CXCL10 (IP-10) Production within the Lymph Nodes Mediates Entry of SIV-Specific CXCR3$^+$ CD8$^+$ T Lymphocytes

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ABSTRACT

Background: Lymph nodes (LN) harbor cells chronically infected by HIV/SIV, especially CD4$^+$ follicular helper lymphocytes which are located within the B cell areas. LN are characterized by low frequency of effector memory CD8 T cells that are, in general, excluded from the follicles. Using a vaccination protocol that combines intramuscular DNA delivery followed by *in vivo* electroporation, we have analyzed the trafficking of virus-specific CD8$^+$ T lymphocytes.

Methods: Eight MamuA01$^+$ rhesus macaques were immunized with plasmid DNA encoding p57^{RR}. Two weeks after the fourth vaccination, the animals were sacrificed, and the dissemination of vaccine-induced T cell responses was monitored throughout the body by immunophenotyping combined with CM9^{RR} tetramer staining, followed by flow cytometry. Chemokine production, including CXCL9, CXCL10 and CXCL11, by lymph node mononuclear cells (LNMC) from the vaccinated animals stimulated *ex vivo* with SEB and IFN-γ was measured by ELISA, multiplex chemiluminescence detection assays (MSD) and intracellular staining.

Results: High magnitude (up to 15% of total CD8$^+$ T cells) of vaccine-induced virus-specific CD8$^+$ T cells were found in peripheral blood from all the immunized macaques. These cells were actively dividing (Ki67), expressed high levels of CXCR3 and efficiently migrated into central and peripheral LN. The CM9-specific CD8$^+$ T cells within the LN also expressed CXCR3 and had an effector phenotype (CD95$^+$ CD28^{low} CD45RA^{low} CD127$^-$ CCR6$^-$ CCR4$^-$) with no significant CCR7 expression, suggesting that these cells could be located outside the T cell areas. LNMC from these vaccinated macaques stimulated *ex vivo* with IFN-γ or SEB released high levels of IP-10 (CXCL10) and CXCL11. A combination of surface and intracellular staining with anti-CXCL9 and IP-10 antibodies revealed that these chemokines were produced by HLA-DR$^+$ B lymphocytes, CD11c$^+$ CD14$^-$ dendritic cells and a subset of cells with the phenotype (CD3$^-$ CD20$^-$ CD14$^-$ CD11c$^-$ HLA-DR$^+$) of follicular dendritic cells (FDC).

Conclusions: Expression of CXCR3 by the vaccine-induced virus-specific CD8$^+$ T cells indicates that these cells can migrate into areas where the chemokines CXCL9, CXCL10 and CXCL11 are produced. Because these chemokines are produced within the LN by cells located in the B cell areas (B lymphocytes and FDC) the data suggest that a CXCR3-dependent and CXCR5-independent pathway of effector cells entry into the follicles exist.

INTRODUCTION

DNA vaccination induces robust T cell responses in macaques

✓DNA vaccination in rhesus macaques induces high magnitude of cytotoxic (GrzB$^+$ CD107$^+$ Perforin$^+$) SIV-specific T cells

Hu, X., *Hum Vaccin Immunother.* 14:2163-2177, 2018; Hu, *Hum Gene Ther.* 29:1029-1043, 2018; Singh, *J. Virol.*, 92. pii: e00281-18, 2018; Hu, *Hum. Vaccin. Immunother.* 13: 2859-2871, 2017; Hu. *J. Immunol.* 197: 3999-4013, 2016; Valentin, A., *Clin. Immunol.* 155: 91-107, 2014; Kulkarni, *PLoS One* 9: e86254. 2014; Patel, *Proc. Natl. Acad. Sci. U S A* 110: 2975-2980, 2013.

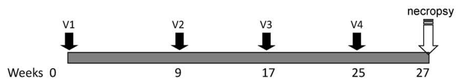
✓DNA-induced SIV-specific T cells efficiently disseminate into mucosal effector sites

Hu, *Hum Vaccin Immunother.* 14:2163-2177, 2018; Valentin, *Clin. Immunol.* 155: 91-107, 2014

✓DNA-induced SIV-specific CD8$^+$ T cells are also present systemically within secondary lymphoid tissues

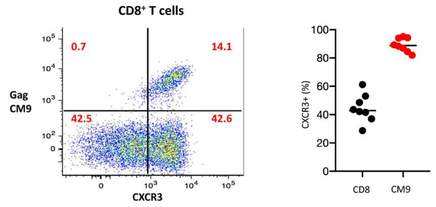
Valentin, *Clin. Immunol.* 155: 91-107, 2014

Gag DNA vaccination of Indian Rhesus Macaques to induce virus-specific CM9 tetramer$^+$ CD8 T cells

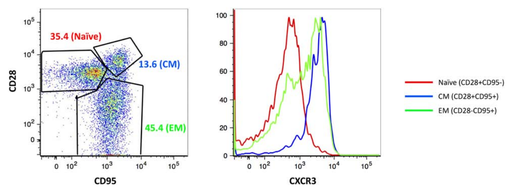


- Vaccination of 8 mamu A*01$^+$ macaques with SIV gag DNA via the intramuscular route by in vivo electroporation
- Analysis of T cell responses in blood and lymph node mononuclear cells collected at 2 weeks post the last vaccination

