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LAMP2 as a marker of EBV-mediated B lymphocyte transformation in the study of lysosomal storage diseases

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Abstract Following the degradative pathway, vesicles loaded with extracellular material, eventually, dock and fuse with lysosomes, acquiring specific membrane markers of these organelles and acid hydrolases responsible for digest their content. The lysosomal-associated membrane protein 2 (LAMP-2), the best characterized lysosomal membrane protein, is found in late stages of endosome maturation and may be used as a marker of lysosome-associated membranes. Lysosomal storage disorders (LSDs) are described by the absence or deficiency in hydrolase activity leading to substrate accumulation within lysosomal components and to the onset of several diseases. It is known that lymphocytes infected by Epstein–Barr virus (EBV) are able to form cytoplasmic vacuoles, which work as a storage compartment for lysosomal acidic hydrolases. At the present study, we validate the EBV as a transforming agent of B lymphocytes in stability studies of long-term stored samples, since the methods used to keep samples in liquid nitrogen and thaw them have all proven to be efficient in samples frozen for up to 2 years. To confirm and investigate some of the most prevalent LSDs in the South of Brazil—Pompe, Fabry and Gaucher diseases—we first measured the enzymatic activity of α-glicosidase, α-galactosidase, and β-glicosidase in those cytoplasmic-formed vacuoles and then looked to LAMP-2 immunoreactivity by employing confocal microscopy techniques.

Keywords Lymphocyte transformation · Inborn errors of metabolism · Epstein–Barr virus · Lysosomal storage disorders · Pompe disease · Fabry disease · Gaucher disease

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Introduction

During endocytosis, extracellular material is actively recognized and internalized into membrane vesicles that are originated from the plasma membrane, the endosomes. After formation, the endosome is conducted by cytoskeleton elements to its final destination—the lysosomes—for degradation [1]. Throughout this process, the vesicle interacts with several components of the endocytic pathway, suffering constant membrane remodeling and changes in the lumenal content, as a result of membrane fusion and fission events. Firstly, the endocytic vesicle interacts with early endosomes, then with late endosomes, until finally to fuse with the lysosomes, a process that gradually acidifies the intra-vesicular content and increase hydrolytic activity. Meanwhile, some membrane components are recycled back to the plasma membrane, where they can be reused for cellular functions [1, 2].

One of the most significant changes that occur during endosome maturation is the reduction in pH levels to approximately 5. The low pH is crucial to the sorting and transport of lysosomal hydrolases from the Trans-Golgi Network to the mature endosome [3]. After transport the inactivated hydrolases are released into the lumen of acidic endosomes, while the receptors remain in the membrane and are eventually recycled back to the Golgi. Endosomes