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The Authors' Reply to TPA-2014-0477

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To the Editor:

We thank Masuda et al. for their discussion of a possible role of ILCs in the engraftment/rejection of allogeneic stem cell transplants. The main conclusion of our manuscript, “Natural Killer Cell Subsets Differentially Reject Embryonic Stem Cells Based on Licensing”, was that allogeneic embryonic stem (ES) cells are sensitive to cytotoxicity from natural killer (NK) cells *in vitro* and rejection *in vivo*. NK cells which expressed Ly49 molecules capable of binding self MHC-I molecules, termed “licensed”, showed higher cytotoxicity and were the dominant NK subset mediating the rejection of ES cells. Anti-ASGM-1 and anti-Ly49 antibodies were used to deplete all NK cells or NK subsets, respectively, to show *in vivo* proof-in-concept that NK cells could reject ES cells. However, it was correctly pointed out that these markers are also expressed on targets outside of NK cells, including ILC1 and ILC3 cells which have been reported to express low levels of Ly49 receptors (1). Thus differential engraftment of ES cells after anti-Ly49 administration may have been partially due to depletion of ILCs. However, a direct role of ILCs is unlikely in this scenario given the lack of cytotoxic function in these cells except for ILC1 which have been reported to exhibit low cytotoxic potential (2). Additionally, these cells are largely localized in mucosal areas and therefore unlikely to mediate rejection of a subcutaneous ES graft. ILCs may play a larger role in the setting of ES engraftment through their production of cytokines and modulation of T cell responses. However, in order to isolate NK cell responses we chose to utilize SCID mice which lack T and B cells. Thus, experimental models isolating ILC contributions to T cell-mediated rejection of allogeneic grafts of undifferentiated cells as well as potentially direct effects would be informative.

As Masuda et al. point out, current therapeutic strategies employing ES- or induced pluripotent stem (iPS)-derived products aim to ensure that undifferentiated cells are excluded which may limit the applicability of our results to current clinical practice. However, the objective of our manuscript was to define the immunogenicity of ES cells in the context of NK cell biology. NK cells have been reported to show increased sensitivity to undifferentiated cell types, with increasing tolerance as these cells differentiate (3). Therefore, NK cells may play an important role in rejecting residual pluripotent cells in the graft and preventing potential teratoma formation. A distinct role for ILCs and the extent of

the effects on overall engraftment in this process remains to be determined as this nascent field progresses.

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