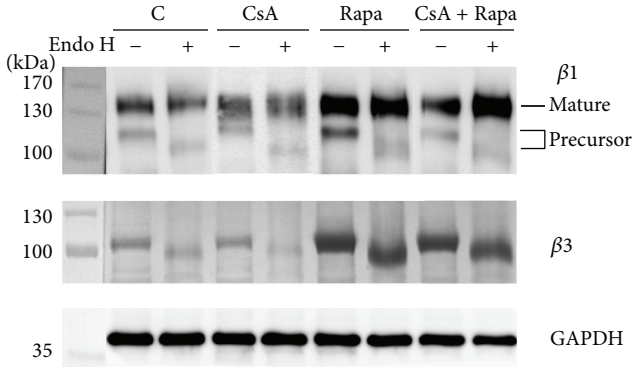

Figure 3: Rapamycin (Rapa) and the combination of cyclosporin A (CsA) and Rapa changes β1 and β3 integrin expression on MLR-activated cells. Peripheral blood mononuclear cells were cultured for 6 days in the presence of CsA (200 ng per 1 mL medium), Rapa (20 ng per 1 mL medium), and the combination of CsA (150 ng per 1 mL medium) and Rapa (12 ng per 1 mL medium) in a two-way mixed leukocyte reaction (MLR) and then lysed in RIPA buffer. Protein extracts (15 μg) were digested with endo-β-N-acetylglucosaminidase H (Endo H) from *Streptomyces plicatus*, SDS-PAGE-separated on 10% gel under reducing conditions, electroblotted onto a PVDF membrane, and probed with specific primary antibodies: mouse monoclonal anti-β1 (Chemicon, MAB2251, clone B3B11) and rabbit polyclonal anti-β3 (Chemicon, AB1932). Antibody-bound integrins were visualized by chemiluminescence. GAPDH was the endogenous control. C: untreated cells.

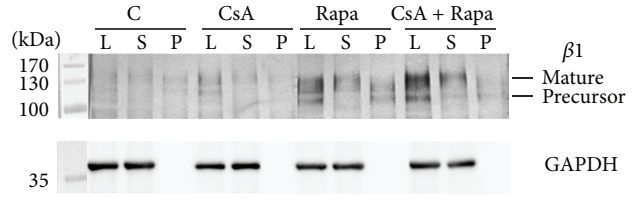

Figure 4: High-mannose/hybrid-type glycans are present mainly on premature β1 integrin subunit. Cell lysate (L), glycoproteins precipitated with GNA-agarose (P), and supernatant collected after precipitation (S), containing proteins not recognized by GNA, were resolved on 10% SDS-PAGE gel under reducing conditions, electrotransferred to a PVDF membrane and destined for β1 integrin subunit immunodetection. GAPDH was the endogenous control. C: untreated cells, CsA: cyclosporin A, and Rapa: rapamycin.

anti-LFA-1 protects against MHC-incompatible graft rejection of fetal small bowel grafts transplanted into mice, making this integrin a potential target for immunosuppression in intestinal transplantation. In turn, the effect of the novel immunosuppressive drug mycophenolate mofetil (MMF) on tumor cells was dose- and cell line-dependent. In kidney carcinoma Caki I cells and pancreatic carcinoma DanG cells treated with 0.1 μM and 1 μM MMF, the expression of integrins of the β1 subfamily ($\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$, $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$) was downregulated; in colonic adenocarcinoma HT-29, $\alpha3\beta1$, and $\alpha6\beta1$ integrins were upregulated in the presence of 1 μM MMF; and in prostate carcinoma DU-145 most of the analyzed integrins ($\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$, and $\alpha5\beta1$) were upregulated under both MMF doses [41]. Dexamethasone (DEX), a glucocorticoid used commonly for topical ocular application, upregulated $\alpha v\beta3$ integrin expression in the N27TM-2 cell line derived from human ocular trabecular meshwork, due to an increase of both the half-life and transcription of β3 integrin mRNA [42]. CsA also upregulated integrin β3 expression but in a dose-dependent manner, resulting in enhanced murine embryonic adhesion and invasion, which promoted embryo implantation [43]. Our work also showed an increase of β1 and β3 integrin expression in the presence of a therapeutic dose of Rapa and under CsA and Rapa combined (Figures 3 and 4). An earlier study demonstrated that integrins β1 and β3 mediate adhesion of murine $CD8^+$ cytotoxic T lymphocytes (CTL) to fibronectin (FN), which increased signal triggering by an association of proline-rich tyrosine kinase-2 (Pyk2) with paxillin and the Src kinases, resulting in MHC I-peptide-driven CTL degranulation [48]. β1 and β3 integrins participate in adhesion of activated T cells to ECM proteins upon TCR triggering or, spontaneously, in secondary lymphoid organs or inflamed tissues, where they become highly exposed to ECM proteins [48, 49]. In this context it is difficult to explain the increase of β1 and β3 integrin expression we observed upon immunosuppressive drug treatment, but we note that the upregulation of integrin expression was accompanied by an increase of high-mannose/hybrid-type oligosaccharides on β1 and β3 integrins, as shown by the reaction with GNA (Figure 2(a)). The total surface expression of the high-mannose/hybrid-type N-glycans was also influenced by the immunosuppressive drugs we used; the combination of Rapa and CsA markedly increased the amount of those structures (Figure 1). Only a few previous studies have addressed the effect of immunosuppressive agents on leukocyte glycosylation. Paul et al. [46] showed that MMF inhibited IL-1-induced expression of GNA-recognized oligosaccharides with terminal mannose (Man) on rat endothelial cells. The ability of MMF to downregulate glycosylation results from MMF inhibition of inosine-monophosphate dehydrogenase, the enzyme that catalyses biosynthesis of (deoxy) guanosine nucleotides necessary for transfer of Man and fucose to glycoproteins [46, 50]. MMF-induced reduction of the expression and glycosylation of some adhesion molecules decreases the recruitment of lymphocytes and monocytes to sites of inflammation and graft rejection [50]. Itraconazole (Ita), one of the mTOR inhibitors, reduced poly-N-acetyllactosamine and tetra-antennary complex-type N-glycans in human umbilical vein endothelial cells (HUVEC) and caused an increase of Man5GlcNAc2 oligomannose structures on vascular endothelial growth factor receptor 2 (VEGFR2). Hypoglycosylation of VEGFR2 strongly inhibited its autophosphorylation after VEGF stimulation [15]. Induction of hypoglycosylation on VEGFR2 in HUVEC cells by Ita was similar to the effects of this drug in macrophages RAW 264.7. Glycosylphosphatidylinositol-anchored glycoprotein CD14 in RAW 264.7 became Endo H-sensitive in the presence of Ita. CD14 with altered glycosylation was delivered to the cell surface, as determined by binding of concanavalin A (Con-A) [51]. Alteration of glycan synthesis by immunosuppressive drugs has also been observed in cancer cells. Treatment of MDA-MB231 breast cancer cells with Rapa upregulated the sialylation of N-glycans on β1 integrin