HLA-B27/hβ2m Drosophila a new model to study HLA-B27 implication in spondyloarthritis

Nadège Jah, Benjamin Grandon, Aurore Rincheval-Arnold, Isabelle Guénal, Sébastien Gaumer, Claudine André, Maxime Breban, Gilles Chiocchia

HLA-B27/hB2m DROSOPHILA A NEW MODEL TO STUDY HLA-B27 IMPLICATION IN SPONDYLOARTHRITIS

Jah N. [1,2] , Grandon B. [1,2.3] , Rincheval-Arnold A. [3] , Guenal I. [3] , Gaumer S. [3] , André C. [1,2] , Breban M. [1,2,4] , Chiocchia G. [1,2]

1 INSERM U1173 Inflammation and infection, France, Faculty of Health Sciences Simone Veil, Montigny-le-Bretonneux; 2 University of Versailles Saint-Quen-tin-en-Yvelines, Inflamex Laboratoire d'Excellence, Paris; 3 Laboratory Genetic and Cellular Biology, Faculty of Health Sciences Simone Veil, Montigny-le-Bretonneux; 4 Service de Rhumatologie, Ambroise Paré Hospital, University of Versailles Saint-Quentin-en-Yvelines, Boulogne, France

Introduction/Aim. Spondyloarthritis (SPA) is a group of chronic inflammatory disorders of the joint affecting primarily the axial skeleton but also peripheral limbs. Extra-articular manifestations are also considered a hallmark of SPA (e.g. inflammatory bowel diseases). SPA is highly inheritable, even though the environment plays an important role in the development of the disease. The most important part of heritability comes from the HLA-B27 allele of the Major Histocompatibility Complex (MHC), that is present in 70-90% of patients, as compared to 6-8% of the Caucasian population. HLA-B27 functions as a classical class I MHC molecule in most instances, in association with the β2-microglobulin (β2m) invariant light chain, by presenting antigenic peptides to CD8+ T cells. However, the mechanism(s) by which HLA-B27 contributes to SPA remain(s) poorly understood despite 45 years of research. Several theories have been proposed, none being yet proven. The "arthritic" peptide theory speculates on the canonical antigen-presenting function of a class I MHC molecule, proposing that particular peptides derived from the joint would be specifically presented to pathogenic CD8+ T cell, resulting in inflammatory disease. The failure to demonstrate the validity of such hypothesis, particularly in faithful animal model, fostered the emergence of novel theories implicating noncanonical characteristics of the HLA-B27 molecule. These include, a tendency of HLA-B27 to misfold, which could result in ER stress and unfolded protein response (UPR) eventually triggering an inflammatory response. However, the folding kinetics of different subtypes does not correlate with their disease association. Moreover, no UPR activation in HLA-B27+ cells from SPA patients could be observed, suggesting that HLA-B27 misfolding may not be fully relevant to pathogenicity. We chose to develop a model, which allows the study of HLA-B27 activities in the absence of its role on adaptive immunity that may mask some of its pathogenic effects.

Materials and Methods. Fruit fly is a well-studied and highly tractable genetic model organism and it is an invaluable system to understand complex molecular mechanisms. Indeed, most basic biological and physiological properties as well as signaling pathways are conserved between mammals and Drosophila, allowing to model genetic aspects of numerous human pathologies. It is being increasingly used to gain insight into the molecular and genetic aspects of inflammation. To further test the hypothesis that expression of HLA-B27 molecule, in association with β2m, triggers cellular disturbance, we produced transgenic Drosophila expressing SPA-associated HLA-B27 subtypes in combination with humanβ2m, speculating that this simplified animal model could facilitate deciphering of the non-canonical cellular and molecular effects of HLA-B27. Using the UAS-Gal4 inducible system, we tested the effect of different patterns of expression of these transgenes and highlighted genetic interaction with essential signaling pathways. We then tested whether our results could be extended to HLA-B27 transgenic rat model.

Results. Drosophila that are transgenic for SPA-associated B*2705 or B*2704 alleles, in combination with β2m, display striking phenotypes, including a wing crossveinless phenotype and a reduced eye size when the HLA-B27/hβ2m transgenes are co-expressed in these tissues. In contrast, neither

Drosophila single transgenic for HLA-B27 alone, nor transgenic for the SPA-non-associated allele HLA-B*0702 with or without hβ2m, developed such phenotypes. Interestingly, the wing phenotype appears to result from a genetic interaction with the BMP pathway and is associated with misregulation of dad, a BMP signaling target homologous to mammalian Smad7. Similarly, smad7 is found up-regulated in dendritic cells from HLA-B27 transgenic rat, as compared to HLA-B7 expressing cells.

Discussion. Interestingly, the BMP pathway is strongly involved in bone renewal, hematopoiesis and regulates the immune response in mammals. Moreover, the misregulation of the BMP pathway observed in our Drosophila model tends to remind of a rare human Mendelian disorder closely reminiscent to SPA, i.e. Fibrodysplasia Ossificans Progressiva.

Conclusions. Altogether, our results highlight how Drosophila can be used as a test-tube to study human diseases. The implication of BMP misregulation remains to be scrutinized to identify crucial steps of SPA development.