Clinical Immunology (2011) 139, 336–349

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

IL-10-conditioned dendritic cells prevent autoimmune diabetes in NOD and humanized HLA-DQ8/RIP-B7.1 mice

Ningwen Tai[a,1], Hisafumi Yasuda[a,b,1], Yufei Xiang[a], Li Zhang[a], Daniel Rodriguez-Pinto[a], Koichi Yokono[b], Robert Sherwin[a], F. Susan Wong[c], Masao Nagata[b], Li Wen[a,*]

[a] Yale University School of Medicine, Department of Internal Medicine, Section of Endocrinology, USA
[b] Kobe University Graduate School of Medicine, Department of Internal and Geriatric Medicine, Japan
[c] University of Cardiff School of Medicine, Center for Endocrine & Diabetes Studies, Cardiff, UK

Received 26 January 2010; accepted with revision 2 March 2011
Available online 9 March 2011

KEYWORDS
DC therapy;
IL-10;
Autoimmune diabetes;
HLA transgenic mouse;
NOD

Abstract This study was to determine whether BMDCs cultured in the presence of IL-10 (G/10-DCs) could promote T cell tolerance and prevent autoimmune diabetes in two different animal models of T1D. Our results showed that G/10-DCs suppressed both insulitis and spontaneous diabetes in NOD and HLA-DQ8/RIP-B7.1 mice. The suppression was likely to be mediated by T cells, as we found that regulatory $CD4^+CD25^+Foxp3^+$ cells were significantly increased in G/10-DC treated animals. In vivo, the G/10-DCs inhibited diabetogenic T cell proliferation; in vitro, they had reduced expression of costimulatory molecules and produced little IL-12/23 p40 or IL-6 but a large amount of IL-10 when compared with DCs matured in the presence of IL-4 (G/4-DC). We conclude that IL-10-treated DCs are tolerogenic and induce islet-directed immune tolerance, which was likely to be mediated by T regulatory cells. This non-antigen-specific DC-based approach offers potential for a new therapeutic intervention in T1D.

1. Introduction

In the most studied animal model of type 1 diabetes (T1D), the Non-Obese Diabetic (NOD) mouse, autoimmune insulitis and diabetes develop spontaneously with some features resembling human T1D. We have developed a "humanized" transgenic model for T1D, HLA-DQ8/RIP-B7.1, to specifically study the effect of human HLA molecules on diabetes development [1–3]. These mice express human MHC class II DQ8 (deficient in mouse MHC class II) on antigen presenting cells and the human B7.1 costimulatory molecule (CD80) on islet beta cells. These HLA-DQ8/RIP-B7.1 mice also develop spontaneous insulitis and diabetes [2,3]. Both $CD4^+$ and $CD8^+$ T cells contribute to the autoimmune process and cytokine imbalances play a crucial role in the process leading to

* Corresponding author at: Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, Mail Box 208020, 330 Cedar Street, New Haven, Connecticut 06520, USA. Fax: +1 203 737 5558.
E-mail address: li.wen@yale.edu (L. Wen).
[1] These authors contributed equally to the study.

1521-6616/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2011.03.003

disease in both model systems [4,5]. Recent studies also suggest that a defect in T regulatory cells additionally promotes disease development [6,7].

Antigen presenting cells (APCs) play a crucial role in T cell differentiation by providing costimulatory signals and cytokines at T cell priming. Dendritic cells (DCs), a major subset of APCs, have the most potent capacity to efficiently prime naïve and memory T cells, thus playing a central role in modulating the magnitude of immune responses including autoimmunity [8–10]. Indeed, DCs are among the first cells infiltrating the pancreatic islets of NOD mice [11,12]. DCs have distinct APC functions dependent on their maturation state. Immature DCs (imDCs) very efficiently internalize and process antigens, whereas mature DCs (mDCs) have a reduced capacity to internalize and process antigen, but express higher levels of costimulatory molecules such as CD40, CD80 and CD86 after stimulation [8,13].

