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*Correspondence: NCI-Frederick, P.O. Box B, Building 535/Room 226A, Frederick, MD 21702-1201; Tel: 301-846-1856; Fax: 301-846-7146; E-mail: bergamaschic@ncifcrf.gov

Improved Efficiency and Delivery of IL-15 DNA Vectors in Combination with IL-15 Receptor Alpha in Mice and Macaques Deliver a Potent Growth Signal for NK and T Cells

Cristina Bergamaschi,[1*] Rashmi Jalah,[2] Margherita Rosati,[1] Antonio Valentin,[1] Viraj Kulkarni,[2] Candido Alicea,[2] Vainav Patel,[1] Gen-Mu Zhang,[1,2] Barbara K. Felber,[2] and George N. Pavlakis[1]

[1]Human Retrovirus Section, and [2]Human Pathogenesis Retrovirus Section, Vaccine Branch, NCI-Frederick, Frederick, MD

Aim: To generate DNA vectors expressing high levels of either human IL-15 or IL-15 Receptor alpha (IL-15Ra), and to study their in vivo biological activity as vaccine adjuvants when expressed in combination.

Methods: Optimized DNA expression vectors for IL-15 and IL-15 Receptor alpha (IL-15Ra) were constructed by eliminating the instability sequences in the mRNA (RNA/codon optimization). In addition, the IL-15 signal peptide was replaced with that from tissue Plasminogen activator (tPA), or other proteins, which dramatically increased secretion (IL-15t). Expression of these vectors was monitored in vitro in transfected cells, and in vivo in Balb/c mice or Rhesus macaques by intramuscular injection. In mice the DNA was alternatively delivered by hydrodynamic injection, which led to very high plasma levels (up to 0.5 µg/ml). Mice were sacrificed 3 to 21 days post-injection, and muscle or plasma IL-15 was measured by ELISA. The phenotype of different lymphocyte subsets in spleen, liver and lung was analyzed by multiparameter flow cytometry.

Results: Co-expression of the optimized IL-15t and IL-15Ra greatly increased the levels of IL-15 in vivo, and resulted in approximately 1000-fold increase compared to the wild type IL-15 cDNA alone. These data show that the complex IL-15/IL-15Ra increases the half life of the cytokine in vivo, as we have also shown in vitro. The high concentration of systemic IL-15 was biologically active as demonstrated by the increased frequency of NK cells in liver and lung, and the dramatic increase of T lymphocytes with properties of effector memory cells in lung, liver and spleen. Rhesus macaques injected with these vectors also express high plasma levels of IL-15, demonstrating the ability of these vectors to achieve high levels of expression in vivo.

Conclusion: The combination of IL-15t and IL15Ra results in the mutual stabilization of the molecules, both in vitro and in vivo. The high level of expression of the optimized vectors and their enhanced activity indicate that they could be useful as adjuvants in DNA-based vaccines and in immunotherapy protocols against cancer.

IL15 Receptor Alpha Appears to Be Mandatory for the Function of IL-15 in vivo

- IL-15 is normally presented in vivo as a cell-associated cytokine bound to IL-15Ra
- IL-15Ra is cleaved by TACE/ADAM17 together with trans-presented IL-15, and the IL-15sRa/IL-15 complexes can also signal upon binding to target cells
- IL15sRa/IL-15 complexes are essential for expanding the IL-15 effects from autocrine or juxtacrine to paracrine or endocrine modes and influence the nature and/or duration of the signaling event and bioavailability of IL-15

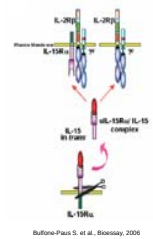


Figure 1

Experimental Procedures

- Generation of DNA vectors for the optimal expression of:
  - human and rhesus macaque IL-15 (IL-15t)
  - human and rhesus macaque IL15 Receptor alpha (IL15Ra)
  - human and rhesus macaque-secreted extracellular portion of IL15Ra (IL15sRa)
- In vitro experiments:
  - Evaluation of human IL-15 levels by ELISA and detection of human IL15Ra by Western blot after transient transfection of 293 cells
- In vivo experiments:
  - Evaluation levels and bioactivity of optimized IL-15 in vivo (mice and macaques)

