Clinical Immunology (2015) **159**, 63–71

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Identification of autoreactive CD8$^+$ T cell responses targeting chromogranin A in humanized NOD mice and type 1 diabetes patients

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Received 23 August 2014; accepted with revision 26 April 2015
Available online 6 May 2015

KEYWORDS
Type 1 diabetes;
Epitope;
Chronogranin A;
Humanized NOD mice

Abstract ChgA has recently been identified as the autoantigen for diabetogenic CD4$^+$ T cells in NOD mice and T1D patients. However, autoreactive CD8$^+$ T-cell responses targeting ChgA haven't been studied yet. Here several HLA-A*0201-restricted peptides derived from mChgA and hChgA were selected by an integrated computational prediction approach, followed by an HLA-A*0201 binding assay. MChgA$_{10–19}$ and mChgA$_{43–52}$ peptides, which bound well with HLA-A*0201 molecule, induced significant proliferation and IFN-γ-releasing of splenocytes from diabetic NOD.β2m^{null}.HHD mice. Notably, flow cytometry analysis found that mChgA$_{10–19}$ and mChgA$_{43–52}$ stimulated the production of IFN-γ, perforin, and IL-17 by splenic CD8$^+$ T cells of diabetic NOD.β2m^{null}.HHD mice. Furthermore, hChgA$_{10–19}$ and hChgA$_{43–52}$-induced IFN-γ releasing by specific CD8$^+$ T cells were frequently detected in recent-onset HLA-A*0201-positive T1D patients. Thus, this study demonstrated that autoreactive CD8$^+$ T cells targeting ChgA were present in NOD.β2m^{null}.HHD mice and T1D patients, and might contribute to pathogenesis of T1D through secreting proinflammatory cytokines and cytotoxic molecules.

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http://dx.doi.org/10.1016/j.clim.2015.04.017

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by T cell-mediated destruction of insulin-producing pancreatic islet β cells [1]. It has been commonly accepted that $CD4^+$ T cells play an important role in initiation and progression of T1D [2]. However, autoreactive $CD8^+$ T cells have been proved to play an indispensable key role in the destruction of islet β cells and development of T1D [3]. $CD8^+$ T cells may be the early initiators that mediated the destruction of islet β cell in nonobese diabetic (NOD) mice. MHC class I molecule-deficient NOD mice do not develop insulitis [4,5], and some islet-derived $CD8^+$ T cell clones transfer diabetes in the absence of $CD4^+$ T cells [6,7]. Several observations in humans also inferred the functional importance of $CD8^+$ T cells in the destruction of human islet β cells [8–11]. Thus, the identification of epitopes from islet β cell antigen recognized by $CD8^+$ T cells in humans not only can provide new tools for a more accurate prediction of T1D, but also is important for the elaboration of new immunotherapeutic strategies.

Interestingly, most β-cell antigens, such as insulin [12,13], glutamic acid decarboxylase (GAD) [14,15], and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) [16,17], can be recognized by both islet-specific $CD4^+$ T cells and $CD8^+$ T cells. Chromogranin A (ChgA) has recently been identified as an autoantigen for diabetogenic $CD4^+$ T cells in both NOD mice [18,19] and human T1D subjects [20]. However, it remains unknown whether ChgA can be recognized by autoreactive $CD8^+$ T cells in T1D. Human histocompatibility leukocyte antigen-A*0201 (HLA-A*0201) is the most commonly expressed HLA class I allele in Caucasians and Asians (50%), and contributes to the susceptibility to T1D [21]. T1D onset is significantly accelerated in HLA-A*0201 transgenic NOD mice with HLA-A*0201-restricted CD8 T cells appearing in early, prediabetic insulitic lesions [22]. Therefore, several studies have used humanized NOD.β2m^{null}.HHD mice to identify β cell autoantigen-derived peptides that are recognized by HLA-A*0201-restricted $CD8^+$ T cells with potential clinical relevance to human T1D [21,23,24]. However limited peptides have been identified as targets recognized by HLA-A2-restricted $CD8^+$ T cells in human T1D. In this study, we tested the autoreactivities of $CD8^+$ T cells against several HLA-A*0201-restricted candidate epitopes from murine and human ChgA protein in NOD.β2m^{null}.HHD mice and HLA-A*0201 positive T1D patients, respectively.

