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RESEARCH HIGHLIGHT

Meet the inlaws: Embryonic stem cell derivatives meet the immune system

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Since the derivation of embryonic stem (ES) cell lines from human blastocysts in 1998 [1], ES cells have emerged as a potential source of cells and tissues that could be used for cell replacement therapy of incurable degenerative diseases. This is due to their remarkable pluripotency, which enables them to differentiate into any adult cell type of the three embryonal germ layers. Indeed, several groups have reported the successful differentiation of ES cells into adult-type cell lineages including, but not limited to: cardiomyocytes, hematopoietic cells, hepatocytes, and neurons [2]. Currently, cell replacement therapy and organ transplantation are limited by a shortage of available donors; however, ES cells possess a high proliferative capacity and can be propagated indefinitely in culture without loss of pluripotency [3], suggesting that they could serve as an unlimited source of cells for the treatment of degenerative diseases.

In addition to establishing efficient protocols for the differentiation of ES cells into functional adult cells, one important aspect that requires attention is the interaction of the newly differentiated cells with both the innate and adaptive immune systems in the allogenic setting. Initial studies on ES cell immunogenicity by us and others, suggested that ES cells are immune privileged and may be readily transplanted across MHC barriers without or with minimal immunosuppression [4-7]. These experiments were based on both *in vitro* and *in vivo* experiments that appeared to suggest poor immunogenicity by ES and ES-like cells. In further support of this idea, *in vivo* studies utilizing a humanized mouse model to characterize human ES cell immunogenicity demonstrated that human ES cells induced considerably weaker allogenic immune responses than adult allografts [8]. However, it is now clear that non-differentiated ES cells cannot be used without prior differentiation and purification as they may cause teratomas [2]. Immunological studies on ES cell derivatives, however, are still lacking.

In this issue, Ladhoff and colleagues ask whether terminally differentiated endothelial cells derived from rat ES cell-like cells stimulate allogenic T-cell and B-cell responses [9]. Furthermore, the authors address whether inflammation augments allogenic immune responses against ES-derived endothelial cells, an important consideration because the application of ES cell therapies will likely cause some basal level of inflammation. First, MHC class I and class II expression on the ES-derived endothelial cells was compared to that of primary rat aortic endothelial cells, their adult counterparts. Interestingly, the ES-derived endothelial cells expressed significantly less class I antigens than the aortic endothelial cells, while the expression of class II antigens was absent on both cell types. The cells were then treated with IFN-γ to simulate inflammation, and although the expression of class I antigens on the ES-derived endothelial cells did increase, the total class I surface density was significantly lower on the ES cell-derived endothelial cells compared to the aortic endothelial cells. Interestingly, the expression of class II antigens remained absent on the ES-derived endothelial cells while expression on aortic endothelial cells was significantly upregulated. These findings suggested that in an inflammatory setting, ES-derived endothelial cells could evade immune recognition by $CD4^+$ T cells. Indeed, the ES-derived endothelial cells failed to stimulate the proliferation of alloreactive $CD4^+$ T cells *in vitro*, even when pre-treated with IFN-γ. In contrast, the adult-derived rat aortic endothelial cells significantly stimulated alloreactive $CD4^+$ T cell proliferation after exposure to IFN-γ. The ability of ES-derived endothelial cells to evade $CD4^+$ T cell recognition also suggested that they may be able to avoid rejection by helper T cell directed-immune responses, such as the generation of allo-antibodies. This notion was confirmed *in vivo* by detecting the levels of IgG-allo-antibody in the sera of immunized

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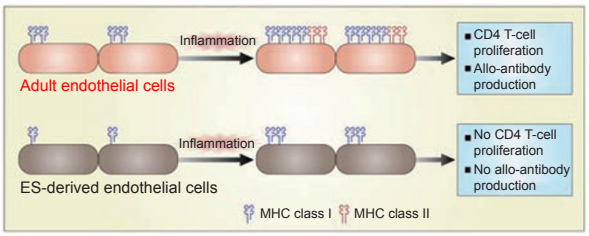

