Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

The central role of antigen presentation in islets of Langerhans in autoimmune diabetes

Boris Calderon, Javier A Carrero and Emil R Unanue

The islets of Langerhans normally contain resident antigen presenting cells (APCs), which in normal conditions are mostly represented by macrophages, with a few dendritic cells (DC). We present here the features of these islet APCs, making the point that they have a supportive function in islet homeostasis. Islet APCs express high levels of major histocompatibility complexes (MHC) molecules on their surfaces and are highly active in antigen presentation in the autoimmune diabetes of the NOD mouse: they do this by presenting peptides derived from molecules of the β-cells. These APCs also are instrumental in the localization of diabetogenic T cells into islets. The islet APC present exogenous peptides derived from secretory granules of the β-cell, giving rise to unique peptide-MHC complexes (pMHC) that activate those non-conventional T cells that bypass thymus selection.

Addresses
Washington University School of Medicine, Department of Pathology and Immunology, St. Louis, MO, USA

Corresponding authors: Unanue, Emil R (unanue@wustl.edu, unanue@pathology.wustl.edu)

Current Opinion in Immunology 2014, **26**:32–40

This review comes from a themed issue on **Antigen processing**

Edited by **Caetano Reis e Sousa** and **Emil R Unanue**

For a complete overview see the Issue and the Editorial

Available online 16th November 2013

http://dx.doi.org/10.1016/j.coi.2013.10.011

Introduction

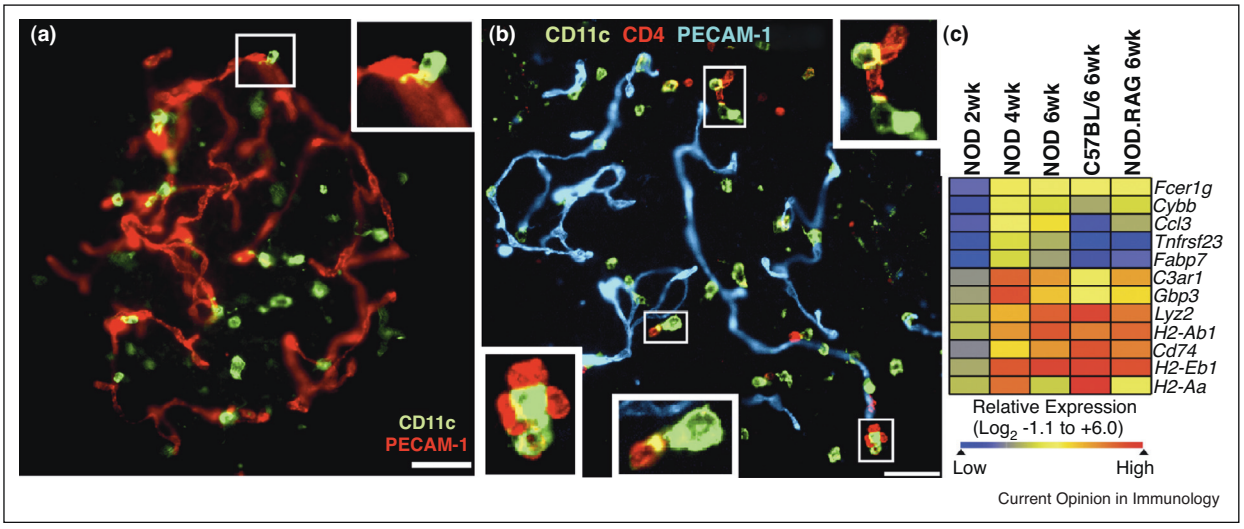
In autoimmune diabetes of the NOD mouse two tissues contain antigen presenting cells (APCs) responsible for presentation of diabetogenic antigens, that is the antigenic peptides that trigger the autoreactive T cells. These two tissues are the islets of Langerhans and the pancreatic lymph node (pLN). We reviewed the APCs of the islets recently [1]. This paper is an update. Normal islets are made up of three sets of endocrine sets: the β-cells, which are the most abundant and generate the secretory granules containing insulin, the α-cells that produce glucagon, and the δ-cells, a minor component that produces pancreatic polypeptide. The islets have a rich vascular network. Within the islets normally resides a non-endocrine myeloid cell belonging to the phagocytic lineage, the center of our analysis.

The early studies on the islet APC and their functional relevance came from studies on allogeneic islet transplantation in mouse experiments. These were experiments first carried out in the laboratories of Paul E. Lacy and Kevin Lafferty, who identified a cell within the islets that expressed Major Histocompatibility Complex (MHC) molecules and that were a target of rejection of islet transplants [2•,3•]. Both laboratories observed that culturing allogeneic islets for a period of time resulted in a prolongation of graft rejection in some strain combinations. These were extensions of the passenger leukocyte observations initially made by George Snell as an element of allogeneic graft rejections [4], but extended by Lafferty's group not only using islets but other tissues like thyroid [5•]. We now know now that the phagocytic cells of the islets are not passenger leukocytes but resident cells with characteristic features and properties; most important are their close adhesion to the vessels and their high content of products of the β-cell secretory granules [6••].