2. Materials and methods

2.1. Mice and T1D subjects

NOD.β2m^{null}.HHD mice were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). The mice were bred and maintained in specific pathogen-free facilities. All animals were treated according to "Principles of Laboratory Animal Care and Use in Research" (Ministry of Health, Beijing, China). All experimental protocols were approved by the Animal Ethics Committee of the Third Military Medical University.

Fresh blood samples were obtained from 10 HLA-A*0201-positive (age [mean ± SD] 10.3 ± 3.4 years, range 5–14 years, 40% female) and 5 HLA-A*0201-negative (10.4 ± 5.0, 5–18, 40%) T1D patients and 8 HLA-A*0201-positive healthy controls (29.6 ± 7.2, 22–41, 50%). All patients with recent-onset T1D (disease duration 5–389 days) were lean and presented at diagnosis with acute onset of symptoms, and have required permanent insulin treatment from the time of diagnosis. These patients were all positive for autoantibodies to islet cell, GAD and/or insulin. HLA-A*0201-positive T1D patients and healthy controls were identified by flow cytometry (BD Bioscience, Franklin Lakes, NJ, USA) using an aliquot of the cells stained with allophycocyanin (APC)-conjugated anti-HLA-A2 mAb BB7.2 (eBiosciences, San Diego, CA, USA). The study protocol was approved by the ethics committee of the Third Military Medical University, and informed consent was obtained from all participating subjects in this study.

2.2. Peptides

To screen candidate HLA-A*0201 binding peptides within murine Chromogranin A (mChgA) (GeneBank accession no. P26339) and human ChgA (hChgA) (GeneBank accession no. P10645), an integrated approach combining three online T-cell epitope prediction algorithms including SYFPEITHI (http://www.syfpeithi.de/), IEDB (http://tools.immuneepitope.org/mhci/), and NetMHC (http://www.cbs.dtu.dk/services/NetMHC/) was used. The peptides scored top 10 were collected in SYFPEITHI method, and the cutoff standard of other two methods is $IC_{50} < 500$ nm. The predicted candidate m/hChgA peptides and control peptides (HIV $Pol_{476–484}$ ILKEPVHGV, $IGRP_{206–214}$ VYLKTNVFL, $mInsA_{2–10}$ IVDQCCTSI, $IGRP_{265–273}$ VLFGLGFAI), were synthesized with purity >95% at Chinese Peptide Company (Hangzhou, China). These peptides were dissolved in DMSO (dimethyl sulfoxide, DMSO) at a concentration of 20 mg/mL, and stored at −80 °C.

2.3. HLA-A*0201 binding assay

T2 cells (1×10^6/mL) were incubated with each peptide (10 μg/mL) and 3 μg/mL beta-2-microglobulin (Sigma-Aldrich, St. Louis, MO, USA) for 16 h at 37 °C. Then the cells were washed and stained with anti-HLA-A2 mAb BB7.2 (BD Bioscience), followed by incubation with FITC-conjugated goat anti-mouse IgG (Beyotime, Jiangsu, China), and analyzed using an FACS Canto cytometer (BD Bioscience).

2.4. Splenocyte proliferation assay

Splenocytes freshly isolated from 12- to 16-week-old diabetic female NOD.β2m^{null}.HHD mice were co-cultured in triplicate with 10 μg/mL indicated peptides in 96-well plates. After incubation at 37 °C for 72 h, [^{3}H] thymidine (1 μCi/well) was added for an additional 16 h of culture, and uptake of [^{3}H] thymidine was determined using a liquid scintillation counter (Beckman Coulter, Brea, CA, USA).

2.5. Mouse or human IFN-γ ELISPOT assays

ELISPOT plates were precoated with anti-mouse or anti-human IFN-γ mAb (MabTech, Stockholm, Sweden) overnight at 4 °C, and blocked with RPMI 1640 plus 10% FBS (HyClone