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The avidity of cross-reactive virus-specific T cells for their viral and allogeneic epitopes is variable and depends on epitope expression

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ABSTRACT

Virus-specific T cells can recognize allogeneic HLA (allo-HLA) through cross-reactivity of their T-cell receptor (TCR). In a transplantation setting, such allo-HLA cross-reactivity may contribute to harmful immune responses towards the allograft, provided that the cross-reactive T cells get sufficiently activated upon recognition of the allo-HLA. An important determinant of T-cell activation is TCR avidity, which to date, has remained largely unexplored for allo-HLA-cross-reactive virus-specific T cells.

For this purpose, cold target inhibition assays were performed using allo-HLA-cross-reactive virus-specific memory $CD8^+$ T-cell clones as responders, and syngeneic cells loaded with viral peptide and allogeneic cells as hot (radioactively-labeled) and cold (non-radioactively-labeled) targets. CD8 dependency of the T-cell responses was assessed using interferon γ (IFNγ) enzyme-linked immunosorbent assay (ELISA) in the presence and absence of CD8-blocking antibodies.

At high viral-peptide loading concentrations, T-cell clones consistently demonstrated lower avidity for allogeneic versus viral epitopes, but at suboptimal concentrations the opposite was observed. In line, anti-viral reactivity was CD8 independent at high, but not at suboptimal viral-peptide-loading concentrations.

The avidity of allo-HLA-cross-reactive virus-specific memory $CD8^+$ T cells is therefore highly dependent on epitope expression, and as a consequence, can be both higher and lower for allogeneic versus viral targets under different (patho)physiological conditions.

1. Introduction

In humans, the estimated T-cell receptor (TCR) repertoire after positive and negative selection in the thymus covers around 10^8 unique TCR clonotypes [1]. If these would only reflect single specificities, the TCR repertoire would be far too restricted to cope with the broad array of mutating pathogens encountered throughout life. T cells therefore have the intrinsic ability to cross-react to multiple viral epitopes, a phenomenon known as heterologous immunity. In recent years, it became clear that virus-specific T cells not only have the ability to cross-react to multiple viral peptides, but also to allogeneic HLA (allo-HLA) molecules. Such cross-reactivity is very common and occurs within all individuals [2]. Heterologous immune responses of virus-specific T cells that are directed against allo-HLA molecules could pose a threat to both hematopoietic and solid organ transplantation. Indeed, animal models have shown that virus-specific T cells can actively hamper tolerance induction and mediate allograft rejection [3]. Recently, we reported the first ex vivo analysis of virus-specific T cells possessing cross-reactivity to donor peptide:HLA antigens in renal allograft recipients. In 13 of 25 transplant recipients, cross-reactivity to donor antigen was demonstrated within T-cell populations specific for viral epitopes [4]. Remarkably, the presence of donor cross-reactive T cells in the circulation

Abbreviations: Allo-HLA, allogeneic HLA; CMV, cytomegalovirus; CRA, ^{51}chromium-release assay; EBV-LCL, Epstein-Barr Virus transformed lymphoblastoid cell line; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; FLU, influenza virus; FRET, Förster resonance energy transfer; HS, human serum; HUVEC, human umbilical vein endothelial cell; IFNγ, interferon γ; ITAM, immunoreceptor tyrosine-based activation motif; mAb, monoclonal antibodies; ME, β-mercaptoethanol; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PE, phycoerythrin; PHA, phytohaemagglutinin; pMHC, peptide-MHC; SPR, Surface Plasmon Resonance; SSO, sequence-specific oligonucleotide; SSP, sequence-specific primer; TCR, T-cell receptor; VZV, varicella zoster virus

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of these transplant patients was not associated with inferior outcomes relative to patients who lacked these cells. However, to determine the clinical consequences of virus-specific T cells with cross-reactivity to allo-HLA, further and more extensive (prospective) clinical studies are necessary.

The potential of allo-HLA cross-reactive T cells to harm an allograft depends on their effector function, which in turn hinges on TCR avidity: the cumulative strength of all non-covalent binding interactions between a T cell and its target cell. Indeed, high avidity donor-reactive cytotoxic T cells have been associated with acute rejection of cardiac allografts [5,6]. Avidity is largely defined by the interaction between a TCR and its peptide-MHC (pMHC) ligand (TCR affinity), but additional cell surface molecule interactions (e.g. TCR dimerization), co-receptor binding and cell adhesion molecules also play an important role. Indeed, TCR affinity and avidity are unmistakably correlated with TCR signal strength and T-cell activation [7–9].

Variation in T-cell ligation has been shown to induce differences in downstream signaling pathways, mainly by altered phosphorylation downstream of the TCR [10,11]. Phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) plays a crucial role in determining “T-cell fate” by promoting different TCR signaling pathways [12]. TCR ligation of a T cell with high avidity for its ligand is more likely to induce a full-blown T-cell response compared to TCR ligation of a T cell with low avidity for its ligand, resulting in more pronounced or even qualitatively different effector functions [13,14]. Accordingly, TCRs generally bind with higher affinity to agonistic peptides compared to partial agonists or antagonists [15]. As a result of thymic selection, the peripheral TCR repertoire expresses low to moderate avidity for self-HLA, thereby avoiding T-cell activation and autoimmunity. Since allo-HLA is not expressed in the thymus, allo-HLA cross-reactivity is not restricted by positive and negative thymic selection. Therefore, TCR avidity for allo-HLA could in theory have a much broader spectrum compared to self-HLA.

