Preclinical evaluation of fms-like tyrosine kinase 3 ligand (FLT3L) in autoimmunity, allergy, and infection

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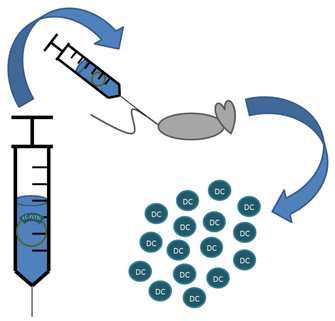
Abstract

The cytokine fms-like tyrosine kinase 3 ligand (FLT3L) is a growth factor that promotes robust expansion of dendritic cells (DC). Although initially pursued as a potent immune stimulator (e.g. tumor vaccination immunotherapy), we and others recently showed that FLT3L expands tolerogenic DC subsets. **We hypothesize that FLT3L may therefore suppress autoimmune and allergic diseases but exacerbate bacterial infection *in vivo*.** A limitation of FLT3L is its short plasma half-life, necessitating repeated injections of recombinant protein. Our lab developed a human immunoglobulin FC-domain-mouse-FLT3L (FC-FLT3L) fusion protein with improved half-life. As a further enhancement, we used hydrodynamic gene transfer (HDT) to induce robust, durable DC expansion *in vivo*, requiring only one i.v. injection of FC-FLT3L DNA. We evaluated FC-FLT3L HDT in mouse models of multiple sclerosis, food allergy against peanut, sepsis, and foodborne infection. In the multiple sclerosis model – experimental autoimmune encephalomyelitis (EAE) – we immunized mice by s.c. injection with myelin oligodendrocyte glycoprotein peptide/complete Freund's adjuvant and i.v. injection of pertussis toxin. To trigger peanut-induced anaphylaxis, we sensitized mice by oral gavage with peanut protein and cholera toxin, then challenged them with peanut protein alone. To induce sepsis, we gave mice systemic administration of endotoxin. To model foodborne infection, we infected mice with the murine gut pathogen *Citrobacter rodentium*. Consistent with our hypothesis, prophylactic FC-FLT3L HDT significantly suppressed clinical EAE. Therapeutic FC-FLT3L HDT also significantly suppressed clinical peanut-induced anaphylaxis. In the bacterial model, fecal colony count was significantly elevated in FC-FLT3L HDT treated mice; 5/8 mice reached end-point criteria (>20% weight loss) compared with 0/8 for the controls. Interestingly, FC-FLT3L HDT increased mortality in experimental sepsis; further studies are ongoing. Taken together, our comprehensive preclinical evaluation of FC-FLT3L HDT indicates that FLT3L can suppress autoimmune demyelinating disease and allergy, but simultaneously inhibits protective antibacterial immunity, likely through tolerogenic DC-induction.

Introduction

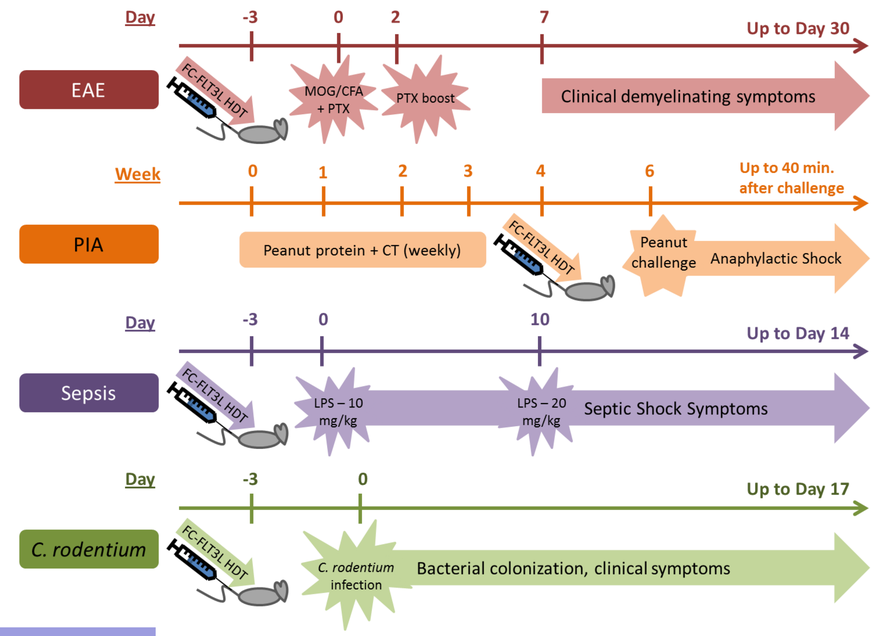


Dendritic cells (DC) are antigen-presenting cells that play a key role in adaptive immune response, by recognizing foreign antigens and inducing either a protective or a tolerogenic response. Using FC-FLT3L HDT, we aim to rapidly expand DC with a tolerogenic phenotype that may suppress symptoms of autoimmunity and allergy.

HDT: In various disease models, mice were injected with FC-FLT3L (10µg) in 2.0 mL physiological saline solution over 3-5 seconds i.v.

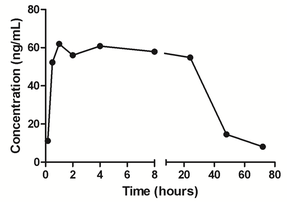
Experimental autoimmune encephalomyelitis (EAE) – mouse model of multiple sclerosis, a demyelinating disease that often results in full-body paralysis.
Peanut-induced anaphylaxis (PIA) – allergic reaction to peanut antigen, resulting from peanut sensitization.
Sepsis – overly-aggressive immune reaction to systemic bacterial endotoxin that results in systemic anaphylaxis.
Citrobacter rodentium (*C. rodentium*) – gut pathogen that acts as mouse model of foodborne infection.

