Expression and Regulation Of Chemokines in Murine and Human Type 1 Diabetes

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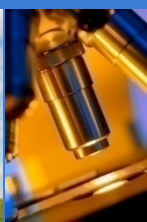

Inflammation and Type 1 Diabetes

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- **T1D is a progressive autoimmune disease that is accompanied by a pronounced inflammatory component.**

- **Many (>25) chemokines and chemokine receptors have emerged as potential contributors to initiation and progression of the T1D.**




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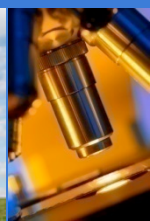

Unbiased and Integrated Approach

Goal: Foundation for the informed selection of potential therapeutic targets within the chemokine/receptor family.

- **Human islet culture system (islets incubated 24h in ± IL-1β, IFNγ and TNFα)**
- **Murine models of virus-induced (LCMV) and spontaneous T1D (NOD)**
- **Histopathological examination of pancreata from diabetic organ donors (nPOD)**




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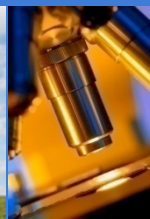
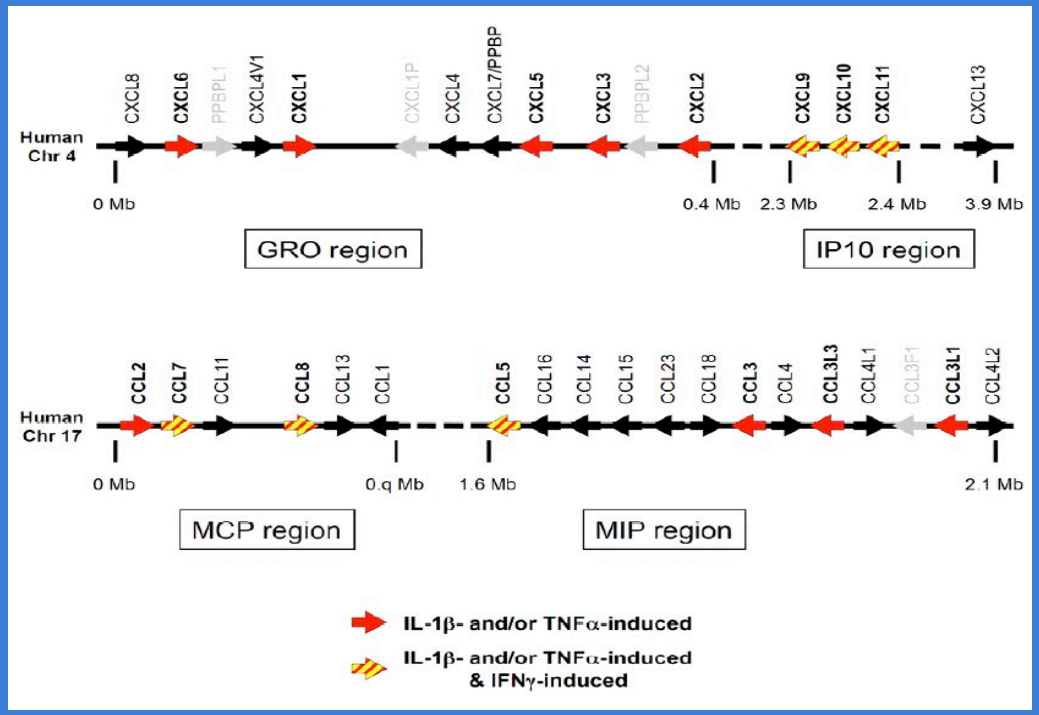


Chemokine Family in Human



Genomic organization of the major human chemokine clusters. 31 human chemokine genes are organized in 4 major clusters on Chr 4 (GRO and IP10 regions) and Chr 17 (MCP and MIP regions).