Figure 1 ES cell-derived endothelial cells are immune privileged. Expression of MHC class I antigens on ES cell-derived endothelial cells is less than that of adult endothelial cells. In response to inflammation, expression of class I and II antigens remains low or absent, respectively, on ES cell-derived endothelial cells, while their expression is markedly increased on adult endothelial cells. In contrast to adult endothelial cells, ES cell-derived endothelial cells do not stimulate allogenic T-cell or B-cell responses.

rats. Immunization with ES-derived endothelial cells, either with or without IFN-γ treatment, failed to induce the formation of allo-antibodies, while immunization with IFN-γ pre-treated adult derived aortic endothelial cells induced robust allo-antibody formation. These results demonstrate that ES-derived endothelial cells fail to stimulate alloreactive T- and B-cell responses, even in an inflammatory setting (Figure 1), and support previous conclusions that ES-derived cells are immune privileged [4, 7, 8].

While the findings of this study suggest that ES-derived cells may be used for the treatment of degenerative diseases without the need for immunosuppression, other questions still remain. First, the mechanisms involved in ES-derived cell immuno-evasion must be further elucidated. Do the ES-derived endothelial cells escape immune surveillance solely due to poor MHC antigen expression, or do they actively inhibit immune responses and induce tolerance? In a recent article, our group demonstrated that ES-derived hematopoietic cells established mixed chimerism across MHC barriers and induced tolerance to donor-type cardiac allografts with minimal host conditioning [10]. This was associated with the generation of intra-graft regulatory T cells. Whether allogenic ES-derived endothelial cells also induce transplantation tolerance remains unclear. Second, it will be important to determine whether ES-derived endothelial cells are capable of evading innate immune recognition, especially by NK cells. NK cells seek and destroy aberrant cells that have down-regulated the expression of MHC class I antigens. The low class I expression by ES-derived endothelial cells marks them as potential targets of NK cell cytotoxicity. While the answers to these questions remain outstanding, Ladhoff and colleagues' findings confirm that ES-derived cells, unlike adult tissues, might not require severe immunosuppressive regimens when transplanted across MHC barriers. In prospective studies, it will be intriguing to determine whether such conclusions can be drawn on human ES-derived tissues. More often than not, the immune system has one more trick up its sleeve.

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References

1 Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al*. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**:1145-1147.
2 Boyd AS, Higashi Y, Wood KJ. Transplanting stem cells: potential targets for immune attack. Modulating the immune response against embryonic stem cell transplantation. *Adv Drug Deliv Rev* 2005; **57**:1944-1969.
3 Williams RL, Hilton DJ, Pease S, *et al*. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature* 1988; **336**:684-687.
4 Fandrich F, Lin X, Chai GX, *et al*. Preimplantation-stage stem cells induce long-term allogeneic graft acceptance without supplementary host conditioning. *Nat Med* 2002; **8**:171-178.
5 Drukker M, Katz G, Urbach A, *et al*. Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci USA* 2002; **99**:9864-9869.
6 Li L, Baroja ML, Majumdar A, *et al*. Human embryonic stem cells possess immune-privileged properties. *Stem Cells* 2004; **22**:448-456.
7 Bonde S, Zavazava N. Immunogenicity and engraftment of mouse embryonic stem cells in allogeneic recipients. *Stem Cells* 2006; **24**:2192-2201.
8 Drukker M, Katchman H, Katz G, *et al*. Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells* 2006; **24**:221-229.
9 Ladhoff J, Bader M, Brosel S, *et al*. Low immunogenicity of endothelial derivatives from rat embryonic stem cell-like cells. *Cell Res* 2009; **19**:507-518.
10 Bonde S, Chan KM, Zavazava N. ES-cell derived hematopoietic cells induce transplantation tolerance. *PLoS ONE* 2008; **3**:e3212.