ELSEVIER

Gene Expression Patterns 6 (2006) 928–934

GEP

www.elsevier.com/locate/modgep

Expression pattern of Filamin-240 in *Drosophila* blood cells

Florentina Rus [a,*], Éva Kurucz [a], Róbert Márkus [a], Sergey A. Sinenko [a,1], Barbara Laurinyecz [a], Csilla Pataki [a], János Gausz [a], Zoltán Hegedűs [a], Andor Udvardy [a], Dan Hultmark [b], István Andó [a,*]

[a] *Biological Research Center of the Hungarian Academy of Sciences, H-6726 Szeged, Temesvári krt. 62, Hungary*
[b] *Umeå Centre for Molecular Pathogenesis, Umeå University, S-901187 Umeå, Sweden*

Received 14 December 2005; received in revised form 18 February 2006; accepted 7 March 2006
Available online 10 March 2006

Abstract

The expression pattern of Filamin-240 was studied in subsets of *Drosophila* blood cells by means of immunofluorescent staining and Western blot analysis with use of an antibody specific to a "filamin-folding domain", a consensus motif profile generated from the 20 existing filamin repeats. Expression of Filamin-240 is restricted to lamellocytes – a special blood cell type of the cellular immune response – and is involved in the regulation of lamellocyte development. In the *cher*^{1} homozygous larvae, which lack Filamin-240 protein, a vigorous lamellocyte differentiation occurs which is further enhanced upon in vivo immune challenge by a parasitic wasp, *Leptopilina boulardi*. By introducing a full-length transgene encoding the *Drosophila* Filamin-240 protein into the *cher*^{1} Filamin-deficient homozygous mutant, the mutant blood cell phenotype was rescued. These data demonstrate that the expression of Filamin-240 is strictly lamellocyte specific in *Drosophila* blood cells and that the protein is a suppressor of lamellocyte development.

Keywords: *Drosophila*; Hemocyte; Blood cell; Lamellocyte; Encapsulation; Filamin; Actin network; Blood cell development; Immune induction

1. Results and discussion

The innate immunity of vertebrates shows remarkable similarities to the innate immunity of invertebrates and a common evolutionary ancestry may be proposed. *Drosophila*, similarly to other invertebrates, is able to mount a rapid and efficient humoral and cellular immune reaction in response to microbial and parasitic infection (Rizki et al., 1985; Hoffmann and Reichhart, 1997; Hultmark, 2003), therefore *Drosophila* has turned out to be a very useful model to study the basic principles of innate immunity.

The blood cells, or hemocytes, in *Drosophila* participate in the immune response (Rizki and Rizki, 1986; Vass and Nappi, 2000; Lavine and Strand, 2002) by synthesis of circulating antimicrobial peptides and phagocytosis of microbes by plasmatocytes, or the encapsulation of larger foreign bodies such as eggs of parasites by specialized flat cells, the lamellocytes (Rizki et al., 1985; Evans et al., 2003; Hultmark, 2003; Márkus et al., 2005). Lamellocytes are essentially absent in healthy uninfected larvae but they may appear in low numbers at the time of metamorphosis. However, immune induction by parasitization with the Hymenopteran wasp *Leptopilina boulardi* initiates the rapid differentiation of lamellocytes, which subsequently adhere to and surround the egg, which begin to melanize, thereby walling it off inside the larvae (Evans et al., 2003). A third class, the crystal cells are involved in melanin deposition in wounds and around foreign objects, a reaction, which follows the encapsulation reaction (Rizki et al., 1980; Rizki et al., 1985).

The further study of hemocyte development and function has been facilitated by the identification of gene

* Corresponding authors. Tel.: +36 62 599 677; fax: +36 62 433 503 (I. Andó).
E-mail addresses: tina@brc.hu (F. Rus), ando@brc.hu (I. Andó).
[1] Present address: Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, 621 Charles E. Young Drive, LSB 2204, Los Angeles, CA 90095, USA.

1567-133X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2006.03.005

products and genes whose expression is restricted to morphologically and functionally distinct classes of blood cells (Kurucz et al., 2003). For these purposes a library of hemocyte-specific monoclonal antibodies has been generated and used (Asha et al., 2003; Vilmos et al., 2004; Zettervall et al., 2004; Williams et al., 2005). One particular antibody, 4B8, reacted with the majority of lamellocytes in a hemocyte overproducing mutant stock, *l(3)mbn-1* (Shrestha and Gateff, 1982) (Figs. 1A and B), or following immune induction of wild-type Oregon-R larvae by the parasitic wasp *L. boulardi* (Figs. 1C and D). The antigen, recognized by the antibody, was detectable on permeabilized hemocytes, but not on native, live cells (data not shown) demonstrating it was expressed in the cytoplasm. Western blot analysis of extracts from *l(3)mbn-1* homozygous larvae yielded two major protein bands, corresponding to 90 and 240 kDa (Fig. 1E, lane 1). In extracts from the wild-type Oregon-R or the hemocyte-deficient dom^1 homozygous larvae, one, 90 kDa protein band was found (Fig. 1E, lanes 2 and 3). In hemocyte extracts prepared from the *l(3)mbn-1* homozygous mutants or from the wasp-infested Oregon-R larvae, a predominant 240 kDa protein was expressed (Fig. 1F, lanes 1 and 2), whereas the expression of the 90 kDa protein is under the level of detection or is very low. In the extracts derived from hemocytes of the non-infested Oregon-R larvae, the expression of these proteins is near the threshold of detection (Fig. 1F, lane 3). Thus, both the cellular expression pattern of the antigen

