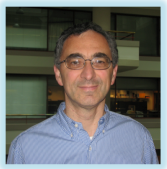
Ca-LVM alginate incorporating CXCL12 provides longterm protection to microencapsulated alloislets in NOD/LtJ mice



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INTRODUCTION AND OBJECTIVE

Survival of an islet allograft is dependent on the abrogation of rejection of donor tissue by recipient effector T-cells and anti-islet antibodies. Several different approaches have been taken to protect transplanted islets from the immune response including micro-encapsulation of islets (Vaithilingam, 2011). Ca-LVM alginate based capsules protect transplanted islets from both mechanical stress and the recipients' humoral immune system. However, the current generation of islet encapsulants do not protect against cell mediated immunity. The chemokine, Stromal cell-derived factor (CXCL12) has been shown to repel recipient effector T-cells and support islet survival(Papeta et al, 2007; Liu et al. 2011). CXCL12 is know to mediate its effects via its interaction with its cognate receptor on T cells and islet cells, CXCR4.

The objective of this work was to develop a new Calcium-LVM alginate encapsulant incorporating the chemokine, CXCL12, to sustain alloislet function and prevent immune mediated rejection.

MATERIAL AND METHODS

The release kinetics of CXCL12 from 2% Ca-LVM alginate capsules were studied. C57BL/6 islets were encapsulated in the Ca-LVM-CXCL12 encapsulant and transplanted into the peritoneal cavity of diabetic NOD/LtJ mice. Control mice received alloislets in unmodified Ca-LVM encapsulant. Graft survival, T cell infiltration into the graft and cell-mediated anti-allogeneic responses were measured.

RESULTS AND DISCUSSION

First we demonstrated that CXCL12 incorporation into microcapsules resulted in sustained release of the chemokine (Figure 1). The Ca-LVM-CXCL12 encapsulant significantly delayed rejection of alloislets compared with control encapsulant ($p<0.05$)(Mean days non diabetic post transplantation; Ca-LVM-CXCL12=136; Ca-LVM=62) (Figure 2). Immuno-histochemical studies of retrieved CXCL12-capsules revealed intact islet morphology in comparison to unmodified capsules in which islets were necrotic (data not shown). Flow cytometric demonstrated CXCR4 expression by CD4, CD8, and regulatory T cells from the spleen of NOD/LtJ mice and increased expression by Tregs of CXCR4 compared to CD4+CD25-T cells and CD8+ T cells (Figure 3). This suggests a potential mechanism for the differential effects of CXCL12 on various T cell subtypes on trafficking to specific sites in vivo.

In addition, we have previously shown that selective attraction of Tregs to CXCL12 is associated with significantly increased levels of CXCR4 expression (the receptor for CXCL12 on the cell surface), and significantly greater chemotactic responses of Tregs to the chemokine in vitro as compared to CD8+T cells (Righi et al., 2011).

Functional studies of T cells infiltrating the peritoneal cavity in alloislet recipients are ongoing and extension of this work into large animal models of xenoislet transplantation are being planned.

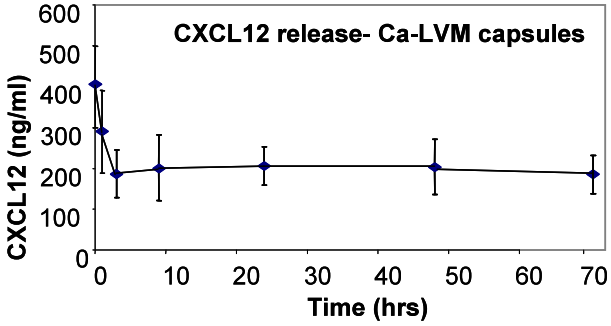


Figure 1. 1µg/ml CXCL12 was mixed with Ca-LVM alginate and microcapsules were placed in a fixed volume of DMEM. The concentration of CXCL12 was measured at specified times in the capsules and medium. This chemokine release curve illustrates favorable steady state release kinetics of CXCL12 from the encapsulant at 1.75ng/ml +/- 0.4ng/ml after an initial rapid release over the first 3 hours.