Methods and Materials



MOG = myelin oligodendrocyte glycoprotein peptide (s.c. injection)
CFA = complete Freund's adjuvant (s.c. injection)
PTX = pertussis toxin (i.v. injection)
CT = cholera toxin (p.o. gavage)
LPS = lipopolysaccharide, bacterial endotoxin (i.p. injection)

Results



PK Measure	*FLT3L**	*FC-FLT3L*
C_{max}(ng/mL)	569 ± 121	61.94
T_{max} (h)	1.5 ± 0.5	1.0
$T_{1/2}$ (h)	5.2 ± 0.4	38.2

Figure 1: FC immunoglobulin-FLT3L fusion improves protein half-life. Wild-type C57Bl/6 mice were injected i.p. with FC-FLT3L (10µg) formulated in PBS. Native FLT3L protein PK data obtained from Robinson *et al.**

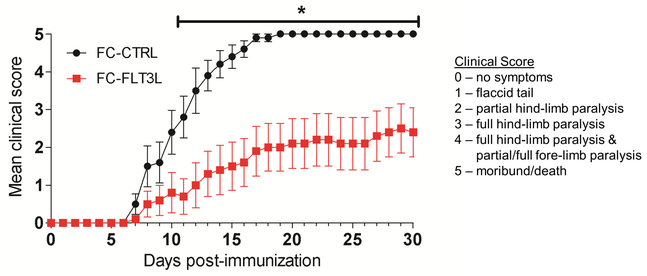


Figure 2: Prophylactic FC-FLT3L HDT suppresses clinical EAE. Prior to disease induction wild-type C57Bl/6 mice were injected with either FC-FLT3L or FC-control via HDT. Mice with clinical score of 4 for 2 consecutive days were euthanized on the third day, and henceforth received clinical score of 5.

N = 10 mice/group (representative of 3 independent experiments)
Mean ± s.e. displayed; *p < 0.05 by Student's *t*-test

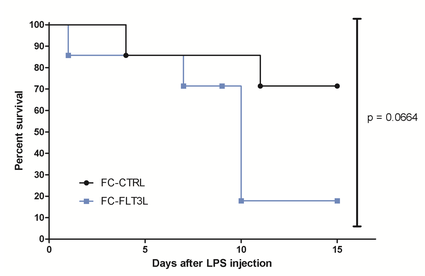


Figure 3: Prophylactic FC-FLT3L HDT increases mortality in sepsis. Sepsis was induced in wild-type C57Bl/6 mice, by LPS injection. Prior to first disease induction mice were injected with either FC-FLT3L or FC-control via HDT.

N = 7 mice/group

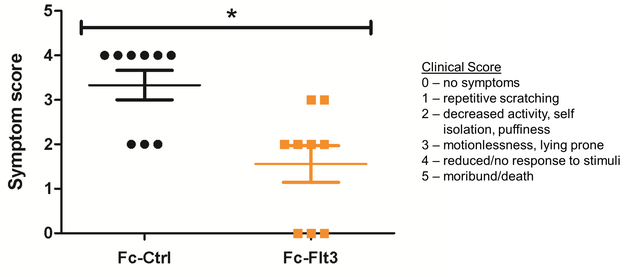


Figure 4: Therapeutic FC-FLT3L HDT suppresses peanut-induced anaphylaxis. After peanut sensitization and before challenge wild-type Balb/c mice were injected with either FC-FLT3L or FC-control via HDT. Maximum clinical score within 40 minutes following challenge was recorded.

N = 9 mice/group; mean ± s.e. displayed; *p < 0.05 by Student's *t*-test

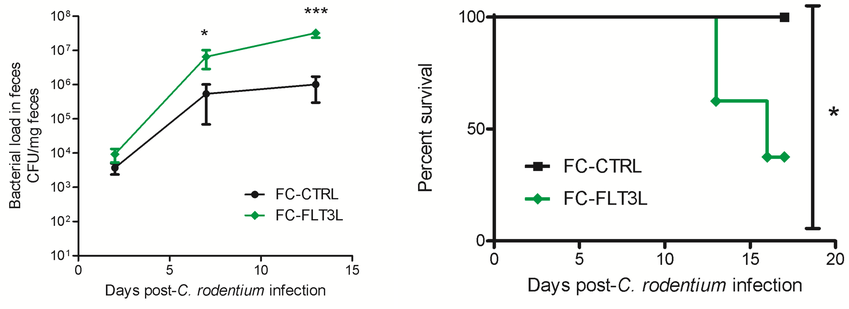


Figure 5: Prophylactic FC-FLT3L HDT increases fecal colony count and mortality in infection by C. rodentium.
Prior to disease induction wild-type C57Bl/6 mice were injected with either FC-FLT3L or FC-control via HDT.

N = 8 mice/group, mean ± s.e. displayed; *p < 0.05, ***p < 0.001 by Student's *t*-test

*Robinson SN, Chavez JM, Pisarev VM, Mosley RL, Rosenthal GJ, Blonder JM, et al. Delivery of Flt3 ligand (Flt3L) using a poloxamer-based formulation increases biological activity in mice. Bone Marrow Transplant. 2003 Mar;31(5):361–9.

Acknowledgements

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Conclusions

FLT3L can suppress autoimmune demyelinating disease and allergy. However, it also has a detrimental effect on protective antibacterial immunity.