CONTRIBUTIONS OF MASS SPECTROMETRY IN THE UNDERSTANDING OF THE DROSOPHILA IMMUNE RESPONSE

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Research on the innate immune response of invertebrates has revealed several similarities with vertebrates. Insects have developed an acute response resembling that observed in humans, implicating effectors, receptors, and regulation of gene expression. Innate immunity is an ancient metazoan feature that allows animals to recognize typical chemical structures presented by invading microorganisms, and to activate a sophisticated set of humoral and cellular responses to control infection. The fruit fly *Drosophila melanogaster* has emerged in recent years as an original and powerful genetic model to study the evolutionary conserved genetic and molecular mechanisms operating in innate immunity. Higher insects such as *Drosophila* control infection by an array of innate immune reactions that include (i) phagocytosis and encapsulation by blood cells, (ii) proteolytic cascades leading to coagulation and melanisation, and (iii) secretion of a cocktail of potent AMPs. *Drosophila* is a well-recognized favorable genetic model system for the analysis of the first line defense against microorganisms. In 1991, when we started researching the immune response of this model, it was quite challenging to investigate, using conventional biochemistry and mass spectrometry (MS) approaches, the molecular mechanisms of the defense reactions in this genetic model for which the genome was not solved and annotated until 2000. Faced with this challenge, we developed refined sample preparations and methodologies – protocols taking advantage of ongoing improvements in MS, two-dimensional gel electrophoresis and bioinformatics - to perform differential analyses of blood (hemolymph) content from immune-challenged *versus* control *Drosophila*. Two strategies were developed virtually in parallel: (1) peptidomic analyses through matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS and high performance liquid chromatography for molecules below 15 kDa, and (2) proteomic studies based on gel electrophoresis, MALDI-TOF fingerprinting and database searches, for compounds of higher molecular mass. The peptidomic strategies led to the detection of an important number of peptides induced in the hemolymph of challenged flies as compared to controls. Of these, almost 30 molecules were characterized, amongst which were antimicrobial peptides. The gel electrophoresis strategy yielded the identity of a series of proteins that were potentially involved in the *Drosophila* immune response, including, to mention but a few of them, proteases, protease inhibitors, and serpins. Together, peptidomic and proteomic analyses serve the understanding of the molecular mechanisms involved in the innate immune reactions of *Drosophila*.

Keywords: Fruit fly, *Drosophila melanogaster*, hemolymph, innate immunity, mass spectrometry, 2D electrophoresis, peptidomics, proteomics