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Specific maternal microchimeric T cells targeting fetal antigens in β cells predispose to auto-immune diabetes in the child

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ABSTRACT

Objective: During pregnancy there is an exchange of cells between the fetus and the mother including T lymphocytes that can persist after delivery. Previous studies have described an increased numbers of maternal cells in children with juvenile diabetes as compared to their unaffected siblings. Our objective was to assess the possibility for these chimeric T cells to trigger an anti-beta cell response.
Research design and methods: We mated OT2 transgenic female mice having T cells specifically targeting ovalbumin to RIP-OVA males expressing ovalbumin in pancreatic β cells. This allowed us to examine RIP-OVA progeny from OT2 mothers to assess the consequences of maternal T cells acquired during gestation or lactation. We quantitatively analyzed the pancreas of RIP-OVA mice from OT2 mothers for islet infiltration and compared them to RIP-OVA mice not exposed to OT2 mothers or to wild-type mice from OT2 mothers.
Results: RIP-OVA mice from OT2 mothers had significantly more peri-insulitis ($p = 0.0083$) compared to wild-type littermates. Similarly RIP-OVA mice from OT2 mothers had more peri-insulitis as compared to RIP-OVA mice from RIP-OVA mothers ($p = 0.0073$). Presence and specific anti-ovalbumin activity of maternal OT2 cells in the offsprings' peripheral lymphoid tissues was found in a separate group of mice. In animals presenting islet inflammation, CD3+ infiltrating cells in the pancreas were however derived from the offspring and not from OT2 mothers. In accordance, OT2 and RIP-OVA double transgenic mice with high levels of auto-reactive T cells had more peri-insulitis and sometimes intense insulitis when they were from OT2 mothers as compared to RIP-OVA mothers ($p = 0.046$).
Conclusions: In highly specific fetal/maternal combinations, maternal T cells with activity against the offspring pancreatic beta cells, presumably chimeric in fetal organs, initiate islet inflammation and may therefore predispose to auto-immune diabetes.

1. Introduction

The immune system distinguishes self from non-self antigens, a remarkable capacity given the infinite number of possible proteins and peptides. It has to react rapidly and aggressively against microbial epitopes in infections, while remaining silent toward self by diverse mechanisms of tolerance that regulate different components of the innate and adaptive immune system. These mechanisms are however mainly focused on controlling the activation and activity of T cells. Auto-immune diseases are diverse and share in common a triggering phenomenon called the rupture of tolerance toward self antigens. There are about 100 different diseases considered auto-immune and even if each of them is considered rare, overall they are found in 5–10% of the population in most developed countries [1] where their incidence is increasing [2]. They might affect several organs in conditions such as lupus or systemic sclerosis. Alternatively, these disorders may be limited to a specific cell type such as in type 1 diabetes targeting the pancreatic islet beta cells that produce

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insulin. Apart from several genetic disorders directly affecting regulatory mechanisms described above, the reasons for the development of such phenomena remain unknown. Microbial infections have been proposed as a potential initial trigger of immune responses that subsequently extend to self antigens by molecular mimicry, bystander activation of auto-reactive T cells or epitope spreading among other mechanisms [3]. Although infectious agents have been associated with some auto-immune diseases, there are to date no clear data on an overall mechanism explaining this rupture of tolerance.

Bidirectional trafficking of maternal and fetal cells is a well described phenomenon and occurs during most if not all pregnancies resulting in the persistence for decades after delivery of low levels of fetal or maternal cells in the mother or in the offspring respectively [4–7]. The consequences of this phenomenon have been the subject of intensive research in the past decade. The cell types exchanged between mother and fetus include leucocytes and notably T cells [8–11] in addition to progenitors of different lineages [7,12] such as hematopoietic [5] or mesenchymal stem cells [13] or endothelial progenitors [14].

The long term persistence of fetal and maternal semi-allogenic T cells suggested the possibility of allogenic immune reactions of these chimeric cells in the host. This was proposed as a potential mechanism triggering auto-immunity in childhood or after childbearing years [15]. Consequently, many authors investigated the association between microchimerism of fetal or maternal origin and auto-immune disorders that could mimic graft vs host disease such as systemic sclerosis (SSc) or juvenile dermatomyositis (DM). Although, SSc was first associated with fetal cell microchimerism later studies showed that the number of circulating fetal microchimeric cells was similar as compared to unaffected siblings [16,17]. In contrast, the presence of maternal microchimeric cells has been also strongly associated with SSc or juvenile DM [18–21]. Therefore, auto-immune disorders may be associated with microchimerism but the causal role of chimeric T cells in triggering the host's immune disease remains putative.

