

ANALYSIS OF LAMELLOCYTE-SPECIFIC MOLECULES IN *DROSOPHILA MELANOGASTER*

PhD thesis

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Introduction

The fruit fly, *Drosophila melanogaster* is rated as an excellent model system to study the innate immunity, because insects and vertebrates share numerous common molecular elements of immune response. The larval immune system of *Drosophila* consists of the fat body, which is the main place of production of antimicrobial peptides, the lymph glands and the posterior hematopoietic tissue, which are responsible for the production and differentiation of hemocytes, and the circulating hemocytes, which have three main morphologic subsets: plasmatocytes, crystal cells and lamellocytes.

In our laboratory, we examine the blood cells or hemocytes of *Drosophila melanogaster*, which have a remarkable role in the immune response. We identified several marker molecules of hemocytes, which help us to follow their differentiation. We assume that these marker molecules would play role in the development or function of the hemocytes, thus we decided to analyze these proteins with molecular methods.

Lamellocytes have an essential role in the encapsulation, which is an immune response against parasitoid wasps or transformed self tissue. During encapsulation, plasmatocytes recognize the foreign tissue and spread on the parasitoid egg, then lamellocytes create a multilayered capsule around it, which will be melanized by crystal cells causing the death of the intruder. Encapsulation reaction shares phenotypic similarities with mammalian granulome formation, but the key components are still unknown, for example the receptors responsible for the parasitoids, or the molecules which achieve the communication between the hemocytes.

Methods

Classical genetic methods with *Drosophila*

In vivo immune induction by parasitoid wasp

Immunization of mice

Competitive epitope analysis

Immuno-histochemistry and indirect immunofluorescence

Fluorescent, light and confocal microscopy

Isolation of hemocytes and preparing of hemocyte lysate

Immunoprecipitation

Western-blot analysis

Silver staining

Molecular biological methods (isolation of genomic DNA, total RNA, polymerase chain reaction, PCR coupled with reverse transcription

Microarray analysis

in situ RNA hybridization

in silico sequence analysis

Results and discussion

Our first aim was to identify the L1 protein, which is expressed by a type of blood cells, the lamellocytes, because we assumed that the L1 molecule would have a function in the encapsulation reaction or the maturation of lamellocytes. We showed that monoclonal antibodies which react with lamellocytes in the same pattern recognize three different epitopes of the L1 protein. We immunoprecipitated the L1 protein from hemocyte lysate, isolated it from acrylamide gel, and analysed its peptides by mass spectrometry, then identified the coding gene in the database. The gene we named as *atilla*, consists of three exons and is localised in the 33D2-D3 cytological region of second chromosome.

In order to examine the function of the gene, we generated the loss of function alleles of the gene by P-element remobilization. Analysis of the *atilla* null alleles revealed that they are homozygote viable and fertile with no phenotype in viability. We tested the immune response of the *atilla* null mutants against parasitoid wasp infection. The results showed that in the absence of the Atilla protein the differentiation and the function of lamellocytes, and the effectiveness of the encapsulation reaction did not change as compared with controls.

We also investigated the function of the *atilla* gene in genetic interaction with the *l(3)mbn-1* mutation, which shows robust lamellocyte differentiation and melanotic tumorous phenotype, and also with the hemocyte specifically driven UAS-DAlk,, which induces lamellocyte formation without melanotic tumorous phenotype. The *atilla* null alleles did not change the phenotype of the single mutations in the interaction, thus we concluded that the *atilla* gene do not play role in the lamellocyte differentiation through the genes examined in the interaction experiments.

We performed a genomic expression screen on the parasitic wasp induced *atilla* null mutant larvae. We collected the genes which showed expressional changes in the *atilla* null mutant larvae compared to the control in all the examined time points: 24, 48 and 72 hours after parasitoid wasp infestation. One third of the selected genes is associated with a known or a potential immune function, but it is still necessary to find their real connection with the *atilla* gene.

