ELSEVIER

Journal of Autoimmunity 25 (2005) 21–32

journal of autoimmunity

www.elsevier.com/locate/issn/08968411

Efficacy of clonal deletion vs. anergy of *self*-reactive CD4 T-cells for the prevention and reversal of autoimmune diabetes

Anca Preda-Pais [a], Alexandru C. Stan [b], Sofia Casares [c], Constantin Bona [c], Teodor-D. Brumeanu [a,*]

[a] *Department of Medicine, Division of Immunology, Uniformed Services University of Health Sciences, 4301, Jones Bridge Road, Bethesda, MD 20814, USA*

[b] *Institute of Neuropathology, Hannover Medical School, Hannover 30625, Germany*

[c] *Department of Microbiology, Mount Sinai School of Medicine, New York, NY 10029, USA*

Received 21 January 2005; revised 31 March 2005; accepted 6 April 2005

Abstract

The *self*-reactive CD4 T-cells play an essential role in triggering and sustaining organ-specific autoimmune diseases. Silencing or elimination of these cells can prevent and reverse an autoimmune process. We have previously showed that a single dose-administration of a soluble dimeric MHC II–peptide chimera (DEF) in double-transgenic mice delayed the onset autoimmune diabetes, and restored the euglycemia in already diabetic mice for a period of 1 week. DEF dimer protection relied on induction of anergy of diabetogenic CD4 T-cells in spleen, and stimulation of IL-10-secreting T regulatory type 1 cells in pancreas. Herein, we show that an octameric form of DEF has doubled the period of protection and reversal of disease by clonal deletion of diabetogenic CD4 T-cells in both the thymic and peripheral compartments. Deletion occurred by activation-induced cell death subsequent to repartitioning and signaling of FAS–FADD apoptotic module in the plasma membrane lipid rafts. Our previous and present data indicated first, that DEF valence translates into various effects on the antigen-specific CD4 T-cells, i.e., Th2 immune deviation, anergy, and apoptosis. Second, the present findings argue for a better efficacy of clonal deletion than anergy of diabetogenic CD4 T-cells for the protection and reversal of autoimmune diabetes.
Published by Elsevier Ltd.

Keywords: Autoimmune diabetes; CD4 T-cells; Clonal deletion; MHC II–peptide chimeras

1. Introduction

Autoimmune diabetes (Type 1 Diabetes, T1D; insulin-dependent diabetes mellitus, IDDM) is a chronic autoimmune disease resulting from the T-cell-mediated destruction of pancreatic β-cells, and loss of insulin secretion [1,2]. The role of CD4 T-cells in pathogenesis of autoimmune diabetes has been overtly demonstrated in various animal models [3–5].

Anti-mitotic agents like Cyclosporin A, anti-inflammatory cytokines, or antibodies directed against inflammatory cytokines or T-cell markers were shown to delay the onset of type 1 diabetes in animal models, but the risk for systemic immune suppression makes their

Abbreviations: HA110-120, the CD4 immunodominant peptide from PR8/A influenza virus; NH_2-PEG_{2500}-NH_2, diaminated polyethylene glycol of 2500 kDa; DEF, genetically engineered soluble dimer of (I-$E^d\alpha\beta$/Fcγ2a/HA110-120)$_2$ chimera; DEF octamer, four units of DEF dimer enzymatically polymerized through NH_2-PEG_{2500}-NH_2 polymer at the galactose sites of the sugar moieties of DEF chimeras; 6.5 mAb, rat IgG2b/k clonotypic monoclonal antibody recognizing the HA110-120-specific TCR; AICD, activation-induced cell death.

* Corresponding author. Tel.: +1 301 295 3608; fax: +1 301 295 3557.

E-mail address: tbrumeanu@usuhs.mil (T.-D. Brumeanu).