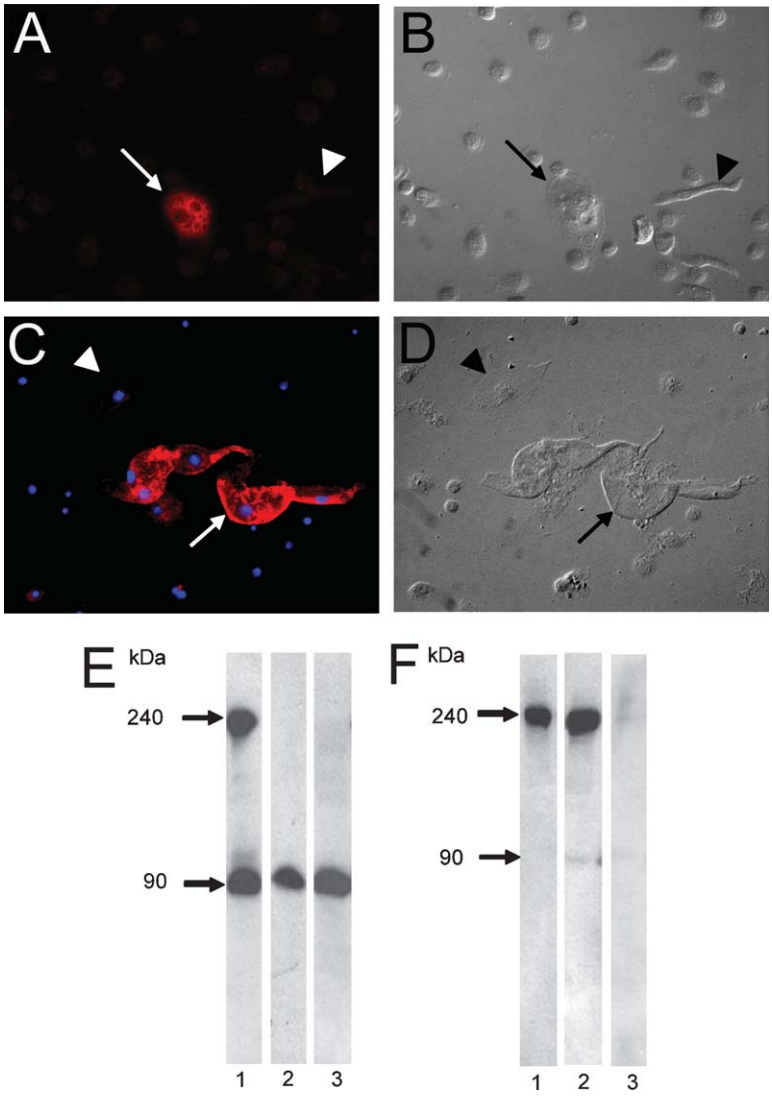

Fig. 1. The 4B8 antibody reacts with lamellocytes. Immunofluorescent detection of the recognized antigen in circulating hemocytes of *l(3)mbn-1* larvae (A) and wasp-infested Oregon-R larvae (C). Panels (B) and (D) are the corresponding Nomarski pictures. Red fluorescence is 4B8 staining, whereas the blue, DAPI-fluorescence, marks the nuclei. Lamellocytes expressing or non-expressing this antigen are marked with arrows and arrowheads, respectively. Western blot analysis on whole larval (E) and hemocyte extracts (F). In extracts from the *l(3)mbn-1* homozygous mutant larvae, two major protein bands corresponding to molecular masses of 90 and 240 kDa (E, lane 1) are seen. In extracts from the wild-type Oregon-R (E, lane 2) or the hemocyte-deficient dom^1 homozygous mutant larvae (E, lane 3), only the 90 kDa protein band is present. In the hemocyte extracts of *l(3)mbn-1* (F, lane 1) or in that of wasp-infested Oregon-R larvae (F, lane 2) a predominant expression of the 240 kDa protein is seen. Slot F, lane 3 is uninfested Oregon-R control.