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Negative regulation of HLA-DR expression on endothelial cells by anti-blood group A/B antibody ligation and mTOR inhibition

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ABSTRACT

Donor-specific antibody (DSA), particularly against HLA class II, is a major cause of chronic antibody-mediated rejection (CAMR) after transplantation, although ABO-incompatible kidney transplantation has recently demonstrated favorable graft outcomes. The condition of no injury even in the presence of anti-donor antibody has been referred to as "accommodation", which would be one of the key factors for successful long-term graft survival. The purpose of this study was to analyze the beneficial effect of anti-blood group A/B antibody ligation on endothelial cells against HLA-DR antibody-mediated, complement-dependent cytotoxicity (CDC). Blood group A/B-expressing endothelial cells EA.hy926 or Human Umbilical Vein Endothelia Cells (HUVEC) were incubated with IFNγ in the presence or absence of anti-blood group A/B antibody or mTOR inhibitor (mTOR-i) for 48 h. The effects on signaling pathway, HLA expression, complement regulatory factors, and CDC were investigated. Expression of HLA-DR on EA.hy926 or HUVEC were successfully elicited by IFNγ treatment, although little or no expression was observed in quiescent cells. Pre-incubation with anti-blood group A/B antibody had resistance to HLA-DR antibody-mediated CDC against IFNγ-treated cells in a concentration-dependent manner. This finding was ascribed to decreased expression of HLA-DR by post-translational regulation and increased expression of CD55/59, which was related to ERK and mTOR pathway inhibition. mTOR-i also inhibited HLA-DR expression by itself. Furthermore, the combination of mTOR-I and anti-blood group A/B ligation had an additive effect in preventing HLA-DR antibody-mediated CDC. Anti-blood group A/B antibody might play a preventive role in CAMR. Inhibition of the ERK and mTOR pathways may contribute to the development of a novel treatment in the maintenance period after transplantation.

1. Introduction

Donor-specific HLA antibody (DSA) can cause antibody-mediated rejection, leading to poor graft outcome in organ transplantation [1–3]. Many therapeutic efforts against B-cells have been attempted to prevent DSA-mediated rejection [4–6]. Moreover, HLA- or ABO-incompatible kidney transplantation (HLA-I or ABO-I) has been safely and effectively conducted thanks to the advancement of potent immunosuppressive drugs and B cell-targeted therapy such as rituximab [7]. However, under similar situations with anti-donor antibody, there seems to be a great difference in long-term graft survival between HLA-I and ABO-I. In particular, achievement of ABO-I is now comparable to ABO-identical or compatible transplantation (ABO-Id/C) [7,8].

It is well-known that the presence of de novo or preformed DSA, especially anti-HLA class II antibody would have a crucial effect on chronic antibody-mediated rejection (CAMR), exerting a negative influence on long-term outcome [9–12]. The medium-term effect of B cell targeted therapies on production of de novo DSA is still controversia [6,13]. Since favorable results have been reported from ABO-I without rituximab pretreatment [14–16], anti-blood group A/B antibody may have some beneficial effect on grafts unlike HLA antibody. Considering that there is no effective prevention or treatment against CAMR at present, the development of an innovative therapy would certainly be a challenging and necessary endeavor.

Accommodation is the most important concept for making a long-term success of organ transplantation. The term "accommodation" means a situation where the transplanted organ is not rejected even

Abbreviations: ABO-I, ABO-incompatible kidney transplantation; CAMR, chronic antibody mediated rejection; CDC, complement-dependent cytotoxicity; CIITA, class II transactivator; DSA, donor-specific antibody; HLA-I, HLA-incompatible kidney transplantation.

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in the presence of an anti-donor antibody [17–19]. Although accommodation has been observed clinically in ABO-I, its mechanisms have yet to be elucidated. Indeed, many researchers have spent their time evaluating antibody ligation-triggered immune reaction, intracellular signaling event and gene regulation [18,20–22]. We previously showed that anti-blood group A/B antibody ligation down-regulated endothelial cell activation–related signaling pathway, ERK, followed by induction of complement regulatory protein, CD55/59 [23], but it was reversed by thrombin and/or complement activation [24]. In contrast, low levels of anti-HLA antibody ligation activated the PI3K/AKT survival signaling pathway, whereas high levels up-regulated the ERK pathway, resulting in endothelial activation and graft rejection [25].

HLA class I molecules are widely expressed in all types of cells, while HLA class II expression is restricted to antigen presenting cells, such as B-cell and dendritic cells, and activated T cell [26]. Cultured endothelial cells do not express HLA class II molecules unless they are exposed to a specific stimulation, particularly IFNγ, which is produced during infection [27] or trauma [28] in vivo. Elegant HLA class II induction models of endothelial cells have demonstrated that IFNγ activates JAK-STAT signaling pathway, followed by the induction of class II transactivator (CIITA). DSA, especially anti-donor HLA class II (DR, DQ) antibody, is reportedly harmful and associated with CAMR in kidney transplantation. Since DSA could target induced HLA class II molecules on activated endothelial cells, the CAMR process might be accelerated. The mechanistic insight into the regulation of HLA class II protein on endothelial cells would provide a novel strategy for effectively preventing or delaying CAMR.

The aim of the current study was to elucidate the properties of activated endothelial cells regarding HLA class II molecule expression. However, although non-activated cultured endothelial cells express no or minimal quantities of HLA class II (DR, DQ) antigens, microvascular endothelial cells of normal kidney cortex in vivo constitutively express DR, but no (or a very low level of) DQ antigens [29,30]. Therefore, we focused our attention on DR expression at first. Under an IFNγ-stimulated condition, we investigated the effect of anti-blood group A/B ligation on antibody-mediated cytotoxicity to induced HLA-DR molecules. Furthermore, anti-blood group A/B ligation-triggered intracellular events in ERK and mTOR signaling pathways were also examined to clarify the underlying mechanisms.

