Enrichment and identification of bioactive milk proteins by proteomic means

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Human milk contains a wide variety of proteins conferring unique qualities for growth promotion, development and well-being of the newborn. Nutrient content, promotion of beneficial gut microflora, enhanced immuno-competence, gut development and maturation as well as anti-microbial activity are examples of human milk benefits [1].
Often, only a specific milk fraction containing a subset of milk proteins is required to provide a defined biological activity. Milk fractionation is an important industrial process for obtaining specific milk pools with particular physico-chemical and biological properties [2,3]. Direct injection of complex protein mixtures, *e.g.* those derived from crude biological fluids like milk, is often incompatible with conventional liquid chromatography (LC), because of column clogging and rapid deterioration of chromatographic performance. We therefore developed and applied restricted access media (RAM), a chromatographic support combining size exclusion and anion exchange in the same resin [4], to rapidly and efficiently fractionate human breast milk and to evaluate the resulting fractions in terms of biological activity. The specific activity of interest was the soluble-CD14 (sCD14)-related immune response [5], which correlates with the presence of sCD14 in human milk [6]. A recent study revealed an interaction between the soluble Toll-like receptor 2 (sTLR2) and sCD14 in plasma and milk, suggesting a novel and specific innate immune mechanism regulating microbe-induced TLR triggering [7]. The biological context of sCD14 activity is of great interest to better understand molecular mechanisms of innate immune responses. The RAM resin was designed to specifically enrich sCD14 and its potential interaction partners. It yielded an sCD14-rich fraction and showed specific sCD14-dependent activity [8]. The characterization of the protein composition of this particular RAM fraction resulted in the identification of novel proteins potentially interacting with sCD14 [9]. In more detail, we report on on-line two-dimensional (2D) LC-MS/MS strategies implemented on different mass spectrometric (MS) platforms and combined with data post-processing to improve confidence in protein identification. Reproducibility and robustness of the approach were evaluated and reinforced by repeating the analysis five times. Loading the three most recent releases of the human EnsEMBL database for the searches highlighted the influence of changing database content on protein identification [9].

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