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- **Cytokine-induced chemokines genes in response IL-1β and/or TNFα (red arrows) or in response IFNγ (yellow/red) arrows**
- **Black arrows = chemokines not differentially regulated by cytokine treatments**
- **Gray arrows = pseudogenes Direction of the arrows = transcriptional orientation.**

CCL5, CCL8, CCL22, CX_3CL1, CXCL9 and CXCl10



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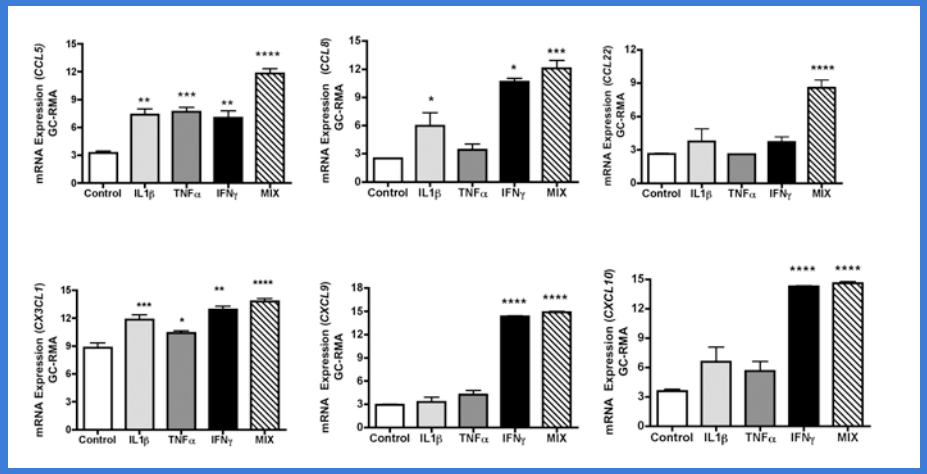
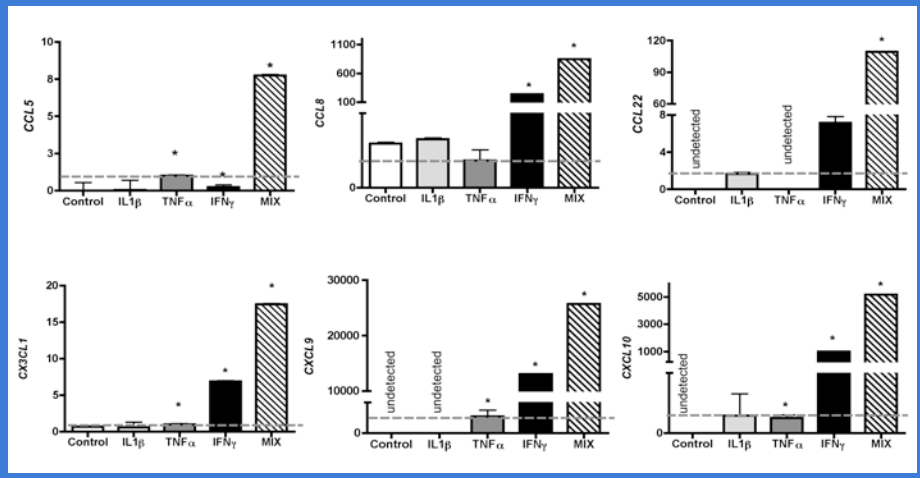
Microarray Analysis and qRT-PCR

CCL5, CCL8, CCL22, CX_3CL1, CXCL9 and CXCl10





A. Chemokine transcripts induced in human islet cells in response to inflammatory stimuli. obtained on **HG U133 Plus 2.0 Affymetrix** chip (n=3-4). Asterisks indicate significant differences between control and cytokine treated islets with $p<0.05$).

B. Validation of *chemokine transcripts by qRT-PCR* . Endogenous **HPRT1** was used for normalization. Data (mean ± SE; 4 donors) was quantified using 2 $^{-\Delta\Delta C_T}$ method. Asterisks indicate significant differences between control and cytokine treated islets ($p<0.05$).