TCR affinity and avidity can be addressed by several techniques: for example, competitive tetramer-staining can be used to estimate the hierarchy of TCR avidity for nominal and allogeneic epitopes [16,17] while techniques such as Surface Plasmon Resonance (SPR) [18], Förster resonance energy transfer (FRET) [19] and the mechanical micropipette adhesion frequency assay [20] can address technical kinetics such as half-life and association / dissociation rates of the TCR-pMHC complex. Yet, a downside of these assays is that they require comprehensive knowledge of the recognized epitope(s), which in case of allo-HLA cross-reactive T cells not only requires identification of the allo-HLA, but also the allopeptide. The latter is laborious and time-consuming and has only been done for two human TCRs with the same viral specificity [16,21]. As a consequence, much is still unknown about TCR affinity and avidity of virus-specific cross-reactive T cells. Here, we aimed to characterize the relative avidity of human cross-reactive virus-specific $CD8^+$ memory T cells for nominal and allogeneic epitopes by using different techniques.

2. Materials and methods

2.1. Collection of responder and target cells

Peripheral blood mononuclear cells (PBMCs) of healthy individuals were obtained after informed consent in accordance with the Declaration of Helsinki. Phytohaemagglutinin (PHA) blasts were generated by incubating PBMCs for 7 days in RPMI 1640 medium (Gibco, Carlsbad, CA) supplemented with penicillin/streptavidin, glutamine, 15% human serum (HS), and PHA (4 μg/mL; Murex Biotech Ltd, Dartford, UK).

Epstein-Barr Virus transformed lymphoblastoid cell lines (EBV-LCLs) were generated by incubating PBMCs for 1.5 hours at 37 °C with supernatant of the EBV-producing marmoset cell line B95.8, and subsequently cultured in Iscove's Modified Dulbecco's Medium (IMDM; Lonza, Basel, Switzerland) supplemented with penicillin/streptavidin, glutamine and 10% fetal calf serum (FCS).

Human umbilical vein endothelial cells (HUVECs) were cultured in M199 medium (Gibco) supplemented with 10% FCS, sodium pyruvate (Gibco), penicillin/streptavidin (Gibco) and β-mercaptoethanol (0.05 M; Sigma Aldrich, St. Louis, MO), and were used at passages P1-4.

HLA typing was performed by sequence-specific oligonucleotide (SSO) and sequence-specific primer (SSP) genotyping at the European Federation of Immunogenetics (EFI)-accredited national reference laboratory for histocompatibility testing at the Leiden University Medical Center, Department of Immunohaematology and Blood Transfusion, the Netherlands.

2.2. Generation of virus-specific $CD8^+$ T-cell lines and clones

Virus-specific $CD8^+$ memory T-cell lines and clones were generated by fluorescence-activated cell sorting (FACS Aria; BD) as previously described [22]. PBMCs were stained with phycoerythrin (PE)-labelled viral tetramers CMV pp65(123-131) HLA-B∗35:01/IPSINVHHY (CMV B35/IPS), FLU MP(58–66) HLA-A∗02:01/GILGFVFTL (FLU A2/GIL), VZV IE62(593-601) A∗02:01/ALWALPHAA (VZV A2/ALW), and EBV EBNA3A(325-333) B∗08:01/FLRGRAYGL (EBV B8/FLR) (Protein facility of the Leiden University Medical Center, Department of Immunohaematology and Blood Transfusion, the Netherlands) and fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies (mAb) against CD4, CD19, CD45-RA, CD14, CD40, CD16 and CD56 (BD Pharmingen, San Diego, CA). The FITC channel (FL1) was used as a dump channel.

2.3. Cold-target inhibition

Cold target inhibition assays were performed to define TCR avidity for syngeneic HLA + viral peptide versus allo-HLA + endogenous (allo) peptide. Hereto, the ^{51}Chromium release assay (CRA) [23] was altered by including not only hot (radioactively-labeled) but also cold (non-radioactively-labeled) targets in different cold / hot target ratios (1:1; 2.5:1; 10:1; 20:1). EBV-LCLs and HUVECs were used as target cells. Both syngeneic HLA + viral peptide and allogeneic HLA + (allo)peptide target cells were employed as hot and cold targets, and different peptide-loading concentrations were used (ranging between 0.01 ng/ml to 1000 ng/ml). All conditions (disregarding validation) were performed in triplicate at effector:target (E:T) ratio 1:1.

2.4. CD8 blocking assays

CD8 blocking was assessed in both IFNγ ELISA and ^{51}Chromium release assay. Virus-specific $CD8^+$ T-cell clones ($5x10^3$) were incubated with or without CD8 blocking antibody FK18 (7.7ug/ml; 1 hour 37 °C) as described previously [24]. For IFNγ ELISA, T-cell clones were co-cultured for 24 hours with EBV-LCLs ($5x10^4$) expressing either self-HLA, self-HLA + viral peptide, or allo-HLA molecules (triplicate wells; 24 hours at 37 °C). Culture medium consisted of IMDM (Lonza) supplemented with penicillin/streptavidin, glutamine, 5% FCS (Lonza), 5% HS, and IL-2 (10 U/mL). After 24 hours, IFNγ production was assessed in a standard enzyme-linked immunosorbent assay (ELISA; U-CyTech, Utrecht, The Netherlands) according to protocol. Different peptide-loading concentrations were used (ranging between 0.01 ng/ml to 1000 ng/ml). For ^{51}Chromium release assay, the experiments were performed with EBV-LCLs and PHA blasts as previously described [23]. All conditions were performed in triplicate at effector:target (E:T) ratio 1:10 (ELISA) and 1:1 (CRA) respectively.