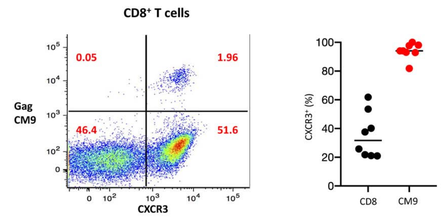
Circulating virus-specific (Gag CM9$^+$) CD8$^+$ T cells induced upon DNA vaccination express CXCR3



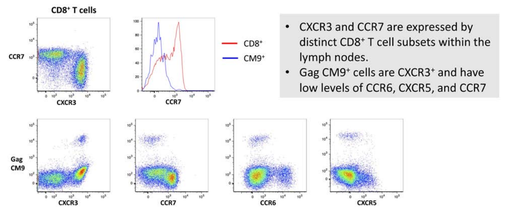
Memory CD8$^+$ T lymphocytes up-regulate CXCR3, a chemokine receptor typically absent on naïve lymphocytes



Virus-specific CD8$^+$ T cells within the lymph nodes also express CXCR3

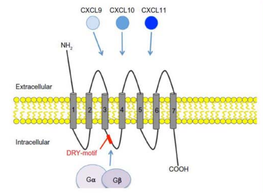


Expression of chemokine receptors by CD8$^+$ T lymphocytes in lymph nodes



- CXCR3 and CCR7 are expressed by distinct CD8$^+$ T cell subsets within the lymph nodes.
- Gag CM9$^+$ cells are CXCR3$^+$ and have low levels of CCR6, CXCR5, and CCR7

CXCR3 is a member of the seven transmembrane family of receptors and binds to CXCL9, CXCL10 and CXCL11



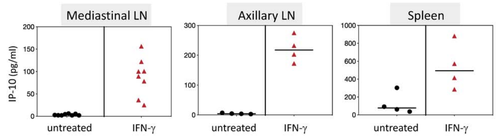
Michlmayr, 2014

- ✓ CXCR3 binds CXCL9, CXCL10 (IP-10) and CXCL11
- ✓ CXCR3 is expressed by memory T lymphocytes (both CD4 and CD8) and NK cells
- ✓ CD8 T cell differentiation towards a terminal effector cytotoxic phenotype goes together with CXCR3 up-regulation and CD127 (IL-7R) down-regulation

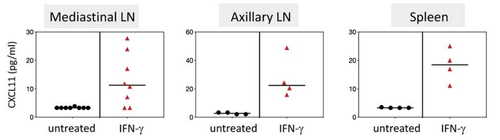
CXCL9, CXCL10 (IP-10) and CXCL11 (I-TAC)

- ✓Are highly inducible by IFN-γ
- ✓ Induce migration of memory but not naïve T cells
- ✓ Share the same receptor (CXCR3)
- ✓ Are produced by different cell populations.
  - ✓CXCL9 is released by stromal cells
  - ✓IP-10 is produced by hematopoietic cells

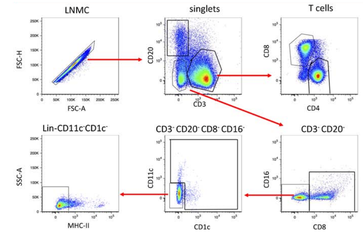
CXCL10 (IP-10) is produced by lymph node cells upon stimulation with IFN-γ *ex vivo*



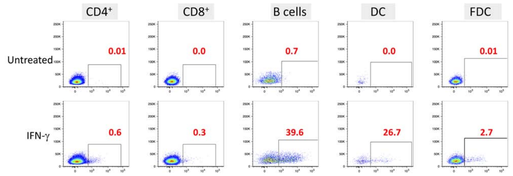
CXCL11 (I-TAC) is produced by Lymph Node cells upon stimulation with IFN-γ *ex vivo*



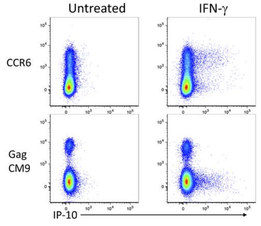
Which cells produce CXCL10? Gating strategy for the analysis of CXCL10 production by intracellular staining



Lymphocytes and myeloid cells, including FDC, produce IP-10 within the lymph node



CXCL10 (IP-10) is not produced by Gag CM9$^+$ CD8 T cells



- The main CD8$^+$ T cell subset producing IP-10 is CCR6$^+$
- Gag CM9$^+$ cells are mainly CXCR3$^+$ CCR6$^-$ and do not produce IP-10

CONCLUSIONS

- **CXCL10 (IP-10) and CXCL11 (I-TAC) are produced within the lymph nodes and, therefore, may be responsible for the homing of CXCR3+ lymphocytes**
- **IP-10 is highly produced by B lymphocytes and cells with a phenotype compatible with FDCs**
- **Although CXCR5 expression is an absolute requirement for resident follicular cells (B lymphocytes, Tfh) just to enter the follicle, down-regulation of CCR7 is critical feature to leave the T cell area**
- **A CXCR5-independent pathway to enter the B cell area exists within the LN and is mediated by CXCR3/IP-10 interaction**