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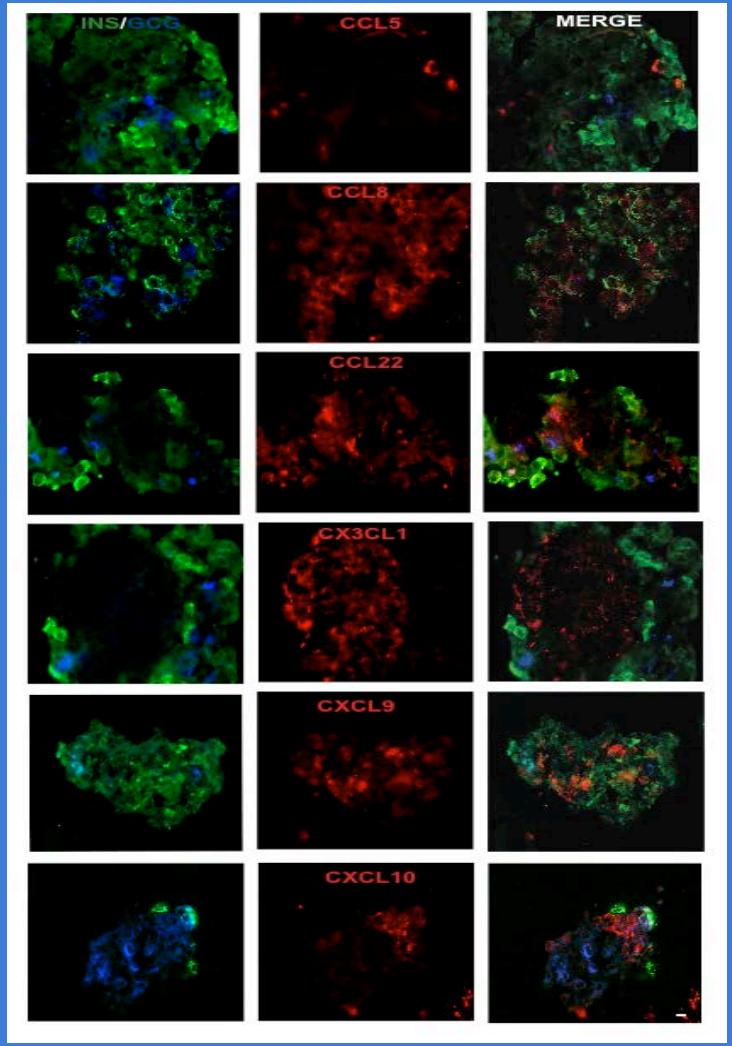
Chemokines in cultured human islets

- Islets cultured for 24h in MIX
- Fixed, embedded and sectioned
- Immunofluorescent staining: Insulin (green/ Cy2), glucagon (Blue/AMCA), and chemokine (red/Cy3)



