We recently demonstrated that anti-inflammatory macrophages (Mph2) suppress T-cell responses and induce Treg by producing Reactive Oxygen Species (ROS). Mph2 may thus be instrumental in down-regulating T-cell responses against allografts. Here we investigated the effect of immunosuppressive drugs, as used after transplantation, on ROS production by Mph2 and subsequent T-cell activation. Macrophages were differentiated from monocytes with M-CSF, in presence or absence of cyclosporine A, FK506, dexamethasone, rapamycin, mycophenolic acid or prednisolone. During the differentiation of Mph2 it was shown that all immunosuppressive drugs, with dexamethasone being the most potent, increased the ROS producing capacity of Mph2. Furthermore, dexamethasone enhanced the T-cell suppressive capacity of Mph2 with regard to proliferation, IFN-γ and IL-4 production. These effects were partly dependent on ROS production. In contrast, rapamycin decreased both the ROS producing and T-cell suppressive capacity. Dex injection in congenic rats with normal ROS production resulted in upregulation of ROS production by macrophages and induced higher numbers of circulating Treg in a ROS-dependent fashion, whereas this was not shown in ROS hampered rats. In conclusion, dexamethasone enhances the macrophage-mediated suppression of T-cell activation mediated by increased ROS production and induces Treg.