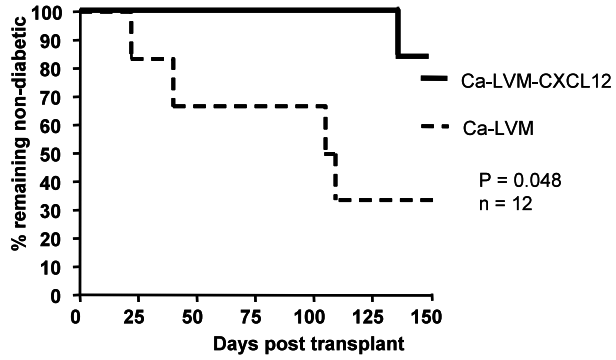
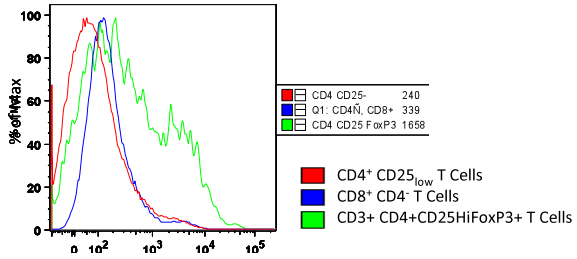


Figure 2. Islets from 6 – 8 week old C57/B6 mice were encapsulated with unmodified Ca-LVM alignate or with Ca-LVM incorporating CXCL12 at a concentration of 1µg/ml. Microencapsulated islets were then transplanted into the intraperitoneal cavity of diabetic NOD/LtJ mice and blood glucose monitored for up to 150 days

post transplant. Incorporation of CXCL12 into a Ca-LVM encapsulant maintained alloislets in a functional state and significantly delayed rejection of allogeneic islets in this experimental system (p=0.048, Gehan-Breslow-Wilcoxon test).



Cell Population	Average % Expression +/- SEM	Average MFI +/- SEM
CD3+CD4+CD25Low	39.93% +/- 13.26%	290.33 +/- 59.04
CD3+CD8+CD4-	56.25% +/- 10.65%	378.67 +/- 48.94
CD3+CD4+CD25HiFoxP3+	67.32% +/- 4.02%	1634.0 +/- 52.77

Figure 3. The mean fluorescence intensity (MFI) of CXCR4 expression on each population was calculated, and a representative histogram illustrates the increased expression by Tregs of CXCR4 compared to CD4+CD25-T cells and CD8+ T cells (histogram). Pooled data for % expression and MFI for each T cell subpopulation confirms that CXCR4 expression is detectable on all subpopulations including Tregs, and that this suppressive T cell subpopulation is enriched for both CXCR4 positivity and the level of CXCR4 expression (p<0.0001, Student's *t* test). This suggests a potential mechanism for the differential effects of CXCL12 on the trafficking of various T cell subtypes to specific sites *in vivo*.

Murine and human ovarian cancer which consititutively expresses CXCL12 is heavily infiltrated with Treg cells. Intratumoral Treg infiltration can be abrogated in the context of murine ovarian cancer by treatment with AMD3100, the highly selective antagonist of the receptor for CXCL12, namely CXCR4. In tumor models, the retention of Tregs but repulsion of anti-tumor Teffector cells was found to be related to the differential expression of CXCR4 on the two T cell subpopulations and their migratory behaviours to the chemokine (Righi et al., 2011). In parallel published and preliminary studies, we have shown that alloislets that have been trangenically engineered to express CXCL12 repel effector T cells from the allograft site and survive and function for significantly longer than wild type alloislets (Papeta et al. 2007). In addition, we have demonstrated that murine alloislets coated directly with CXCL12 and transplanted under the renal capsule of recipient diabetic mice resulted in prolonged graft function which was associated with the significantly increased accumulation of FoxP3+ Treg cells at the graft site in comparison to alloislets which were not directly coated with CXCL12 ($p < 0.01$ – data not shown). In addition, we have shown that Treg cells derived from NOD/LtJ mice differentially migrate to CXCL12 in comparison to CD8 T effector cells in vitro (data not shown). These published and preliminary data support the view that release of CXCL12 by islets at a specific donor site results in the differential accumulation of Tregs at and repulsion of effector T cells from that site.

Shi et al. demonstrated that cotransplantation of Treg cells with alloislet grafts results in prolonged islet function in the NOD/LtJ model (Shi et al. 2012). In addition, other islet prosurvival effects of CXCL12 on islets have been defined including chemokine mediated repulsion of effector T cells from the islet graft microvasculature (Sharp et al., 2008).

CONCLUSIONS

Incorporation of CXCL12 into Ca-LVM encapsulant prolongs the functional life of alloislet grafts in diabetic NOD/LtJ mice. This new approach appears clinically relevant to both allo and xenoislet transplantation in humans with type I diabetes. CXCL12 incorporation into the islet graft has been shown to be associated with the selective accumulation of Tregs at the allograft site which we propose contributes to a local immune suppressive micro-environment.

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