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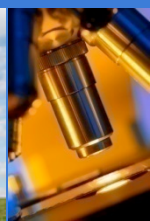
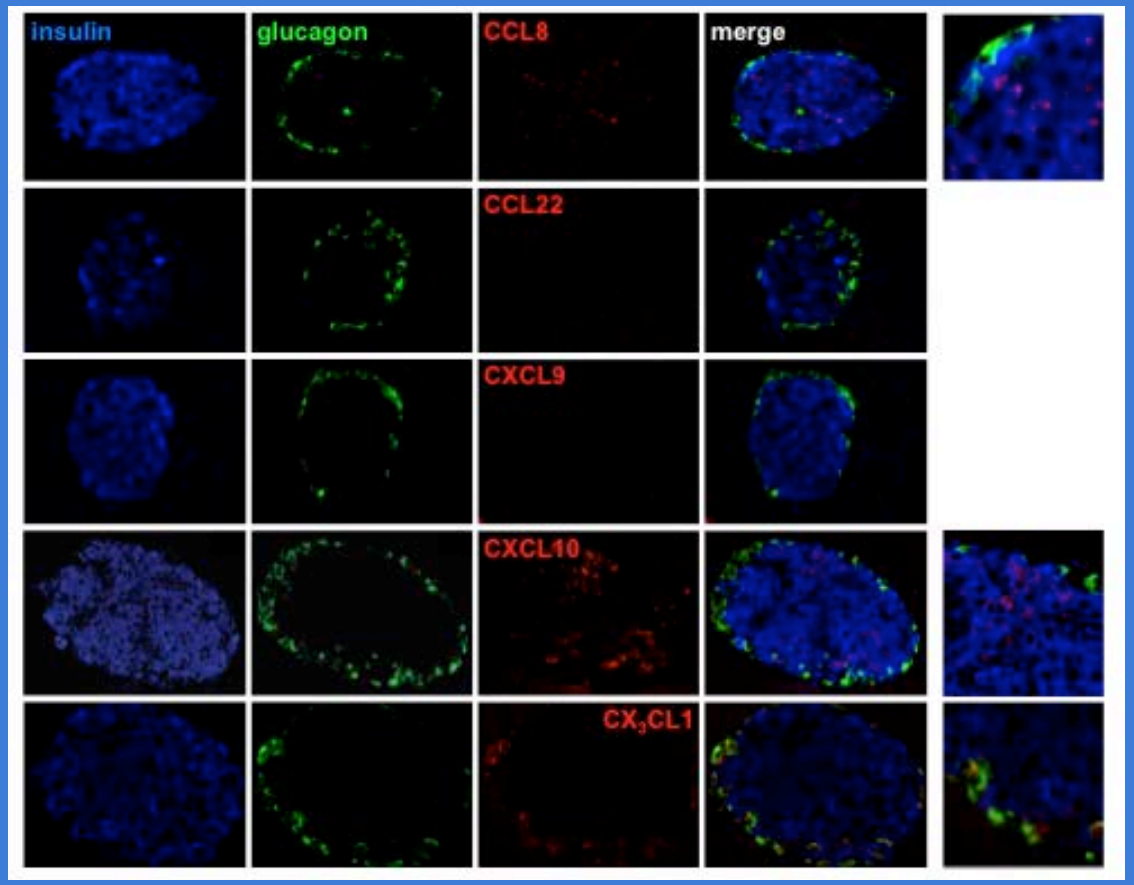


Chemokines in the LCMV Mouse Model (RIP-GP)

- 7-8 week old RIP GP mice
- Single IP dose of 10^5 pfu LCMV (harvested day 7 post-infection)
- Minimal or absent expression of Ccl22 and Cxcl9
- Expression of Ccl8 and Cxcl10 in β-cells and Cx_3cl1 α-cells



