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Autoimmune-Mediated Vascular Injury Occurs Prior to Sustained Hyperglycemia in a Murine Model of Type I Diabetes Mellitus

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Background. **Accelerated cardiovascular disease in patients with type I diabetes (TID) is a well-described condition and serious clinical obstacle. At present, the notion that early atherogenesis is largely dependent on sustained hyperglycemia remains in question. We hypothesize that an alteration in T lymphocyte homeostasis may result in early vascular inflammation, which might amplify subsequent blood vessel injury in euglycemia.**

Methods. **A murine model of carotid arterial ligation was employed to induce neointimal hyperplasia (NIH) in C57/Bl6 (non-autoimmune) and non-obese diabetic (NOD) mice. Additionally, adoptive transfer of NOD splenocytes into immunodeficient NOD mice (NOD.scid) was undertaken to evaluate the influence of restored autoimmunity on NIH development.**

Results. **Interestingly, compared with C57/Bl6 mice, the NOD demonstrate a significant increase in neointimal area. Conversely, the NOD.scid mice (immunodeficient control) reveal almost no evidence of vascular injury. While evidence of early vascular inflammation can be detected in the injured NOD vasculature, uninjured contralateral vessels and those of the NOD.scid have minimal T cell infiltration. Following reconstitution of autoimmune responses *via* NOD splenocyte adoptive transfer, accelerated vascular pathology is restored.**

Conclusions. **These observations suggest that autoimmunity, in the setting of impending hyperglycemia, may contribute to accelerated vascular inflammation and subsequent pathology.** © 2011 Elsevier Inc. All rights reserved.

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Key Words: **neointimal hyperplasia; inflammation; autoimmunity; adoptive transfer.**

INTRODUCTION

Pathogenesis of accelerated cardiovascular disease in patients with type I diabetes (T1D) is under intense investigation. At present, there appears to be a clear correlation between sustained hyperglycemia and atherogenesis [1, 2]. However, soluble markers of inflammation remain after intensive hyperglycemic therapy, suggesting a vascular pathology initiated prior to, or concurrent with, the development of T1D [3]. Autoimmune destruction of pancreatic β-cells is facilitated by T lymphocyte infiltration and robust production of reactive oxygen species. While destruction of these β-cells results in T1D, it is unclear whether the vasculature is an early target of this immune dysregulation.

The non-obese diabetic (NOD) mouse is a well-established model of spontaneous T1D and has been used extensively by investigators to dissect the immune components associated with disease pathogenesis. Not surprisingly, immunodeficient NOD mice, or mice that have mutations in costimulatory pathways, fail to develop T1D [4]. While there has been little application of this model to the investigation of diabetic vascular complications, we have previously identified a distinct vasculopathy (paradoxical vasoconstriction) in the NOD during the prediabetic phase [5]. Importantly, non-autoimmune mice failed to demonstrate this paradoxical vasoconstriction. We hypothesize that a breakdown in T cell homeostasis triggers early

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inflammation and endothelial dysfunction that may amplify vascular injury regardless of glycemic status.

In this study, we employ a low shear-stress model of carotid arterial injury to characterize the natural history of luminal pathology prior to the onset of spontaneous T1D in the autoimmune model. Secondly, we sought to determine the influence of a competent immune response in this process.

METHODS

Animals

All experimental protocols were approved by the University of Colorado Animal Review Committee. Age and weight-matched animals of the following strains were used in all experiments: C57/Bl6, NOD, and NOD.scid (severe combined immunodeficient). NOD and NOD.scid breeding mice were initially acquired from The Jackson Laboratory or the Barbara Davis Center for Childhood Diabetes (Denver, CO). Experimental animals were monitored for diabetes by checking urine glucose levels (Diastix; Bayer, Berkley, CA, USA) and hyperglycemia was confirmed using a One Touch Ultra glucometer (Life Scan, Milpitas, CA). In an effort to remove the potential pathologic influence of sustained hyperglycemia on endothelial injury, all mice underwent carotid ligation and subsequent histologic evaluation prior to the development of hyperglycemia. Glucose levels were closely monitored both pre- and post-procedure for the duration of the experiment. Mice that developed T1D (blood glucose levels > 15 mM) prior to histologic evaluation were excluded from the studies.