Figure 2

IL15 Receptor Alpha Stabilizes IL-15

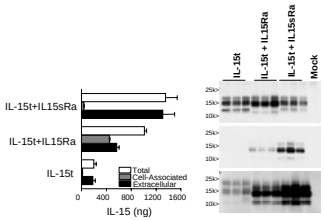
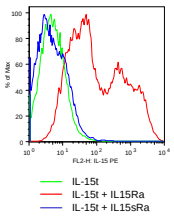


Figure 3

IL-15 Receptor Alpha Co-expression Results in Surface IL-15



	Total IL-15 (ng in supernatant)
IL-15t	192
IL-15t+IL15Ra	628
IL-15t+IL15sRa	1757

Figure 4

IL-15 Co-expression Stabilizes IL-15 Receptor Alpha

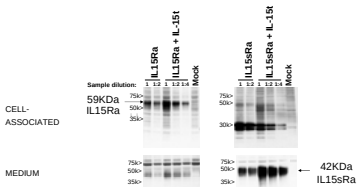


Figure 5

IL15Ra or IL15sRa Greatly Increases IL-15 Plasma Level In DNA-Injected Mice

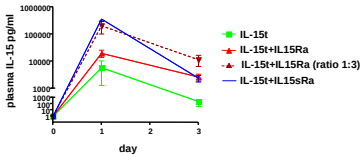
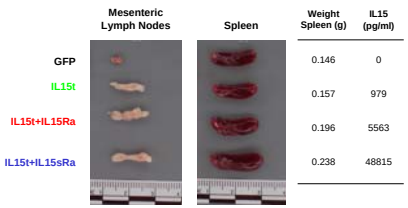


Figure 6

Increased Size of Spleen and Lymph Nodes in the Presence of IL-15/IL15Ra Complexes



	Weight Spleen (g)	IL15 (pg/ml)
GFP	0.146	0
IL15t	0.157	979
IL15t+IL15Ra	0.196	5563
IL15t+IL15sRa	0.238	48815

Figure 7

IL-15/IL15Ra Complexes Are Bioactive in vivo and Result in an Increased Number of NK and T Cells in Lung, Liver and Spleen in Mice

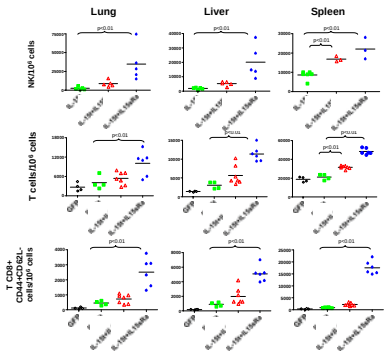


Figure 8

Increased Frequency of Effector Memory T Cells in Lung of DNA-injected Mice

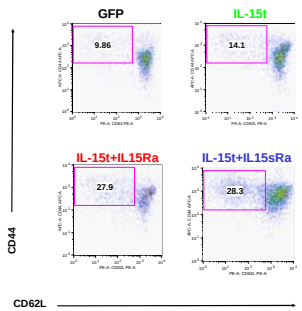


Figure 9

IL-15 Levels Correlate with Bioactivity in Mouse Tissues

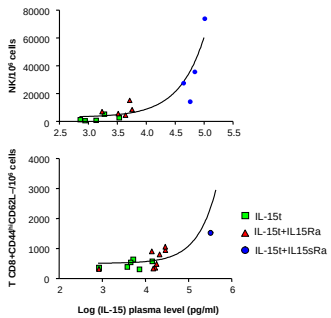
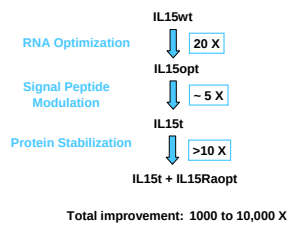


Figure 10

SUMMARY: Optimization of Human IL15



Total improvement: 1000 to 10,000 X

Figure 11

Conclusion

- Co-expression of IL-15 with IL-15Ra allows interaction of the two proteins early during secretion and results in a great increase in the stability of both proteins in vitro
- Mutual stabilization of IL-15 and IL15Ra leads to an improved IL-15 production, stability, tissue availability and bioactivity in vivo
- IL-15/IL15Ra increases dramatically the number and activation of NK and T cells in tissues

IL15Ra should be considered a part of the active IL-15 cytokine rather than a part of the receptor

Future Directions

- Delivery of IL-15/IL15Ra may allow the targeted and efficient expansion of immune system cells in vitro and in vivo
- Applications in vaccines and immunotherapies