2. Material and methods

2.1. Cell culture and materials

Blood group A or B antigen-expressing EA.hy926 cells (HLA-A; *24:02, *25:01, HLA-B; *15:01, *18:01, HLA-Cw; *03:03, *12:03, HLA-DRB1; *11:02, *13:42, HLA-DQB1; n/d, HLA-DPB1; n/d), the human endothelial-like immortalized cell line derived from the fusion of human umbilical vein endothelial cells (HUVEC) with the lung carcinoma cell line A549, were established as previously described [23]. EA.hy926 cells were maintained in DMEM supplemented with 10% FBS (Hyclone, Logan, UT). HUVECs were obtained from the Lonza Corporation (Walkersville, MD) and cultured in EBM2 endothelial cell medium (Lonza). Mouse monoclonal anti-HLA class I antibody (IgG2) (W6/32),

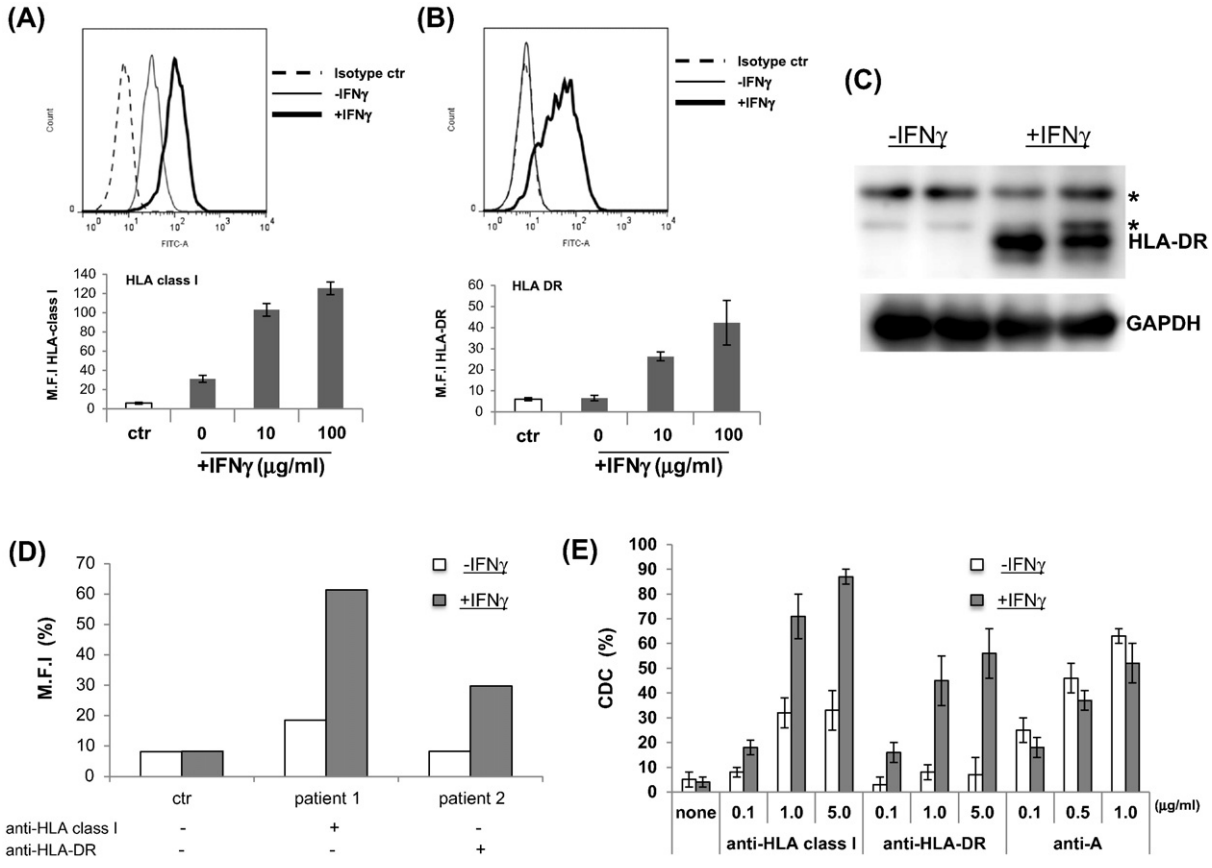

Fig. 1. Establishment of endothelial cells expressing a high level of HLA-DR. (A, B, and C) EA.hy926/A cells were treated with IFNγ for 48 h. Cells were collected and subjected to flow cytometry with FITC-labeled anti- HLA class I (A) or HLA-DR (B), and Western blotting with anti-HLA-DR antibody (**C**). Dot, thick, or bold line represents staining with isotype control (ctr), non-treated cells, or IFNγ treated cells, respectively. (D) EA.hy926/A cells were incubated with human serum obtained from anti-HLA antibody holding volunteers. Total IgG was measured by flow cytometry with anti-human IgG-FITC antibody. (E) Stimulated or unstimulated EA.hy926/A cells with IFNγ were incubated with the indicated amount of anti-HLA (0.1, 1.0, or 5 μg/mL), or -blood group A (0.1, 0.5, or 1.0 μg/ml) antibody for 1 h, followed by 5% complement incubation for 30 min. Cell cytotoxicity was measured by MTT assay. Data represent mean ± SEM (n≧3).