Antigenic Modulation of CD6 by Itolizumab is a Novel Mechanism for Effector T Cell Inhibition

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Methods

CD6 antigenic modulation by Itolizumab

PMNCs (pancreatic bed mononuclear cells) were thawed and incubated with Itolizumab or isotype (hIgG1) at 37°C. Surface level of CD6 was measured by flow cytometry using an anti-CD6 detection antibody that does not compete with Fc receptors (FcRs). Itolizumab binding to Fc receptors is a prerequisite for cleavage event is mediated by a serine protease that requires cell membrane mobility.

Concentration decrease in surface CD6 and increase in soluble levels of CD6 is observed following treatment with Itolizumab (A). Surface and soluble levels of CD6 on memory CD4+ T cells (CD4+CCR7-CD45RA-) decreased while soluble levels detected in the cell supernatant increased in a time-dependent manner following treatment with 10μg/ml Itolizumab. Levels of CD6 on CD4+ T cells treated alone or in combination with monocytes were measured by western blot and are shown in (B). Itolizumab-induced loss of surface CD6 is inhibited in the presence of AB123 (1:1000) fluorescent hydrolase, a serine protease inhibitor, and monoclonal (mAb) on the inhibitor of cell proliferation. (C) CD6 protein levels is reduced in total cell (lyste) following treatment with Itolizumab. Moreover, treatment with 10μg/ml inhibits loss of cellular CD6 induced by Itolizumab. (B) Soluble CD6 is only detected in the supernatant of Itolizumab treated PBMCs (PBMCs) in the absence of AB123. This suggest that Itolizumab induces cleavage of CD6 from the surface of T cells and that this event is mediated by a serine protease that requires cell membrane mobility.

Conclusions

Our results reveal a novel mechanism of antigenic modulation by Itolizumab in which CD6 is cleaved from the T cell surface and released in a soluble form. Cleavage of CD6 occurs via a membrane-bound serine protease and requires to be dependent upon the engagement of Itolizumab with Fc receptor(s) present on monocytes and NK cells. The loss of cell surface CD6 in T cells with reduced responses to TCR-mediated stimulation and allogeneicity, sCD6 is a sensitive composite of HLA that is primarily driven by alloreactive T cells. Here we show that in vitro, Itolizumab generates T cells that are hyporesponsive and less alloreactive to stimulation by a mismatched unrelated donor. This further validates the potential for CD6 to be a therapeutic target for treating tGVHD and for Itolizumab to be an effective treatment for alleviating disease pathology.

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Disclosures

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Stephen Connelly is currently an employee, stockholder, and officer of Equillium.

References


Additional Information

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Stephen Connelly, MD, who is the sponsor of this poster.