77D	dotty staining of PL only with triton.
39	strong for PL, some LM granules staining
Z19E6	weak for PL
Z15H2	very weak for PL
X3U65	very weak for all cells
X32H2	medium staining, better for PL than LM
X27D1	general weak staining
X7C4	general weak staining
X5B6	nuclear staining of PL and few LM
31c2	dotty staining of some PL
31d4	weak dotty staining of some PL
32c2	all cells stain weak
35b3	all cells stain weak
36d4	fiber like staining of PL more than LM
37c5	all cells stain weak
38b6	few cells stain stronger than the rest
21d3	all cells stain weak
23d5	all cells stain weak
25b6	weak staining, PL more than LM
26c4	staining of membrane structure stronger for LM than PL
36c1	few cells stain weak
29c1	weak staining, some cells stain more than others
24a5	strong dotty staining, some cells more than others
14a5	strong dotty staining, some cells more than others
12b2	soft spotty staining, some cells stain more than others

Antibody	<i>I(3)mbn</i> blood cell observation
7c1	strong sandy background probably plasma protein.
7b1	stains PL more than LM
8b1	sandy staining of PL more than LM
11a1	medium staining of all cells
11d2	weak staining of all cells
12b4	one side staining of PL and LM
14b5	weak staining of all cells
29a6	some cells stain weak
8b6	spotty staining of some cells more than others
4b3	weak staining of all cells
4a4	strong one side membrane staining of some PL and LM
4a2	strong membrane staining of PL and LM
2c1	weak staining of all cells
1d4	weak staining of some cells
32	weak nuclear staining
73	stains small spots on the cells

*Negative antibodies: 106, Z20C11, 7, 9H2, 75, 31A4, 7b5, 73, 30, 16, X1B12, X28G4, X11G9, X7B813, 34D, 33a4, 26a2, 2c3, 28c3, 15a2, 16c3, 19c4, 20d3, 3a5 and 109.

5. Immunohistochemistry of whole mount larvae - was performed on wild type 3rd instar larvae stained by specific first antibody (Table 2) and a mouse anti IgG FITC labelled secondary antibody. Observation was conducted by differential interference contrast (Nomarski) and illumination. Hematopoietic organ(**HO**).

Table 2: Immunohistochemistry of whole mount larvae

Antibody	Wild type whole mount observation
12F6	staining of the cuticle and gut
4c1	staining of the gut and may be of the HO
4b8	negative
X14E7	negative, HO ? Negative
77D	staining of the mouth part, gut and cuticle
39	negative, may be staining of the HO
X32H2	negative
X5B6	all most all nuclei
31c2	staining of the cuticle and gut and may be of the HO
31d4	staining of the cuticle and gut
36d4	staining of the cuticle, gut and spots on the imaginal discs
38b6	negative
26c4	negative
24a5	negative
14a5	negative
12b2	negative
7b1	negative
8b1	staining of the gut and may be of the HO
4a4	negative
4a2	staining of basal lamina, fat body and may be the HO

6. Lectin labelling - blood cell monolayers of the *l(3)mbn* mutant - were stained with different lectins labelled either with FITC or TRITC (Table 3). Observation was conducted by phase contrast and illumination. Full name and specificity of lectins are given in Appendix 2

Table 3: Lectin labelling - blood cell monolayers of the *l(3)mbn* mutant

Lectin	<i>l(3)mbn</i> blood cell observation
AH	weak sandy cytoplasmic staining of PL more than LM
AP	weak and spotty staining of all cells
BP	weak spotty staining
EC	negative
GM	granular staining of some cells, LM more than PL, negative with triton
HP	membrane and dots stain on PL more than LM, better with triton.
LC	cytoplasmic sandy staining of PL more than LM
MP	weak cytoplasmic staining of all cells
PA	very weak staining of all cells
PV	negative
SC	weak cytoplasmic staining of all cells, stains stronger with triton, PL more than LM.
TP	PL stain stronger than LM, better with triton
TV	strong staining, LM more than PL
UEA I	weak membrane staining of all cells, with triton strong cytoplasmic and membrane staining.
VV	stains some LM more than PL.

7. Lectin labelling of whole mounts from wild type 3rd instar larvae - were stained with different lectins labelled either with FITC or TRITC (Table 4). Observation was conducted by phase contrast and illumination. Full name and specificity of lectins are given in Appendix 2.

Table 4: Lectin labelling of whole mounts from wild type 3rd instar larvae

Lectin	Wild type whole mount observation
AH	general staining, HO stained the fat body not
AP	
BP	general staining, not including the fat body
EC	
GM	week general staining
HP	general staining
LC	general staining
MP	general staining
PA	
PV	
SC	stains the brain
TP	general staining
TV	strong general staining
UEA I	general staining
VV	general staining

8. Cell culture work - the *Drosophila* hematopoietic cell line *mbn*. Maintenance procedures and observation of the cells.

9. Meeting Prof. Gateff - During my stay at Prof. Tina E. Trenzcek's lab I got the opportunity to meet Prof. Elisabeth Gateff. Prof. Gateff was among the first to investigate and characterize *Drosophila* hematopoiesis. The mutants *l(1)mbn*, *l(3)mbn* and the *mbn* cell line used in this work were generated and studied by her. This meeting contributed a lot to my understanding of *Drosophila* hematopoiesis and the avenues of research in the future of this study.

Conclusion:

As evident from this report my stay in Prof. Trenzcek's lab was extremely productive. Most of the experimental goals were accomplished. I should now be able to both apply these methods myself to study the histopathology upon EPN challenge, and to train others in using them to complement our analysis of the humoral response of *Drosophila* to EPN. Once established in *Drosophila*, we will extend this research to insect pests.

Appendix 1: Genotypes of strains used:

- *l(3)mbn-1(E1)/TM3-Sb, Ser, e*
- *y,l(1)mbn(SO),mbn+/y,l(1)mbn/Y*
- Gal4-e33c/TM3-Ser, GFP
- UAS-GFP/UAS-GFP
- Gal4-Dlmo/Gal4-Dlmo
- Gal4-AP/CyO
- *domino/CyO, GFP*

Appendix 2: Full name and specificity of lectins:

Lectin	Organism of origin	Specificity
AH	<i>Arachis hypogaea</i>	Gal α 1GalNAc or Gal-(Sial2-6)GalNAc
AP	<i>Abrus precatorius</i>	Gal α or Gal α Gal α
BP	<i>Bauhinia purpurea</i>	Gal-GalNAc (not Sial)
EC	<i>erithrina corallodendron</i>	
GM	<i>Glycine max</i>	GalNAc α
HP	<i>Helix pomatia</i>	GalNAc α A substance
LC	<i>Lens culinaris</i>	Man α Glc
MP	<i>maclura pomifera</i>	Gal α i3GalNAc or GalNAc α (2 sites)
PA	<i>Phytolacca americana</i>	Glc Nac
PV	<i>Phaseolus vulgaris</i>	
SC	<i>Canavalia ensiformis</i>	Man α or Glc α better oligo-Man
TP	<i>Tetragonolobus purpureas</i>	
TV	<i>Triticum vulgaris</i>	Glc Nac and sialic
UEA I	<i>Ulex europa</i>	Fuc