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A study of the immune properties of human umbilical cord lining epithelial cells

YI-JUN CAI[1,*], LIN HUANG[1,*], TAK-YEUNG LEUNG[2] & ANDREW BURD[1]

[1]*Division of Plastic, Reconstructive and Aesthetic Surgery, Department of Surgery, and* [2]*Department of Obstetrics and Gynaecology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, SAR*

Abstract

Background aims. Our previous study has demonstrated the stem cell-like properties of human umbilical cord lining epithelial cells (CLECs) and their capability for epidermal reconstitution in organotypic skin culture; however, the immunogenicity of these cells has not been clearly defined. We assessed several aspects of the immune properties of CLECs *in vitro*. *Methods.* We examined CLECs for their immunoregulatory function in a mixed lymphocyte culture experiment. We characterized the expression patterns of the major histocompatibility complex (MHC), co-stimulatory molecules and the pro-/anti-inflammatory cytokines and growth factors in CLECs by means of reverse transcription-polymerase chain reaction, Western blotting, flow cytometry and FlowCytomix multiple analyte detection assays. *Results.* CLECs were found not to induce but to suppress the proliferation response of the peripheral blood mononuclear cells in a mixed lymphocyte culture assay. They did not express the MHC class II antigen HLA-DR but the non-classic MHC class I antigens HLA-G and HLA-E and lacked the expression of the co-stimulatory molecules CD40, CD80 and CD86. In addition, they produced less interleukin-1β and transforming growth factor-β1 but more interleukin-4 and hepatocyte growth factor than did adult keratinocytes, a pattern in favor of wound healing with less inflammation response. *Conclusions.* Our data suggest that CLECs have an immunosuppressive function in addition to their low immunogenicity. This could be at least partially explained by their expression of HLA-G and HLA-E molecules associated with immune tolerance and absence of HLA-DR and co-stimulatory molecules. The demonstration that CLECs produce a favorable pattern of cytokines and growth factors for wound healing provides further support for their potential clinical application in allogeneic cell therapy.

Key Words: *cell therapy, immune property, umbilical cord epithelial cells, wound healing*

Introduction

Human umbilical cord lining epithelial cells (CLECs) have been demonstrated to have stem cell-like properties in our previous study (1). They display some stem cell-specific markers for epithelial as well as pluripotent stem cells, such as CK19, p63, OCT-4, SOX-2, SSEA-4, TRA-1-60 and Nanog. They also present a high proliferative potential and passaging ability in culture. In an organotypic skin culture model, CLECs are capable of generating a fully stratified epithelium, which indicates their clinical application potential in epidermal reconstitution. However, the major obstacle in the use of these cells as potential universal donor cells for epidermal reconstruction may be associated with their allogenic origin, which may lead to the graft-versus-host rejection in cell transplantation (2).

The immunogenicity of a donor cell is largely dependent on the expression of the major histocompatibility complex molecules (MHC) on the cell surface. MHC is also known as human leukocyte antigen (HLA) in humans. The HLA antigens may act as both a stimulus and a target in T-lymphocyte-mediated immune response. Of note, MHC class II molecules, especially HLA-DR, have been cited as a key identifying antigen in the "self" and "non-self" immune recognition mediated by T-helper cells, which is closely related to the immune rejection in allogenic cell/organ transplantation. On the other hand, the proliferation of T lymphocytes in an inflammatory immune response also requires the combined interaction with co-stimulatory molecules, for example, the B7 family members including B7-1 (CD80), B7-2 (CD86) and B7-3 and/or cell adhesion molecules such as CD40 (3,4).

*These authors contributed equally to this work.

Correspondence: **Andrew Burd**, MD, Division of Plastic, Reconstructive and Aesthetic Surgery, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China. E-mail: andrewburd@surgery.cuhk.edu.hk

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The expressions of CD80 and CD86 on antigen-presenting cells (APCs) are necessary for T-cell proliferation, differentiation, cytokine production, survival and memory development through interaction with the CD28 molecule on the T cell (5). The CD40 pathway plays a critical role at many levels of the sensitization and effector phases of allograft rejection, including T-cell priming, T-cell–mediated effector function and the activation of macrophages and natural killer (NK) cells (6). Recent studies have indicated that Wharton's jelly-derived mesenchymal stromal cells (MSCs) do not express the MHC-II and co-stimulatory molecules (CD80, CD86 or CD40), which may be the key mechanisms for MSCs to escape immune recognition by the recipient lymphocytes in allogenic transplantation (7,8).