We analysed the expression pattern of Atilla during the ontogenesis. Immuno-precipitation and immune staining of different tissues showed that the Atilla protein is presented in the embryonal, in the larval and also in the adult stage. It appears on hemocytes only in larvae, but the Atilla protein is also expressed in many organs (gut, trachea, salivary gland, heart tube, endothelium).

The Atilla protein contains a GPI-anchoring site directly before its transmembrane domain, which serves as an alternative site of membrane binding. GPI-anchored proteins frequently occur in lipid rafts, which are special places for assembling receptor complexes. The colocalisation of the Atilla protein with lipid raft marker CTB suggests its association to lipid rafts. The Atilla protein is categorized in the family of u-PAR/Ly6 on the basis of its cystein-rich domain in the extracellular region. The members of this family are usually small polypeptides which have a GPI-anchor. They are expressed widely and have multiple roles, but most of them have a function in the immune response or in tumorous progress.

We have found structural homologs of the *atilla* gene by BLAST search with a protein sequence, in which similar protein domains can be obtained. Interestingly, no u-PAR/Ly-6 family members have been described in *Drosophila* yet. These genes are localised in the genome in miniclusters, containing two to five homologs. We named these genes

atilla-like genes. The deletion of the genomic region containing five *atilla*-like homologs recombined with the deletion of *atilla* and the neighbouring *atilla*-like gene did not affect the differentiation or function of the lamellocytes.

Our second aim was to analyse the origin of lamellocyte production, using *Atila* as a lamellocyte marker. We analysed the time-scale of appearance of the lamellocyte in the lymph glands and the circulation, and found that lamellocytes appear in the circulation earlier and in higher amount than in the lymph glands, which suggest their other place of origin. We have also separated physically by ligature the two compartments, which contain the two potential places of origin, the lymph glands, localized anteriorly in the larva, and the sessile tissue localized in the posterior part of the larva. We have analysed the number of hemocytes and lamellocytes as well as the efficiency of encapsulation reaction in the two compartments after wasp infestation and ligature. We have found that lamellocytes were produced only in the posterior part of the larva; however the lymph gland in the anterior part was uninjured. The encapsulation and melanization of the wasp eggs occurred also only in the posterior part. We described that lamellocytes can come from the posterior larval hematopoietic tissue, after wasp infestation.

Our third aim was to clarify the role of the L5 antigen, namely Filamin in lamellocyte differentiation. We have shown that lamellocytes express the 240 kDa isoform of Filamin encoded by the *cheerio* gene. The loss of function of *cheerio* caused lamellocyte differentiation in larvae without wasp infestation. We could rescue the phenotype of the *cheerio* mutant by transforming it with the cDNA of the *cheerio* gene. We proved that Filamin-240 is the suppressor of lamellocyte differentiation..

Publications:

Andó István, Laurinyecz Barbara, Nagy István, Márkus Róbert, Florentina Rus, Váczi Balázs Zsámboki János, Fehér László, Elisabeth Gateff, Dan Hultmark, Kurucz Éva: **Ősi örökségünk: a veleszületett immunitás. A *Drosophila* sejtes immunitása.** *Magyar Immunológia*, 2003, 2(4):39-45

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Oral presentations:

- 2002 32th Congress of the Hungarian Immunological Society,
Kaposvár, Hungary
- 2002 BRC of HAS, Straub Days, Szeged, Hungary
- 2003 33th Congress of the Hungarian Immunological Society,
Győr, Hungary
- 2004 BRC of HAS, Straub Days, Szeged, Hungary
- 2005 6th Hungarian Genetics Congress and 13th Cell- and Developmental
Biology Days, 2005 13th Symposium on Signals and Signal
Processing in the Immune System,
Balatonöszöd, Hungary
- 2009 15th Symposium on Signals and Signal Processing in the Immune
System, Balatonöszöd, Hungary

Posters:

- 2002 3th Hungarian Conference on Cytometry, Budapest, Hungary
- 2005 19th European Drosophila Research Conference, Eger, Hungary
- 2006 4th International Conference on Innate Immunity, Corfu, Greece
- 2007 36th Congress of the Hungarian Immunological Society,
Hajdúszoboszló, Hungary