0896-8411/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.jaut.2005.04.003

use questionable in asymptomatic humans. Thus, targeting preferentially the *self*-reactive (antigen-specific) T-cells appears to be an attractive therapeutic approach [6]. Among the immune specific therapeutics, the synthetic peptides were shown to prevent or ameliorate the disease in animal models [7], but their short life in vivo requires recurrent administration at considerable high doses to reach the therapeutic effect. We have recently shown that a new class of antigen-specific ligands, namely soluble MHC/peptide complexes genetically engineered to express a *self*-peptide, (i) have longer half-life in vivo [8], (ii) are able to engage both the TCR and CD4 co-receptor on T-cells in an antigen-specific manner [9], and (iii) their modulatory potency on T-cell function can be detected at 1000-fold lower amounts on a molar basis than that of synthetic peptides [9]. In contrast to the synthetic peptide HA110-120, at low TCR/CD4 occupancy the soluble DEF reagent induces antigen-specific Th2 polarization by a negative regulation of STAT4 [9], whereas at high receptor occupancy induces anergy by a blockade of TCR signaling [10]. In a TCR-HA/RIP-HA double-transgenic (dTg) mouse model for autoimmune diabetes, the DEF dimer prevented diabetes when administrated in the prediabetic stage, and restored euglycemia in the recent-onset diabetic mice by induction of IL-10 secreting T regulatory cells (TR-1) in pancreas and anergy of diabetogenic CD4 T-cells in spleen [11]. However, the protection by DEF dimer required repeated administrations every 5–7 days, which indicated a transient immunomodulatory effect of this reagent. We have enzymatically engineered highly multimerized DEF reagents by cross-linking the carbohydrate moieties of DEF dimer at the galactose sites via diaminated polyethylene glycol NH_2-PEG_{2500}-NH_2 [8].

Herein, we present evidence that the octameric form of DEF chimera provides a prolonged protection and better survival of TCR-HA/RIP-HA dTg prediabetic mice than its dimeric form, when administered at the early onset of hyperglycemia. The mechanism by which DEF octamer protected against diabetes relied mainly on the clonal deletion of HA-specific (diabetogenic) T-cell precursors in thymus subsequent to activation-induced cell death (AICD) of these cells through the reorganization and signaling of Fas apoptotic receptor and FADD adapter in plasma membrane lipid rafts.

2. Materials and methods

2.1. Mice

The RIP-HA$^{+/+}$ transgenic (Tg) mice on B10D2/BALB/C background (H-2^d) express the hemagglutinin protein (HA) of PR8/A/34 influenza virus in the pancreatic β-cells under the rat insulin promoter [11]. The TCR-HA$^{+/+}$ Tg mice on BALB/c background (H-2^d) express the 14.3d (α4Vβ8.1) TCR recognizing the HA110-120 CD4 immunodominant epitope of HA of PR8 virus in the context of I-E^d class II molecules [12]. The TCR-HA$^{\pm}$, RIP-HA$^{\pm}$ dTg mice were obtained by crossing TCR-HA and RIP-HA single Tg mice, and genotyped by PCR using specific primers for TCR-HA transgene (forward: 5′TAGGAGAAAGCAATGGAG AC3′ and reverse: 5′GTACCTGGTATAACACTCA G3′), and for HA transgene (forward: 5′CTACCATGC GAACAATTCA3′ and reverse: 5′TCACTACAGAGA CATAGCA3′). The RAG-2 mice on BALB/c background (Jackson Labs) and RAG-2 RIP-HA$^{+/+}$ Tg mice (maintained in our laboratory) were used for adoptive cell transfer experiments. Mice were housed at USUHS in a pathogen-free facility according to the federal and local regulations.

2.2. DEF ligands

The soluble dimeric MHC II–peptide chimera (DEF dimer) consists of I-E^{d}α and I-E^{d}β extracellular domains stabilized through a murine Fcγ2a fragment at the C-termini of I-E^{d}β chains, and expresses the HA110-120 (SFERFEIFPKE) immunodominant CD4 epitope of HA of PR8 influenza virus covalently-linked to the N-terminus of I-E^{d}β chains [13]. The soluble DEF octamer was enzymatically engineered by covalent cross-linking of DEF dimer with diaminated PEG_{2500} (Sharewater, Birmingham, AL), and it was purified by size exclusion chromatography on a Superose 6 column, as described [13]. Control DEF dimer and octamer molecules (cDEF) lacking the HA110-120 peptide were genetically engineered, and, respectively, enzymatically multimerized by diaminated PEG_{2500}. Aliquots of cDEF reagents (DEF dimer and octamer) were loaded in vitro (30 min incubation at 37 °C in 0.1 M citrate buffer, pH 4.5) with a mutant HA peptide (HAm1, SFEGFEIFPKE), and used as specificity controls. The HAm1 peptide does not induce in vitro proliferation and cytokine secretion of HA-specific CD4 T-cells when loaded on APC, and does not affect the response of these cells upon restimulation with the HA110-120 wild peptide. The cDEF reagents were purified as described for DEF reagents [13].

2.3. Therapeutic protocols

Groups of 27–29-day-old euglycemic (prediabetic) dTg mice and 33–36-day-old hyperglycemic dTg mice at the recent onset of disease (21 mice per group) were injected in the tail vein with three consecutive doses (one dose per day) of 200 μl saline (control group), 20 μg DEF dimer, or 20 μg DEF octamer in 200 μl saline. In a parallel experiment, four groups of eight prediabetic mice (28–30-day-old) were injected with empty DEF