GM-CSF and IL-4 are commonly used to generate BMDC in vitro [14]. GM-CSF expands the myeloid subtypes of DC whereas IL-4 is the major Th2 cytokine and enhances DC maturation [15]. Mature BMDCs, cultured with GM-CSF and IL-4 (G/4-DC), suppressed autoimmune diabetes development in NOD mice [16]. IL-10 is an immunoregulatory cytokine that has multifunctional effects on many cell types [17], including DC maturation and function [18,19]. It is interesting that transgenic expression of IL-10 under the insulin promoter in NOD mice accelerated diabetes [20,21]. This suggests that the function of IL-10 is more complex. However, systemic administration of IL-10 or IL-10 gene transfer prevents diabetes in NOD mice and viral vector-mediated IL-10 gene delivery also showed a promising suppression of spontaneous diabetes in NOD mice [22–24]. Recent studies in patients with cancer or HIV suggest that DC-based therapy is safe and practical as autologous DCs can be used after conditioning in vitro without obvious toxicity [25,26]. Furthermore, transfer of antigen-pulsed autologous imDCs [27,28] led to the specific inhibition of antigen-specific $CD8^+$ T cell effector function and the appearance of antigen-specific IL-10 producing cells [28]. Although this study suggested that imDC should be used with caution when trying to enhance tumor or microbial immunity, it provides important in vivo evidence that this approach might be useful in humans to suppress autoimmunity.

In this study, we took an unbiased non-antigen-specific approach, to avoid the risk of promoting islet-specific immune response(s), since multiple autoantigens are involved in T1D development. We demonstrate that IL-10-conditioned and non-antigen-specific imDCs induced immune tolerance in the periphery and prevented autoimmune diabetes in two models of T1D. Thus we suggest that this approach could be translated to prevent and possibly treat human T1D.

2. Materials and methods

2.1. Mice

NOD, DQ8/RIP-B7.1, NOD.Scid, NOD.BDC2.5, SJL and BALB/c mice were maintained in the specific pathogen-free (SPF) animal facilities of Yale and Kobe Universities. The cumulative incidence of diabetes (blood glucose greater than 250 mg/dl or 13.9 mmol/l) of our NOD mouse colony is over 80% in females by 30 weeks of age and ~50% in DQ8/RIP-B7.1 mice by 35 weeks of age. All experiments were conducted, with ethical approval of Yale University and Kobe University Graduate School of Medicine.

2.2. Antibodies and reagents

All the antibodies used in this study were purchased from BD PharMingen (San Diego, CA) or eBioscience (San Diego, CA) unless otherwise stated. Anti-CD3 (hybridoma 2 C11 supernatant) and anti-CD40 (hybridoma FGK45 supernatant) were kindly provided by the late Dr. C.A. Janeway, Jr. FITC-dextran (mw 40,000) was purchased from Sigma (St. Louis, MO). Recombinant murine IL-10 was purchased from PeproTech (Rocky Hill, NJ). GM-CSF was derived from J558L cells, stably transfected with a GM-CSF construct. IL-4 was derived from an IL-4 producing cell line [29].

2.3. Isolation of bone marrow-derived dendritic cells (BMDCs) and purification of T cells

BMDCs were generated from BM cells of young NOD or DQ8/RIP-B7.1 mice. The BM cells were cultured in the presence of GM-CSF (1%) and IL-10 (20 ng/ml) or IL-4 (100 U/ml) in Bruff's medium (Invitrogen) supplemented with 5% FCS. Culture medium was replenished every 2 days. On day 6, non-adherent and loosely adherent cells were harvested which contained ~70% $CD11c^+$ DCs. In some experiments, $CD11c^+$ cells were further purified with a magnetic cell sorter autoMACS (Miltenyi Biotec Germany) and the purity of $CD11c^+$ cells was over 95%. The BMDCs cultured with GM-CSF alone, GM-CSF and IL-4 or GM-CSF and IL-10 were designated as G/0-DCs, G/4-DCs and G/10-DCs, respectively.

We purified total T cells or $CD4^+$ T cells using magnetic beads by negative selection according to the manufacturer's instructions. The purity is routinely 90–95% as verified by flow cytometry. $CD4^+CD25^+$ T cell purification was performed by positive selection with magnetic beads (Stemcell Technologies, Canada) and the purity is ~95% as verified by flow cytometry using different mAbs from the cell purification.

2.4. Antigen processing and presentation of BMDCs

To test antigen processing and presentation function of the BMDCs, BDC2.5 $CD4^+$ T cells and insulin B15-23-reactive $CD8^+$ T cell clone 6426 [30] were used in T cell proliferation assays. A $CD4^+$ T cell hybridoma, 17–119, responsive to GAD_{65} peptide (247–266) and restricted to HLA-DQ8, was also used and IL-2 production was measured by CTLL assay to determine the response of 17–119 to the GAD peptide.

2.5. CFSE dilution assay

T cell proliferation in vivo was examined by CFSE dilution assay [31]. In brief, purified BDC2.5 $CD4^+$ T cells were labeled with CFSE and injected intravenously into NOD mice. In vivo proliferation of BDC2.5 $CD4^+$ T cells was examined by dilution of CFSE using flow cytometry after 3 days.