Concomitant with the Lacy and Lafferty functional studies, examinations of either histological sections or highly purified islets established that islets contained a set of myeloid cells as a normal inhabitant [1]. Hume, Gordon and associates had identified macrophages by the presence of the F4/80 molecule in all endocrine tissues including islets [7].

More recently, the features of the islet APCs were examined by a few laboratories [6••,8••,9••]. Some of the recent studies were done on islets of NOD mice, the main strain used to examine spontaneous diabetes [6••]. We, as well as others, initially classified the islet APC as part of the DC lineage, but our most current findings point to them as macrophages. Most of the islet APCs are characterized as positive for F4/80, MHCII, CD11b and CD11c. Most do not express *Zbtb46*, a protein that identifies the DC lineage [10,11] (our unpublished findings), and depend on colony stimulating factor -1 (CSF-1), see below. Moreover, a transcriptome analysis of isolated islets of NOD and non-diabetic mouse strains of mice at different ages showed the appearance of a gene signature consistent with tissue macrophages [12••] (Figure 1, panel c). A small and variable number of islet APC are made by DC, but these are restricted to the CD103 DC subset. The CD103 DC are associated with the CD8 alpha DC, both of which are developmentally regulated by the batf3 transcription factor [13]. Many of the present studies have not distinguished among subsets of islet APCs, so we best refer to them as 'APC'.

Figure 1



Examination of islets for myeloid cells, T cells and myeloid transcriptional analysis. **(a)** Islet of eight week-old NOD.*Rag1*$^{-/-}$ mouse showing myeloid cells (CD11c+) and blood vessels (PECAM-1+). Note the close apposition of CD11c+ cells with vessels. Inset shows the apposition between islet myeloid cell and vessel. **(b)** Islet from eight week-old NOD female mice stained for blood vessels (PECAM-1+), islet myeloid cells (CD11c+), and T cells (CD4+). Insets show contacts between islet myeloid cells and CD4 T cells. White bars represent 50 μm. Panels (b) and (c) taken from Ref. [12] with permission. **(c)** Microarray identification of myeloid gene signatures in islets of Langerhans. The heat map shows a hierarchically clustered gene subset that is enriched for macrophage/myeloid genes in NOD mice (two, four and six weeks), C57BL/6 and NOD.*Rag-1*$^{-/-}$ (6 weeks). Color intensity is based on the row normalized $\log_2$ scaled relative expression. The subset of genes represented in panel (c) is derived from the analysis detailed in Ref. [12].

There are important characteristics of the islet APCs. First, there is a high expression of both class I and class II MHC molecules as well as costimulatory molecules, making them suitable for presenting antigens [6••]. By all criteria these are activated APC. Second, most of them are associated with the intra-islet blood vessels, positioned next to them: by two photon microscopy on isolated islets, one can visualize them attached to the vessel wall emitting projections within the islet. Of interest is that some of these project into the islet lumen [14].

The islet purification process becomes an important technical aspect when evaluating intra-islet leukocytes. Contamination of non-islet leukocytes is a major hurdle for islet examination due to the lack of rigorous islet purification. The routine protocols uses two purification steps, one being the separation of islets from the acinar and surrounding tissue component by Ficoll gradient centrifugation, following the collagenase treatment; the second step is handpicking the Ficoll purified islets to ensure a purify islet preparation. Omitting the handpicking step consistently results in leukocyte contaminations from non-islet structures. Moreover, frequently small lymphoid aggregates are found in the intra-lobular fat tissue and contaminate the islets preparation. For 100% purity, isolated islets need to be distinguished from these aggregates by selecting them under a microscope using Dithizone which selectively stains islets in red.

The trophic role of the islet APC

In the normal steady state, islet APCs have a supportive role, maintaining the health, the homeostasis of the islet endocrine cells. (Homeostasis is the famous word first enunciated by Walter B. Cannon, based on Claude Bernard's famous concept of the *milieu interieur*: At the cellular level it can be defined as those cell activities that keep the tissue under a balanced steady state [15].) The trophic function of islet APC was first demostrated when examining the *Csf*^{op}/*Csf*^{op} mice [16••]. These mice have a spontaneous mutation in the *Csf1* gene that results in a non-functional molecule, and therefore in a defect in macrophage differentiation. The mice are osteopetrotic as a result of the absence of osteoclasts required for the removal of bone matrix. Banel-Boucharap *et al.* showed an absence of pancreatic macrophages in such mice and a number of functional abnormalities: reduced β-cell proliferation in late fetal life and a reduction in β-cell mass of about 30% in the adult [16••]. We confirmed these findings, proving that the isolated islets had a major loss of the macrophages: about 40% of islets did not have any, and the rest had one to two compared to about 10 in islets from normal mice. Islet mass was likewise reduced [6••]. These findings pointed to an important trophic support role that macrophages have in maintaining tissue homeostasis [17,18••,19••], the nature of which in regards to islet biology has not been determined. The islet macrophage may be a contributor to the local production of the vascular endothelial cell growth factor (VEGF) required for islets angiogenesis [20]. VEGF and its receptors (VEGFRs) are