Differential Systemic Effects of IL-15 Treatment Delivered by Four Different Routes in Rhesus Macaques: Impact on SIV-specific CD8+ T Cells

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ABSTRACT

Background: Heterodimeric interleukin-15 (hetIL-15) is a native stable form of the cytokine that activates and expands cytotoxic T and NK cells. We have reported that hetIL-15 treatment delivered subcutaneously in SHIV infected macaques results in significant decrease in viral RNA within peripheral lymph nodes (LN) and plasma viral loads. In this study, we have expanded the analysis of the hetIL-15 effects on virus-specific CD8$^+$ T cells, as well as the general lymphocyte population, in immunized MamuA01+ rhesus macaques treated with hetIL-15 subcutaneously (sc), intraperitoneally (ip), intravenously (iv) and intramuscularly (im).

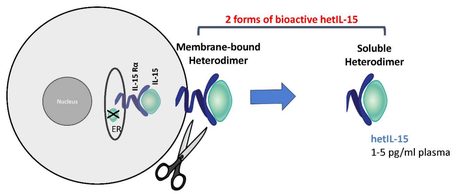
Methods: Eight DNA-immunized rhesus macaques received injections of hetIL-15 over 2 weeks using increasing doses of cytokine (step-dosing) by four different routes (sc, ip, iv and im). At the end of the treatment, hetIL-15 effects on different lymphocyte populations were monitored by multi-parametric flow cytometry.

Results: All four protocols resulted in systemic expansion of CD8+ T lymphocytes and NK cells with higher granzyme B content. These cells were found in both effector sites, such as liver, vagina and rectum, and secondary lymphoid tissues. A significant increase in cytotoxic effector memory CD8$^+$ T cells was found in lymph nodes from all hetIL-15-treated macaques. CM9 tetramer staining demonstrated that the increase of CD8$^+$ effector T cells in lymphoid organs included actively proliferating SIV-specific T cells with higher granzyme content. Some effects of hetIL-15 treatment were restricted to the specific delivery route. Macaques treated ip showed the highest levels of proliferation in CD8$^+$ lymphocytes obtained from the gastrointestinal tract (duodenum, jejunum, ileum and colon), although the proliferating T cells from the gut did not show any increase in granzyme content.

Conclusions: Step-dose administration of hetIL-15 by four different routes is well-tolerated and results in systemic activation and expansion of virus-specific cytotoxic leukocytes that infiltrate LN and peripheral effector sites. The differences observed between LN and the gastrointestinal tract suggest that tissue-specific homeostatic mechanisms may modulate the response of the tissue-resident lymphocytes to hetIL-15. These results suggest that hetIL-15 could be useful in promoting the entry of cytotoxic T cells into areas of chronic HIV replication and contributing to a functional cure of the infection.

INTRODUCTION

IL-15 is a heterodimeric cytokine (hetIL-15)



- Co-expression of IL-15 and IL-15Rα produces a stable heterodimer
  - Natural way of cytokine production *in vivo*
  - The secreted heterodimeric cytokine has extended half-life and systemic effects (similar to hormones)

Bergamaschi, J Biol Chem. 2008

Functions of hetIL-15

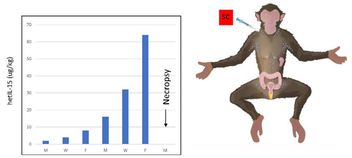
Lymphocyte Expansion and Differentiation
CD8, CD4, NK, γδ T lymphocytes
Important for both, innate and adaptive immunity

Enhanced Activation, Cytotoxicity
Increases expression of granzymes, perforin
Enhances ADCC

Migration
Rapid extravasation of lymphocytes to tissues
Increases CD8 Effectors in tumors

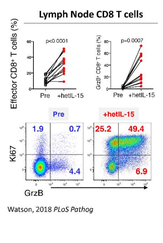
Use hetIL-15 to expand cytotoxic lymphocytes and increase the killing of HIV-infected cells by both innate and adaptive immune mechanisms

Dose escalation of hetIL-15 delivered subcutaneously (sc) in rhesus macaques



Six hetIL-15 injections with increased cytokine amount, delivered MWF in a two-weeks regimen. Each hetIL-15 dose stimulates lymphocyte growth generating new targets for the next increased dose and preventing the toxicity associated with high levels of free cytokine

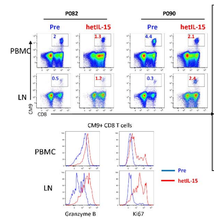
hetIL-15 effects in rhesus macaques



Watson, 2018 *PLoS Pathog*

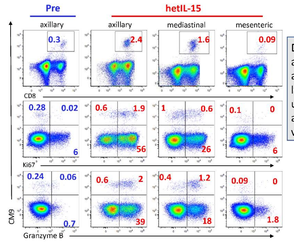
- Expands cytotoxic CD8$^+$ T lymphocytes and NK cells in all the organs
- CD8$^+$ T cells and NKs express higher levels of GrB and accumulate within LN
- **Promotes entry of CTLs within the B cell follicles**
- **Reduces plasma viremia and cell-associated RNA within the LN of SHIV-infected macaques**