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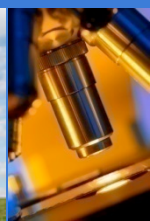
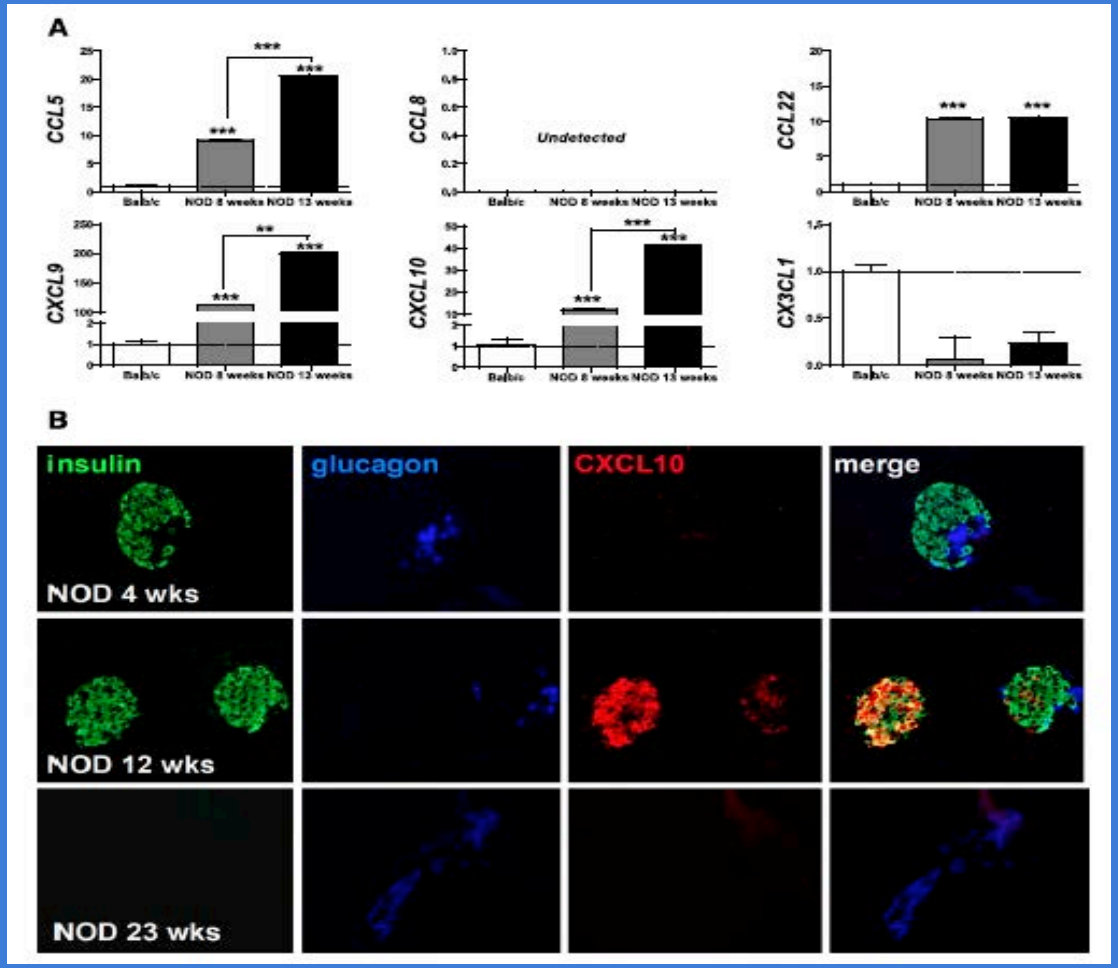


Cxcl10 Expression in Female NOD Mice

A. Chemokine (CCL5, CCL8, CCL22, CX3C11, CXCL9 and CXCl10) mRNA transcript expression in islets isolated from Balb/c (n=6) and 8 and 13 weeks old female NOD mice (n=6) by qRT-PCR.

B. Cxcl10 production by pancreatic β-cells in female NOD mice.



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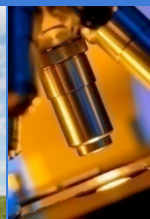

Case Description for T1D

Case	Age	Gender/ Ethnicity	C-peptide (ng/ml)	AutoAb	BMI	Clinical History
6036	49 (34 w T1D)	F/AA	<0.05	mIAA+	25.5	Ketoacidosis **Pancreatitis** Alcohol/Drug abuse, GI ulcers
6052	12 (1 w T1D)	M/AA	0.18	ICA512 + mIAA + IA2ic +	20.3	Diabetic ketoacidosis CMV positive
6087	17.5 (4 w T1D)	M/C	<0.05	mIAA+ ZnT8 +	21.9	Amputation, Colon resection, Cataract Glaucoma, Hypertension, CHF



