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# Involvement of graft-derived interleukin-15 in islet allograft rejection in mice ☆

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**Abstract**

The possibility that islets play a role in graft rejection during islet transplantation for type-1 diabetes patients holds promise for ex vivo islet manipulation and for specific anti-rejection therapy. Interleukin (IL)-15 is a T cell growth factor and chemoattractant that is expressed by non-T cells. Intragraft expression of IL-15 is elevated during acute rejection in patients and in mice, and systemic blockade of IL-15 in mice prolongs allograft survival. However, the source of IL-15 in these conditions is undetermined. Since epithelial cell-derived IL-15 promotes lymphocyte proliferation in culture, we sought to determine whether islet-derived IL-15 promotes rejection in mice.We designed antisense oligodeoxyribonucleotide molecules that target mouse IL-15. Uptake of FITC-labeled antisense molecules and efficacy of IL-15 inhibition in IFNγ-stimulated islets were evaluated. Islets exhibited typical cytoplasmatic distribution of antisense molecules and produced IL-15 levels that were comparable to non-stimulated cells. Antisense-treated islet allografts, that were transplanted across multiple minor-histocompatibility-antigen mismatched strains of mice, were accepted at a higher rate than control-antisense treated islets or untreated islets (88.9% vs. 37.5% and 20%, respectively). Our results suggest that islet-derived IL-15 may be involved in acute islet allograft rejection.
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## 1. Introduction

There is increasing evidence that islets are the producers of chemokines and cytokines, which have the potential of recruiting an immune infiltrate against an islet allograft, as well as supporting immune cell viability during such a response while amplifying the inflammatory response against the graft [1]. Carrying such an endogenously damaging profile of secreted molecules is one of the many barriers for successful islet transplantation for type-1 diabetes patients today. Since a period of potential ex vivo manipulation is granted during islet transplantation, it is of value to learn which rejection-promoting agents are derived directly from islets.

Interleukin (IL)-15 is a T cell growth factor that shares several biological activities with IL-2 [2,3]. Unlike IL-2, however, IL-15 is widely expressed by a variety of non-T cells, including fibroblasts, epithelial cells and pancreatic insulin-producing β-cells [2,4,5]. In addition, coinciding with the wide distribution of IL-15 receptor (IL-15R), the biological activities of IL-15 are more diverse than those of IL-2, and include several non-redundant functions: IL-15 is necessary for differentiation of NK cells and for maintenance of CD8-positive memory T cells [6,7]. IL-15

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is also an anti-apoptotic factor for various cell types [7,8], a potent T cell chemoattractant and an enhancer of pro-inflammatory cytokine production by macrophages [2,5]. In light of the widespread distribution of IL-15 expression and its broad-acting immune-related activities, it is not surprising to find that IL-15 is involved in various immune mediated disorders [3,4,9–15]. An important example is the involvement of IL-15 in rheumatoid arthritis, where IL-15 was found to mediate macrophage production of TNFα via IL-15-activated T cells, as well as enhance synovial T cell proliferation and promote local cytokine release [16,17]. Accordingly, neutralization of IL-15 activity by soluble IL-15Rα prevented disease progression in mouse model of collagen induces arthritis [18].

In acute allograft rejection, it is recognized that T cell growth factors other than IL-2 are involved in the process of rejection. IL-2 knockout (KO) mice and IL-2/IL-4 double-KO mice readily reject allografts [19,20]. Also, the process of allograft rejection occurs despite antibody-mediated blockade of IL-2 or IL-2Rα in mice, and antibody-mediated blockade of IL-2Rα in kidney and heart transplant patients [21–24].

In the context of transplant rejection, IL-15 is likely to provide lymphocyte stimulation and maintenance in the absence of IL-2. Levels of intragraft IL-15-mRNA in human kidney, heart and lung transplants, as well as mouse islet grafts, correlate with acute rejection [4,15,22,25,26]. Recently, donor-derived IL-15 was proven critical for the immune response in mouse model of graft-versus-host disease [27]. Since T lymphocytes are not producers of IL-15 [5], it is possible that engrafted cells endogenously produce functional IL-15, most probably the response to violent peri-operative conditions and an inflammatory environment.