Subcutaneous hetIL-15 treatment results in reduction of Gag CM9$^+$CD8$^+$ T cells in peripheral blood with simultaneous expansion within secondary lymphoid organs (axillary LN)



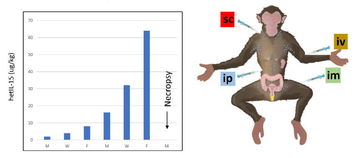
- The CM9$^+$ cells are stimulated to proliferate (Ki67) and contain higher levels of granzyme B
- The increased frequency of CM9+ T lymphocytes within the LN is the result of two independent events: local expansion and homing from the periphery

CM9$^+$ CD8$^+$ T cell distribution in LN is heterogeneous in rhesus macaques after subcutaneous hetIL-15 treatment



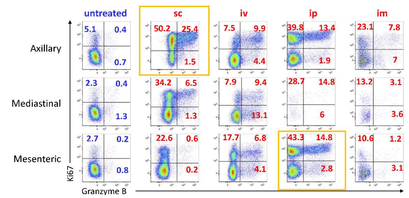
Differences in proliferation and granzyme B content among the general CD8$^+$ T lymphocyte population upon hetIL-15 treatment are also reflected in the virus-specific CM9$^+$ T cells

Could the systemic hetIL-15 effects be influenced by the delivery route? Comparison of four different routes

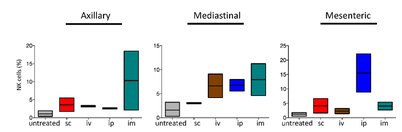


Six hetIL-15 injections with increased cytokine amount, delivered MWF in a two-weeks regimen. Each hetIL-15 dose stimulates lymphocyte growth generating new targets for the next increased dose and preventing the toxicity associated with high levels of free cytokine

hetIL-15 effects in LN depend on the anatomical location and the route of cytokine administration. Subcutaneous delivery gives the highest effects in the draining axillary LN, while intraperitoneal delivery provides the strongest responses in the mesenteric area

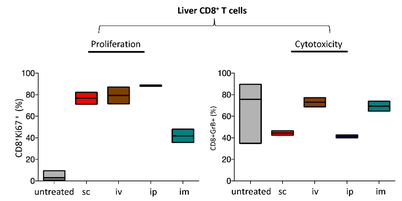


hetIL-15 treatment results in increased infiltration of Natural Killer (NK) cells within all the LN: The intraperitoneal route has the strongest effects in the mesenteric area

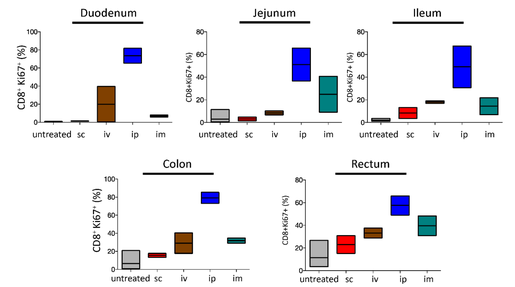


- hetIL-15 treatment increases GrB content and CD16 density on NK cells
- The activated NK cells mediate higher levels of ADCC activity

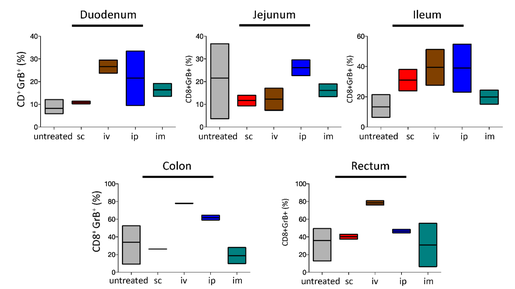
hetIL-15 treatment triggers the proliferation of CD8+ T cells within the liver: Resident CD8$^+$ T cells have a constitutive cytotoxic phenotype with high granzyme B content



hetIL-15 stimulates proliferation of CD8$^+$ T cells in the gut. Animals treated intraperitoneally show the highest responses throughout the gastrointestinal tract



hetIL-15 increases the cytotoxic phenotype of CD8$^+$ T cells located in the ileum segment of the gastrointestinal tract



CONCLUSIONS

- **Step-dose delivery of hetIL-15 by four different routes (subcutaneous, intravenous, intramuscular and intraperitoneal) is well tolerated and has systemic effects in rhesus macaques**
- **All four delivery methods induce expansion and activation of CD8$^+$T lymphocytes and NK cells in all the organs analyzed**
- **Cytokine distribution from the injection site by draining lymphatic vessels probably contributes to the strong local effects associated with certain routes (higher proliferation and GrB content in the draining LN upon subcutaneous delivery)**
- **Responses in the gastrointestinal tract are higher upon intraperitoneal delivery**
- **Adjustment of dosing and delivery route may be beneficial in the therapeutic use of hetIL-15**

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