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CD28^{NULL} T CELLS FROM MYOSITIS PATIENTS ARE CYTOTOXIC TO AUTOLOGOUS MUSCLE CELLS IN VITRO

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Background/purpose T cells are implicated in the disease pathogenesis of dermatomyositis (DM) and polymyositis (PM) but their specificity and effector functions in the inflamed muscle remain unclear. The authors have previously demonstrated that CD28^{null} T cells are the dominating T cell subset in affected muscle of patients with myositis. These cells are apoptosis resistant, pro-inflammatory and both CD4⁺CD28^{null} and CD8⁺CD28^{null} T cells from myositis patients contain perforin, hence are potentially cytolytic. Here, the authors investigated whether CD28^{null} T cells are directly cytotoxic to autologous muscle cells in vitro.

Method Autologous muscle cell - T cell co-cultures were performed in four patients with definite DM or PM and with >10% of CD4⁺CD28^{null} T cells in peripheral blood. Biopsy specimens were obtained from tibialis anterior muscle. Muscle biopsies were enzymatically digested to obtain myoblasts and differentiated into myotubes. PBMC were isolated into CD4⁺CD28^{null}, CD4⁺CD28⁺, CD8⁺CD28^{null} and CD8⁺CD28⁺ T cell populations by flow cytometry. Before co-culturing, myotubes were labeled with calcein and T cell subsets were stimulated with mitogen PHA. The ratios used for T cells versus myotubes were mostly 5:1 and 30:1 (depending on cell yield). Co-culture supernatants were harvested after 24 h, and calcein release, mirroring muscle cell lysis (myotoxicity), was measured. The results are expressed as percentage of maximal lysis with detergent Triton X-100, and were confirmed by morphological changes.

Result The authors could demonstrate myotoxicity of CD4⁺CD28^{null} T cells towards autologous muscle cells (PM1, 34.5%) and the effect was dose dependent (PM2, 43.4% (low ratio), 70% (high ratio); DM1, 15%, 25%; DM2, 2%, 9%). Surprisingly, the myotoxicity of CD8⁺ T cells were lower than that of CD4⁺ T cells. Still, the authors could see a dose response also for CD8⁺CD28⁺ T cells (PM1, 12.1% (low ratio), 39% (high ratio); PM2, 11.9%, 26.8%; DM1 0.7%, 5.1%; DM2

0.6%, 3.8%). The corresponding CD28⁺ T cell subsets demonstrated variable myotoxicity and our preliminary data suggest that this is directly dependent on cytokine output from these populations. Overall, myotoxicity was also reflected by morphological abnormalities in myotubes.

Conclusion Herein, the authors demonstrate for the first time that the muscle-dominating CD28^{null} T cells harm autologous muscle cells from myositis patients. More experiments are needed to establish whether direct cytotoxicity or cytokine-mediated effects is the dominant pathway for myotoxicity. Irrespective of that outcome, these data strengthen the notion that CD28^{null} T cells may cause muscle fiber destruction and hence directly contribute to the chronic inflammation in myositis.