Recently, patients with juvenile diabetes have been reported to have increased levels of maternal cell microchimerism when compared to their unaffected siblings [22] and there are strong suggestions of a prominent role of the maternal perinatal environment in the predisposition to juvenile diabetes among the offspring [23,24]. In the present study, using maternal–fetal combinations of transgenic mice expressing ovalbumin in pancreatic beta cells or an anti-ovalbumin peptide T-cell receptor in T cells, we investigated the possibility for chimeric cells transferred during gestation or lactation to trigger auto-immune diabetes. Our results suggest that maternal microchimeric T cells with an anti-fetal beta cell activity may initiate peri-insulitis that may constitute an early predisposition to diabetes.

2. Materials and methods

2.1. Mice

RIP-OVA mice express *low* levels of ovalbumin under the control of the rat insulin promoter restricting expression to pancreatic beta cells at a level below 0.03 ng [25]. In this model, OVA-specific T cells are not deleted or activated by cross-presentation in the thymus or draining lymph nodes and were therefore retrieved in periphery.

OT2 (RAG2tm1Fwa-Tg(TcraTcrb)425Cbn) mice are transgenic for the TCR alpha- and beta-chains specific for chicken OVA 323–339 peptide in the context of I-A^b. OT1 mice are transgenic for the TCR alpha- and beta-chains specific for chicken OVA 257–264 peptide in the context of H2-K^b. These two TCR transgenic mice had a RAG2−/− background (provided by O. Lantz, Institut Curie, Paris, France). RAG2−/− (B6.129-$Rag2^{tm1Fwa}$) mice (Ly5.2 background) were also used in mating experiments. Ly5.1 (B6.SJL-Ptprca Pep3b) mice are congenic (homozygous) for the CD45 locus (CDTA, Orléans, France). All animals were maintained in standard pathogen-free conditions and had access to food and water ad libidum. All animal protocols were performed according to current French regulations of animal welfare.

All RIP-OVA mice at 6 weeks of age or 4 weeks after delivery received an inoculation of 200 mg/kg of ovalbumin peptide 323–339 in Complete Freund Adjuvant (CFA), followed a week later, by a boost of ovalbumin peptide in Incomplete Freund Adjuvant (IFA). This vaccination aimed at stimulating OT2 lymphocytes. One week later, mice were sacrificed for evaluation. Similarly, in adoptive transfer experiments, unsorted splenocytes from OT1 or OT2 mice on a RAG background were stimulated 4 days in vitro with specific peptide antigens and then injected in recipient mice.

2.2. Flow cytometry

Upon sacrifice, spleen, thymus and bone marrow were collected for flow cytometry. A million cells from suspended tissues were stained with a mix of the following: anti-CD45.1 coupled to FITC, anti-CD4 coupled to APC, anti-CD8 coupled to PerCP-Cy5, anti-TCRβ coupled to PE, anti-Valpha2 coupled to FITC, anti-IgM coupled to PE and anti-CD19 coupled to APC (Beckton Dickinson Pharmingen). Before staining, all cells were incubated with unlabeled anti-CD32 (24G2) antibody (Beckton Dickinson Pharmingen). Cells were analyzed on a FACSCalibur® cytometer using the Cell Quest Pro® software (Beckton Dickinson) and results were analyzed using Flowjo®.

2.3. Elispot

Elispot assays for γ-interferon secretion were performed as previously described [10]. In each well 500,000 responder total splenocytes were incubated for 24 h in plates (Millipore, Molsheim, France) precoated with anti-γ-IFN antibody (Becton Dickinson), in the presence of 100,000 syngenic bone-marrow derived dendritic cells loaded with various amounts of the ovalbumin peptide. After 24 h incubation, plates were washed and revealed with a complementary biotin-coupled anti-IFN-γ antibody (Becton Dickinson). Streptavidine-conjugated horseradish peroxidase was added to the wells, and, after washing, revelation was performed by adding the substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP) + nitro blue tetrazolium (NBT) (R&D Systems, Abingdon-Oxon, UK).

2.4. Histology and immunostaining

Pancreas was collected, fixed in formaldehyde overnight before being embedded in paraffin [26]. Five μm thickness sections were either stained with hematoxylin and eosin or further pre-treated in Citrate buffer pH 6 at 98 °C during 15 min, blocked with 20% Normal Goat Serum and incubated with a rabbit anti-CD3 (1/200, RM9107-R7, Neomarkers) combined with mouse anti-insulin (1/1000) for 1 h. Goat anti-rabbit FITC (1/200) and goat anti-mouse Texas red (1/200) were used for double staining (Jackson Immunoresearch). Slides were then counterstained with 0.3 μg/ml DAPI and mounted. The stained sections were then observed under the microscope (Leica, Deerfield, IL) with a QImaging digital camera (Media Cybernetics, Silver Spring, MD).

2.5. Evaluation of pancreas infiltration

Each pancreas section was then carefully scanned for the presence of insulitis or peri-insulitis on H&E labeled as well as on