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Involvement of LIGHT in atherosclerosis and the large scale production of soluble LIGHT receptor TR2 for in vivo application

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The TNF superfamily member LIGHT has been shown to participate in inflammatory disease by inducing T cell mediated immune responses through interaction with its receptor TR2. Studies of LIGHT blockade have shown attenuating effects on allograft rejection and graft versus host disease. The high expression of LIGHT and TR2 on immune cells present in atherosclerotic lesions and their role in endothelial cell activation and platelet adhesion suggest their involvement in the inflammatory process of atherosclerosis. A significant role for LIGHT in atherosclerosis remains to be tested. In this work the effects of LIGHT on endothelial cells were initially demonstrated. Human umbilical venous endothelial cells (HUVEC) were isolated from human umbilical cords and cultured in endothelial growth medium. The cells were stimulated with recombinant human LIGHT to test its potential to cause endothelial activation. In consistency with previous studies LIGHT was demonstrated to induce expression of leukocyte adhesion molecules ICAM-1, VCAM-1 and E-selectin. LIGHT also induced a strong expression of vWF on endothelial cells, thereby exposing the mechanism behind LIGHT mediated adhesion of platelets to endothelial cells reported in earlier studies. Another interesting finding was LIGHT induced expression of Endoglin in endothelial cells. A significant role for Endoglin in the pathogenesis of atherosclerosis is yet to be determined, but increased expression of Endoglin in atherosclerotic lesions has been shown by immunohistological studies. The LIGHT-Endoglin interaction, demonstrated in the current study warrants for further investigation. The main work of this study then consisted of the large scale production of a soluble mouse LIGHT receptor to test the role of LIGHT in atherosclerosis in a disease model in vivo. A recombinant adenoviral vector was produced in large amounts and at high titre to express a mouse TR2 – human IgG fusion protein (mTR2-hIgG) in large cultures of CHO-cTA-CAR cells. The adenoviral/CHO cell protein production system provided a rapid way (weeks) to produce milligram quantities (milligrams) of recombinant protein at relatively low cost. We achieved a protein yield of 12,5 mg/L with a protein purity of 95%. In parallel mouse LIGHT cDNA was cloned and transfected into HEK 293 cells. The produced mTR2-hIgG protein was demonstrated to bind specifically and with high affinity to mouse LIGHT using flowcytometry analysis. The functional fusion protein can now be applied in vivo to determine the role of LIGHT in the pathogenesis of atherosclerosis.