In this set of experiments, we sought to determine the influence of autoimmunity (*versus* immunodeficiency) on vascular pathology. We employed the NOD.scid mice as the immunodeficient control. NOD and NOD.scid are genetically identical, except NOD.scid mice lack an adaptive immune system. We also employed the C57/Bl6 animals as a non-autoimmune strain for comparison purposes.

Murine Model of Low Shear-Stress Injury

Cessation of carotid arterial flow, as a vascular model of endothelial shear-stress injury, was carried out as previously described [6]. Briefly, general anesthesia was achieved by intraperitoneal injection of Avertin [250 mg/kg body weight, supplemental dose 75 mg/kg]. A midline incision was made in the neck with the subcutaneous tissue retracted cephalad. The carotid artery was gently dissected free from the nerve and jugular vein and ligated at the level of the bifurcation with 6-0 prolene suture. The skin was then closed with 5-0 prolene suture in running fashion. As the contralateral carotid artery remains patent, the animals did not suffer from ischemic stroke and remained healthy for the duration of each experiment. Importantly, this model provides a severe and acute alteration in biomechanical stress, which directly affects the vascular proliferative response. In an effort to limit the possible inconsistency of a balloon-type injury or wire denudation, we chose arterial ligation for this set of observations.

Splenocyte Adoptive Transfer

While under anesthesia with Avertin, NOD.scid mice received 2×10^7 NOD spleen cells injected intravenously into the retro-orbital sinus (ROS). This was done concurrently with the carotid ligation.

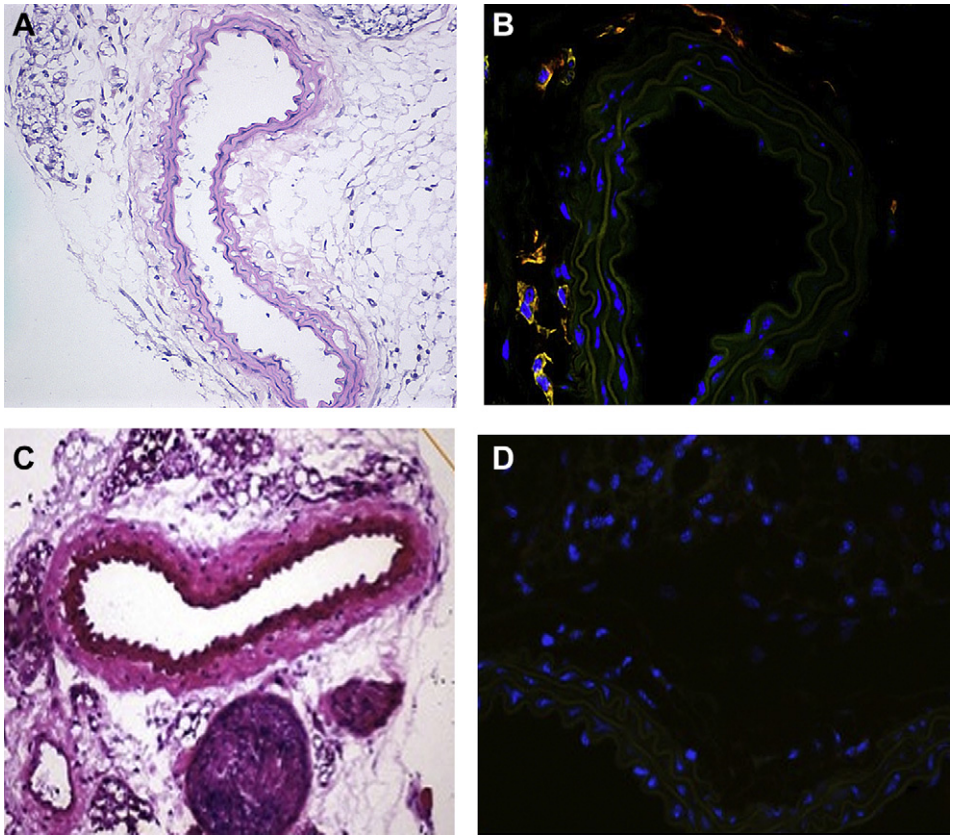

FIG. 1. Localization of CD3+ T cells to the NOD vasculature. Seven days post-carotid ligation the NOD vessels show no evidence of structural injury (A). However, using immunofluorescence numerous adventitial/perivascular T lymphocytes can be identified (B). The contralateral uninjured arteries demonstrate no evidence of structural injury at 7 d (C) [10×]. Similarly, the adventitia do not reveal the presence of T cells (D) [10×].

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