HLA-B27 Transgenic Rat OX62$^+$ DCs Exhibit Multiple Cellular Deficiencies and the Tolerigenic CD4$^-$ Subset Suffers Reduced Viability

M Dhaenens①, I Fert ②, S Glatigny②, S Haerinck①,C Hacquard-Bouder②, C André②, D Elewaut℘, D Deforce①, M Breban②.
Pharm. Biotech., Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium①
INSERM U567 Hôpital Cochin, Paris, France②
Dep. of Rheumatology, Ghent University Hospital℘

Background: Several lines of rats transgenic for HLA-B27 and human β2-microglobulin develop an inflammatory disease that strikingly resembles human SpA. It is hypothesized that disease in HLA-B27-transgenic rats arises as a consequence of interaction between antigen-presenting cells expressing high levels of HLA-B27 and peripheral T lymphocytes, and may result from a rupture of tolerance towards gut bacteria.
Methods: We used 2D PAGE and iTRAQ to compare the protein expression profile of HLA-B27 dendritic cells (DCs) to that of healthy HLA-B7 expressing and nontransgenic (NTG) rat DCs. MHC II surface expression and apoptotic sensitivity were quantified using flow cytometry.
Results: Three protein sets from the proteome analysis were indicative for aberrant cellular processes. First, all proteins involved in protein processing and MHC I assembly were upregulated in B27 DCs, illustrating the higher pressure on the ER due to misfolding of the HLA-B27 heavy chain. Second, all proteins directly influencing actin-dynamics were downregulated. We showed earlier that this not only influences motility, but also plays an important role in deficient immunological synapse formation.
Third, the key thiol protease Cathepsin S involved in MHC II synthesis was downregulated, which led us to quantify RT1-B and RT1-D surface expression. Downregulation concerned both CD4$^+$ and CD4$^-$ OX62$^+$ HLA-B27 DC subpopulations and maturation enlarged differences in both population bias and expression intensity. Deficient actin dynamics could also contribute to this lower MHC II surface expression. Study of sensitivity to MHC class II-mediated apoptosis by antibody stimulation showed that compared to NTG, both B7 and B27 CD4$^+$ DC were more prone to apoptosis but did not mutually differ. In contrast, overnight culturing resulted in a higher cell death in B27 than in control CD4$^-$ DC, even without antibody stimulation. Interestingly, decreased actin dynamics could also be involved in DC apoptosis.