Combination of ImmTOR Tolerogenic Nanoparticles and IL-2 Mutein Synergistically Inhibits the Formation of Anti-AAV Antibodies

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Abstract

We have previously demonstrated that biodegradable ImmTOR nanoparticles encapsulating rapamycin, an inhibitor of the mTOR pathway, has the ability to mitigate immunogenicity of AAV vectors and enable re-dosing. However, delayed immune responses can result in breakthrough of anti-AAV antibodies in some animals, particularly at higher vector doses. We have investigated the combination of ImmTOR with a regulatory T cell (Treg)-selective interleukin-2 (IL-2) mutant molecule (IL-2 mutein). Treg-selective IL-2 muteins have been shown to expand all pre-existing Tregs, unlike ImmTOR which induces antigen-specific Treg. Here we demonstrate that a single dose of ImmTOR administered the same day as an IL-2 mutein acts synergistically to promote massive expansion of antigen-specific Treg. The combination of ImmTOR and IL-2 mutein, termed ImmTOR-IL, provided more durable inhibition of antibody responses to co-administered AAV gene therapy vectors, even at sub-optimal doses of ImmTOR and at vector doses of up to 5E13 vg/kg. These results show that the combination of ImmTOR and IL-2 mutein has the potential to provide more effective and durable antigen-specific immune tolerance to mitigate immunogenicity of AAV gene therapy vectors and enable dose-sparing of ImmTOR.

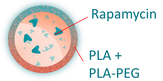
Introduction

Currently systemic AAV gene therapy cannot be re-dosed due to the formation of neutralizing anti-AAV antibodies. Tolerogenic ImmTOR nanoparticles have been shown to induce antigen-selective immune tolerance to co-administered biologic drugs[1]. ImmTOR induces tolerogenic antigen-presenting cells that present co-administered antigen in a manner that results in the formation of antigen-specific Tregs. Preclinical studies have demonstrated the ability of ImmTOR to mitigate the formation of anti-AAV antibodies and enable vector re-dosing in mice and nonhuman primates[2,3]. ImmTOR has also been shown to inhibit capsid-specific CD4 T cell[2] and CD8 T cell (abstract #708) responses and enhance transgene expression at the initial dose[3]. Treatment with ImmTOR provides hepatoprotective effects in a concanavalin A-induced model of liver inflammation[4]. ImmTOR has demonstrated dose-dependent inhibition of anti-drug antibodies against a fungal derived uricase enzyme[5] and is currently in Phase 3 clinical trials.

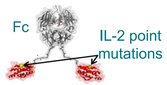
Interleukin-2 (IL-2) is a key cytokine that regulates immune responses. Tregs constitutively express a high affinity IL-2 receptor. IL-2 molecules engineered to be selective for the high affinity receptor induce non-selective expansion of total pre-existing Tregs[6].

Here we demonstrate that ImmTOR-IL, the combination of ImmTOR and an IL-2 mutein, shows profound synergistic activity in inhibiting the formation of anti-AAV antibodies in mice.

Methods and Materials



ImmTOR – Biodegradable synthetic nanoparticles comprised of rapamycin, a small molecule inhibitor of the mTOR pathway, embedded in a matrix of poly(lactide) and pegylated poly(lactide).



IL-2 mutein – Engineered IL-2-Fc fusion protein designed to selectively activate the high affinity IL-2 receptor that is constitutively expressed on Tregs[6]

ImmTOR-IL – Combination of ImmTOR + IL-2 mutein

AAV8-SEAP – Adeno-associated virus-8 encapsidating human secreted alkaline phosphatase transgene

OTII T cells. Transgenic CD4 T cells expressing T cell receptor specific for ovalbumin

Experiment 1 – 2X10^7 ovalbumin-specific OTII cells were adoptively transferred into C57BL6 mice. The next day groups of mice (n=3) were left untreated or treated with a single dose of various combinations of ovalbumin, ImmTOR (100 µg), or IL-2 mutein-Fc fusion protein (9 µg), as indicated. Spleens were harvested 7 days after treatment and CD4+, CD25+, Fox p3+ total Tregs and antigen-specific OTII Tregs were assessed by flow cytometry.

Experiment 2 - C57BL6 mice were treated with 2.7E12 vg/kg AAV8-SEAP on day 0 and 5.0E12 vg/kg on Day 56. Groups of mice (n=5) received no immunotherapy or were treated with IL-2 mutein-Fc fusion protein 9 µg, sub-optimal (50 or 100 µg) or therapeutic (200 µg) doses of ImmTOR, or the combination of IL-2-Fc fusion protein + ImmTOR on Days 0 and 56. Anti-AAV8 IgG antibodies were assessed on various days as indicated.