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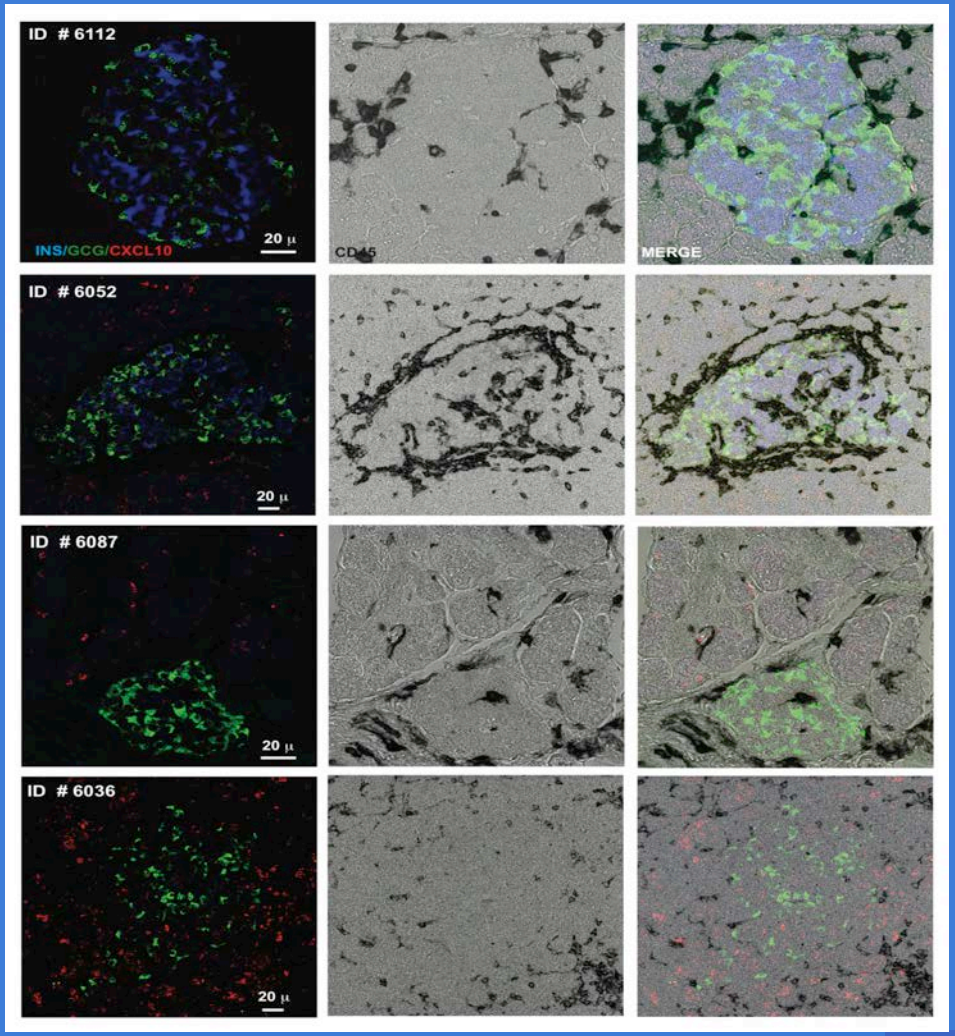
CXCL10 in Human Pancreata



In situ *CXCL10 expression in T1D and healthy control pancreata.* Combined insulin, glucagon, CD45 and CXCL10 stains.

- **Absence of CXCL10 staining in the healthy control (case ID #6112).**
- **CXCL10 present in T1D samples (ID #6087 and #6052), in close association with infiltrating leukocytes (CD45+).**
- **Acinar CXCL10 most prominent in diabetic donor (ID #6036) with clinically confirmed pancreatitis.**

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Conclusions

- Remarkable concordance and synergy between experimental models.
- Cytokine synergy in human islets at the level of gene expression.
- CXCL10 emerges as the key chemokine in both mouse and human.
- Concomitant acinar and endocrine inflammatory involvement can be seen in early T1D.
- Treatment of diabetes: a) Common pathophysiological pathways that targets natural disease course (like inflammation), b) Immnunomodulatory therapeutic regimens for specific components of chemokine/receptor system.




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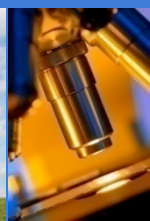

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