There is also increasing evidence that the cells derived from human extra-embryonic tissues have immune-privileged properties. The cells from various parts of the term placenta and the umbilical cord have been shown to have low immunogenicity and are able to exert immunomodulatory function by different mechanisms (7,9–11). Two non-classic MHC class I molecules, HLA-G and HLA-E, are shown to be responsible for the immune tolerance observed in pregnancy, in which the immune accommodation between the mother and the child is viewed as an unusually successful allograft. The expression of HLA-G is highly tissue-restricted, and it was initially found in the cytotrophoblast. However, it has been recently demonstrated that HLA-G is also a crucial immunosuppressive molecule secreted by adult human MSCs (12). HLA-G acts through targeting all of the major immune cell subsets including $CD4^+/CD8^+$ T cells, B cells, NK cells and APCs (13), whereas HLA-E mainly functions as a ligand for the inhibitory NK cells to prevent cell lysis (14).

Regarding the immune response that may be involved in allogenic epidermal cell transplantation, the literature in early years has demonstrated that human keratinocytes participate directly or indirectly in the regulation of T-cell activation and proliferation in the recipient skin (15,16). The keratinocytes lack or express low levels of HLA-DR, B7-1 and B7-2, leading to ineffective antigen presentation in the host immune response. Meanwhile, on stimulation by the inflammatory cytokine interferon-gamma (IFN-γ), the keratinocytes could induce immune tolerance in T cells (17). In addition, the keratinocytes are shown to create a favorable cytokine and growth factor milieu that contributes to an accelerated wound-healing process (18). Vascular endothelial growth factor (VEGF), transforming growth factor beta1 (TGF-β1), fibroblast growth factor-basic (FGF-basic) and granulocyte colony-stimulating factor (G-CSF) are largely secreted by keratinocytes.

In the present study, the immune properties of CLECs have been assessed in an *in vitro*–mixed lymphocyte co-culture assay and also through examining the expression patterns of HLA and co-stimulatory molecules and the expression profile of inflammatory cytokines and growth factors in the cells. These all aim to investigate the potential beneficial effects of CLECs in allogeneic cell therapy.

Methods

Tissue collection

Human umbilical cord tissues were collected from cesarean section of full-term births, with written consent of the mothers in the Department of Obstetrics and Gynaecology, Prince of Wales Hospital, the Chinese University of Hong Kong. Surgical discarded normal skin tissues were collected from the Department of Surgery, Prince of Wales Hospital, with informed consent of the patients. All these procedures were approved by the Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee.

Cell culture

Primary culture of CLECs from human umbilical cord tissues and adult keratinocytes (AKs) from surgical discarded skin tissues were performed as previously described (1,19). Three primary cell lines of each were analysed in this study. Both cell types were cultured and expanded in Gibco Epilife medium supplemented with S7 (Invitrogen, Carlsbad, CA, USA). Cells were passaged on reaching 70–80% confluence. Cells at various passages were cryopreserved in Epilife complete medium supplemented with 10% dimethyl sulfoxide and 10% fetal bovine serum for the following experiments.

Mixed lymphocyte culture assay

In this experiment, the responder cells were human peripheral blood mononuclear cells (PBMCs) prepared by centrifugation of heparinized blood over a lymphocyte separation medium density gradient (Ficoll-isopaque, Sigma, St Louis, MO, USA). The stimulator cells included autologous PBMCs as a negative control, allogeneic PBMCs as a positive control and the testing CLECs. The PBMCs were co-cultured with mitomycin C-treated stimulator cells in 96-well plates. Culture of PBMC alone (without stimulator cells) and CLEC alone were set as additional controls. Three blood donors were used