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Transplant Immunology 15 (2006) 187 – 197
Transplant Immunology
www.elsevier.com/locate/trim

HLA class I antibody mediated accommodation of endothelial cells via the activation of PI3K/cAMP dependent PKA pathway

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Received 29 August 2005; accepted 9 September 2005

Abstract

Allografts transplanted across ABO incompatibility or human leucocyte antigen (HLA)-sensitization undergoes antibody (Ab) mediated hyperacute rejection. Depleting anti-graft Ab from the recipient by plasmapheresis prior to transplantation can prevent this Ab-mediated rejection. Under these conditions, allografts have been shown to function even when the Ab rebound in the recipients. We have developed an in vitro model using human aortic endothelial cells (EC) and elucidated the ability of W6/32 HLA class I monoclonal Ab to provide signals following binding to MHC class I molecules. Using this model, we show that ECs undergo caspase 3-dependent cell death by apoptosis upon exposure to saturating concentrations of W6/32 and complement. In contrast, exposure of ECs to sub-saturating concentrations of W6/32 conferred resistance towards Ab/complement-mediated lysis that has been termed accommodation. Accommodated ECs exhibited a significant increase in the expression of anti-apoptotic genes Bcl-xL, Bcl-2 and Heme Oxygenase-1 and the induction of Phosphatidylinositol 3 kinase (PI3K) and cyclic adenosine monophosphate (cAMP) dependent protein kinase A activities that facilitate the phosphorylation of Bad at positions Ser^{136} and Ser^{112}. In conclusion, exposure of sub-saturating concentrations of HLA class I Ab results in the induction of signals downstream that confers resistance to endothelial cells against Ab-complement mediated cell death. Together, the observations made in this study will provide the basis for delineating the molecular mechanisms involved in mediating accommodation and developing strategies to induce accommodation in grafts prior to transplantation in highly sensitized patients.

Keywords: Antibodies; Endothelial cells; MHC; Signal transduction; Transplantation

1. Introduction

An ever-growing gap between the number of patients requiring transplantation and the number of donor organs available has become a major problem throughout the world. To overcome this, transplantations across human leucocyte antigen (HLA)/ABO blood group barriers are being performed with increasing frequency [1,2]. The major immunologic barrier to the successful transplantation of HLA/ABO-incompatible organs is the presence of antibody (Ab) against the donor antigens [3]. In clinical practice, great deal of efforts are taken to avoid hyper acute rejection (HAR) by ensuring allografts and recipients are matched for blood groups and the recipients are not sensitized towards donor HLA antigens. However, the persistence of circulating Ab against donor HLA antigens and/or other endothelial antigens may be responsible for a delayed form of rejection known as acute humoral rejection (AHR) [4].

Depleting anti-graft Ab from the recipient by plasmapheresis or immuno-adsorption prior to transplantation can prevent AHR and HAR [5–8]. However, despite chronic immunosuppression, anti donor Ab usually returns and persists after successful HLA/ABO-incompatible transplantation [6,8–10]. In some patients the graft continues to function well despite the continued presence of the Ab and the persistence of the target antigen in the kidney—a situation termed accommodation [6,8]. Reliable animal models for accommodation in xenotransplantation have been described by different groups [11–13]. The endothelial cells (EC) lining the graft are resistant to Ab/complement-mediated lysis and this was brought about by

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doi:10.1016/j.trim.2005.09.005

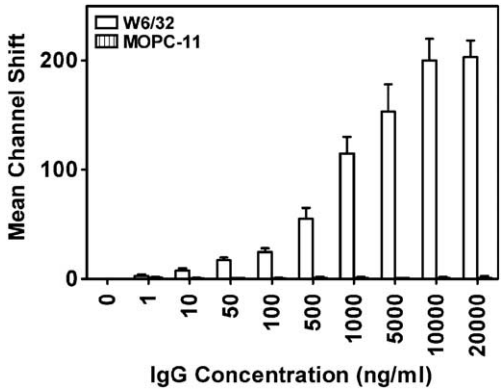

Fig. 1. Binding of W6/32 mAb to ECs. ECs were treated with various concentrations (1, 10, 50, 100, 500, 1000, 5000, 10,000, 20,000 ng/ml) of W6/32 mAb. The extent of binding of the Ab was detected by flow cytometry using FITC conjugated goat α mouse IgG. The results are represented as mean channel shift ±SD of 4 separate experiments.

the expression of the anti-apoptotic and anti-inflammatory genes such as Bcl-xL, Bcl-2 and heme oxygenase-1 (HO-1) [14,15]. The changes in the expression of these proteins have been attributed towards rendering ECs resistant to apoptosis and further damage [14–17].

