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Drosophila Nimrod proteins bind bacteria

Research Article

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Abstract: Engulfment of foreign particles by phagocytes is initiated by the engagement of phagocytic receptors. We have previously reported that NimC1 is involved in the phagocytosis of bacteria in *Drosophila melanogaster*. We have identified a family of genes, the Nimrod gene superfamily, encoding characteristic NIM domain containing structural homologues of NimC1. In this work we studied the bacterium-binding properties of the Nimrod proteins by using a novel immunofluorescence-based flow cytometric assay. This method proved to be highly reproducible and suitable for investigations of the bacterium-binding capacities of putative phagocytosis receptors. We found that NimC1, NimA, NimB1 and NimB2 bind bacteria significantly but differently. In this respect they are similar to other NIM domain containing receptors Eater and Draper.

Keywords: *Phagocytosis • Receptor • Innate immunity • Drosophila*

1. Introduction

Phagocytosis is an ancient, evolutionarily conserved mechanism which in multicellular organisms is involved in tissue remodeling, the clearance of apoptotic cells and the elimination of microorganisms from the body [1]. In *Drosophila melanogaster*, phagocytosis is performed by specialized blood cells, the professional phagocytes known as plasmatocytes. The initial step of phagocytosis is the recognition of microbes or altered self-structures on the target particle. In *D. melanogaster*, a broad range of molecules have been reported to take part in this function, including the epidermal growth factor-like NIM repeat-containing receptors, such as Eater, Draper and NimC1 [2-4]. We have previously shown that the NimC1 receptor, a member of the Nimrod protein family, is involved in the phagocytosis of bacteria [2] and proposed that the *Nimrod* gene cluster is a functional module in innate immunity [5]. Members of the *Nimrod* gene cluster are located in close proximity to each other in the *Drosophila* genome and a number of them are up-regulated in response to an immune challenge, suggesting their co-regulation in the course of the innate immune response [5,6]. Nimrod proteins contain a signal peptide, 1-16 characteristic EGF-like domains, called NIM domains, which have a well-conserved consensus sequence [2], and a short conserved CCxGY motif, immediately preceding the first NIM domain. On the basis of their domain structure, NIM domain-containing proteins can be classified into three categories. The single NIM domain containing transmembrane protein NimA [2], the 1-8 NIM domain containing NimB-type proteins (NimB1, NimB2, NimB3, NimB4 or NimB5) which lack transmembrane domains, and the NimC-type (NimC1, NimC2, NimC3 or NimC4) transmembrane proteins with 2-16 NIM domains [2]. Although the *eater* and *draper* genes are not located in the *Nimrod* gene cluster, the encoded proteins exhibit the characteristic NIM domain structure. Eater and Draper receptors have been demonstrated to bind bacteria [7-9]. NimC1 has been reported to be involved in the phagocytosis of bacteria [2], however no biochemical assays were performed to prove the interaction of NimC1 or other Nimrod proteins with bacterial cells. In order to study the bacterium-binding properties of Nimrod proteins we have now developed an immunofluorescence and flow cytometry-based analysis with which to investigate the bacterium binding of native NimC1 and the recombinant NimA, NimB1, NimB2 and NimC1 proteins.

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