Experiment 3 - C57BL6 mice were treated with a single high vector dose of 5E13 vg/kg on day 0 . Groups of mice (n=5) received no immunotherapy or were treated with a single dose of ImmTOR, three monthly doses of IL-2 mutein-Fc fusion protein 9 µg, or the combination of IL-2-Fc fusion protein + ImmTOR. Anti-AAV8 IgG antibodies were assessed on days indicated.

Results

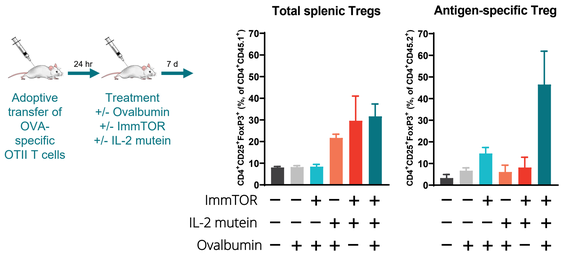


Figure 1. IL-2 mutein acts synergistically with ImmTOR to induce and expand antigen-specific Tregs

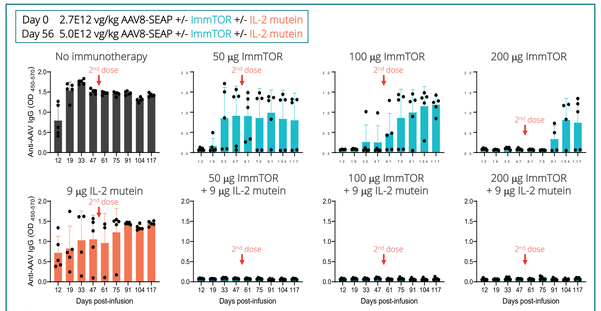


Figure 2. Combination of ImmTOR + IL-2 mutein synergistically inhibit the formation of anti-AAV antibodies

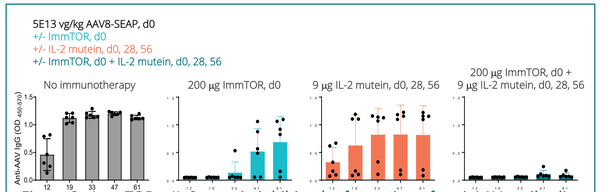
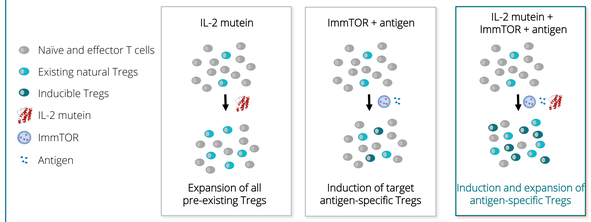


Figure 3. ImmTOR + IL-2 mutein inhibits the formation of anti-AAV antibodies against a high dose of AAV vector

Discussion

Toxicity of high doses of AAV is a major challenge for the AAV gene therapy field. Many groups are working on developing more efficient capsids and transgene cassettes that would allow for smaller doses. A complementary strategy is to enable multiple smaller doses of AAV. Currently AAV gene therapies can only be administered once due to the formation of high titers of neutralizing antibodies. ImmTOR has shown the ability to mitigate the formation of anti-AAV antibodies and enable vector re-dosing. However, antibody responses increase with vector dose. The combination of ImmTOR with Treg-selective IL-2 muteins provides profound synergistic activity in increasing antigen-specific Tregs and inhibiting anti-AAV antibodies, even at high vector doses of 5E13 vg/kg. The ability to re-dose AAV may change the paradigm of AAV gene therapy from 'one-and-done' to 'slower-and-lower'[7].



Conclusions

- ImmTOR + Treg-selective IL-2 mutein (ImmTOR-IL) acts synergistically to induce and expand antigen-specific Treg
- ImmTOR-IL improves the durability of inhibition of anti-AAV antibody formation to repeated vector doses even at some optimal doses of ImmTOR
- The combination also shows synergistic inhibition of anti-AAV antibody formation in response to a high vector dose of 5E13 vg/kg

	IL-2 mutein	ImmTOR	ImmTOR-IL
Induce Treg	✗	✓	✓✓
Expand existing Tregs	✓	✗	✓✓
Antigen-specific	✗	✓	✓✓✓
	Expansion of all pre-existing Tregs	Inducton of target antigen-specific Tregs	Induction and expansion of antigen-specific Tregs

References

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