Several studies examine the involvement of IL-15 in graft rejection by way of neutralization (reviewed in [28]). Antagonist IL-15/Fc fusion protein prevented costimulation blockade-resistant rejection in mice [29] and achieved long-term engraftment in combination with rapamycin and an agonist IL-2/Fc fusion protein [30], as well as graft tolerance [31]. Similarly, soluble mouse IL-15Rα prolonged survival of fully vascularized, minor-histocompatibility Ag-mismatched cardiac allograft [32]. Although these studies clearly establish a function for IL-15 in graft rejection, they could not identify the *source* of IL-15 production, and, therefore, could not determine whether graft-derived IL-15 was involved.

We recently demonstrated that endogenous IL-15, as produced by primary human renal tubular epithelial cells, is the main mediator of lymphocyte proliferation in vitro [33]. The fold-increase observed in IFNγ-induced IL-15 levels in human tubular epithelial cells was comparable to the fold-increase in proliferation of lymphocytes in response to IFNγ-pretreated epithelial cells. The observed proliferation of lymphocytes, in the absence of recombinant cytokine, was the result of elevated endogenous epithelial-cell derived IL-15, as demonstrated by anti-IL-15 antibody. The proliferative response of lymphocytes, which is typically blocked by cyclosporine A, was intact in the presence of endogenous IL-15, similar to previously reported observations obtained with recombinant IL-15 [34]. Therefore, there is potent production of IL-15 by non-immune cells that is highly relevant to current immunosuppression protocols.

In the present study, we specifically focused on the involvement of islet-derived IL-15 on the survival of islet allografts in a mouse model of acute islet allograft rejection. Graft expression of IL-15 was specifically inhibited by anti-IL-15-antisense prior to transplantation of islets into minor-histocompatibility-complex mismatched hyperglycemic recipients.

## 2. Materials and Methods

### 2.1. Cells

Primary tubular epithelial cells were prepared as previously described [35]. NIH-3T3 cells were grown in DMEM with 10% fetal calf serum (Bet-Haemek, Israel). Splenocytes were isolated from B10.BR mice for alloanbtibody detection assay, as previously described [32].

### 2.2. hIL-15-Antisense

Antisense DNA molecules complementary to hIL-15-mRNA were initially designed to target hIL-15 transcripts in order to comply with experiments in human cells. A computerized prediction of the two-dimensional folding of hIL-15-mRNA sequence (GenBank Accession No. BC018149) was performed using software provided by Rensselaer Polytechnic Institute's School of Science (http://www.bioinfo.rpi.edu). The entire stretch of mRNA was used as potential target for antisense binding, including the open reading frame (ORF), and the untranslated regions (UTR), 5′-UTR and 3′-UTR. Non-hybridizing open-loop regions, which exhibited consistent folding-patterns across multiple computerized folding results, served as target sequences for 20-mer complementary single-strand DNA sequences (Oligodeoxyribonucleotides, ODN). The list of potential antisense molecules was narrowed to exclude CpG sequences, hair-pin forming sequences and sequences which are complementary to genomic elements other than IL-15-mRNA, while permitting sufficient purines to facilitate effective strand hybridization. The resulting sequences were synthesized (Sigma) and assayed for activity on primary cultures of human tubular epithelial cells. Briefly, primary tubular epithelial cells were preincubated for 24 h with various ODN sequences (2 μM), washed and added IFNγ (5 ng/ml) and the ODN sequences at the above concentration. IL-15 expression was examined by RT-PCR in total RNA extraction after 6 h or by ELISA in supernatants collected after 48 h. Cells treated with a control non-specific ODN sequence at identical concentrations and timetable, were

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