Numerous studies have shown that HLA class I molecules can transduce signals that regulate various aspects of cell metabolism, including activation and cell growth or cell cycle arrest and apoptosis [18–20]. Ligation to HLA class I molecules expressed on ECs by HLA Ab lead to tyrosine phosphorylation of intracellular proteins like Src, paxillin and focal adhesion kinase [21], generation of inositol phosphates, increased fibroblast growth factor receptor cell surface expression [22,23] and enhanced EC proliferation [22,24,25]. Further, Jin et al. have shown that ligation of HLA class I molecules expressed on ECs by HLA class I specific monoclonal antibody W6-32 leads to the activation of PI3 K/Akt signaling pathway and induction of Bcl-xl and Bcl-2 [26].

There are many clinical reports of accommodation in transplanted allografts [6,8]. However, the events that lead to accommodation of such grafts are yet unknown. Furthermore, the physiological stimuli required for initiating accommodation are unknown. To elucidate the mechanisms underlying the phenotypic changes consistent with accommodation, we previously developed an in vitro model of accommodation using polyclonal HLA class I Ab and human aortic ECs. Using this model, we demonstrated that ECs exposed to sub-saturating concentrations of polyclonal HLA class I Ab are resistant to activation and Ab/complement-mediated cell death [27]. However, polyclonal HLA class I Ab obtained from transplant patient sera contains antibodies with varying affinity and avidity towards HLA. Therefore the phenotypic changes in EC induced by polyclonal HLA class I Ab may vary based on the affinity and avidity of the Ab pool. Further, development of a strategy to induce accommodation in transplanted grafts in a clinical setting would be far more efficient if the affinity and avidity of the HLA class I antibody used to induce accommodation were uniform. In order to characterize and standardize the phenotypic changes in ECs using HLA class I Ab with similar affinity and avidity, we determined the effects of HLA class I specific monoclonal Ab (mAb) W6/32 on endothelial accommodation in this study. Using this model, we have shown that ECs exposed to sub-saturating concentrations of monoclonal HLA class I Ab W6/32 are resistant to Ab/complement mediated cell death. The resistant ECs exhibit significant activation of Phosphatidylinositol 3 kinase (PI3K) and cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA) pathway, which results in the down regulation of Bad phosphorylation and an up-regulation of anti apoptotic genes Bcl-xL, Bcl-2, and HO-1.

2. Objective

The aim of this study was to develop an in vitro model using human aortic endothelial cells (EC) and understand the mechanism underlying HLA class I monoclonal Ab W6/32 mediated accommodation in the background of allotransplantation.

3. Materials and methods

3.1. Ab and reagents

Anti-Bcl-xL, anti-Bcl-2, and anti-HO-1 mAb was purchased from BD PharMingen (San Diego, CA). Fluorescein Isothiocyanate (FITC)-conjugated

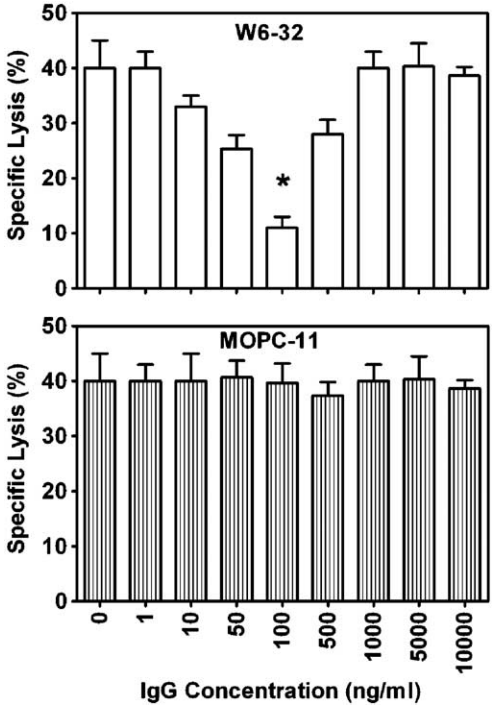

Fig. 2. Inhibition of complement-mediated lysis of ECs pre-treated with a Sub-sat of W6/32 mAb. ECs were pre-treated with various concentrations of (1, 10, 50, 100, 500, 1000, 5000, 10,000 ng/ml) W6/32 mAb or MOPC-11 mAb once daily for 3 days. The ECs were labeled with ^{51}Cr and re-exposed to 10,000 ng/ml of W6/32 mAb in the presence of class I rabbit complement. The release of ^{51}Cr in the cell supernatants was detected using a β-scintillation counter. The results are expressed as % lysis±SD of 4 separate experiments. There was a significant difference between lysis of cells pretreated with 100 ng/ml of W6/32 mAb and all the other cultures ($p<0.05$, Student's t-test).