

Tyrosine Kinase Flt3/Flt3-Ligand Signaling in the Modulation of Immune Responses in Experimental Arthritis

Akademisk avhandling

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av

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Avhandlingen är baserad på följande delarbeten.

- I. Mattias N. D. Svensson, Sofia E. M. Andersson, Malin C. Erlandsson, Ing-Marie Jonsson, Anna-Karin H. Ekwall, Karin M. E. Andersson, Anders Nilsson, Li Bian, Mikael Brisslert, Maria I. Bokarewa.
Fms-Like Tyrosine Kinase 3 Ligand Controls Formation of Regulatory T Cells in Autoimmune Arthritis. *PLoS ONE* 2013; 8(1): e54884.
- II. Mattias N. D. Svensson, Kersti Månsson, Karin M. E. Andersson, Ing-Marie Jonsson, Mats Bemark, Mikael Brisslert, Maria I. Bokarewa.
Germinal center B cells require Flt3-mediated activation of Stat6 for IgG1 class switch recombination. *Manuscript*
- III. Mattias N. D. Svensson, Malin C. Erlandsson, Ing-Marie Jonsson, Karin M. E. Andersson, Maria I. Bokarewa
Impaired signaling through the Fms-Like tyrosine kinase 3 receptor results in increased osteoclast formation and joint destruction during experimental arthritis. *Manuscript*



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Tyrosine Kinase Flt3/Flt3-Ligand Signaling in the Modulation of Immune Responses in Experimental Arthritis

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Rheumatoid arthritis (RA) is an autoimmune, chronic systemic inflammatory disorder that primarily affects flexible joints resulting in severe joint destruction and disability if left untreated. Today, advances in treatment have significantly improved the outcome for patients, although the pathogenesis of RA remains relatively unknown. Signaling through the tyrosine kinase receptor fms-like tyrosine kinase 3 (Flt3) has been suggested to play a part in the RA pathogenesis. Flt3 is primarily expressed on hematopoietic stem cells and lymphoid progenitors in the bone marrow and has an important role in early B-cell development and formation of dendritic cells (DC). Furthermore, the ligand for Flt3 (Flt3L) serves as a regulator of regulatory T-cell (Treg) homeostasis and has been suggested to support differentiation of bone-resorbing osteoclasts.

This thesis aimed to investigate the effect of Flt3/Flt3L signaling on the immune system during development of arthritis using an experimental animal model of human RA. Our study shows that Flt3 signaling supports formation of DCs and Treg cells during arthritis development. Treg expansion associated with Flt3L treatment resulted in a reduced production of inflammatory cytokines, reduced levels of antigen-specific antibodies and reduced bone destruction. On the contrary, lack of Flt3L was associated with reduced Treg formation resulting in loss of control over T-cell proliferation, and bone destruction during arthritis. Flt3L was found to positively influence the transcription of the osteoclast-regulating factor IRF8, and could by this mechanism influence osteoclast formation. Impaired signaling through Flt3 resulted in low IRF8 expression, accumulation of osteoclasts in the arthritic joint and an increased loss of femoral trabecular bone. Conversely, Flt3L treatment was associated with increased IRF8 expression, reduced osteoclast formation and restoration of trabecular bone formation in mice lacking Flt3L (Flt3LKO). Finally, we could identify a previously unacknowledged role for Flt3 in peripheral B-cell responses. We demonstrated that Flt3 was re-expressed on activated B-cells following LPS stimulation *in vitro* and on a population of germinal center B-cells *in vivo*. By using Flt3LKO mice we could identify an important role for Flt3L in class switch recombination (CSR) to IgG1. B-cells from Flt3LKO mice were found to have reduced activation of Stat6 after IL-4 stimulation, resulting in impaired initiation of CSR to IgG1 and highly reduced formation of IgG1+ B-cells and IgG1 production.

In summary this thesis shows that Flt3L has an important function in regulating DC and Treg homeostasis and function during arthritis. Furthermore, Flt3L has a regulatory role on osteoclast development and on trabecular bone formation. Finally, signaling through the Flt3 receptor on activated B-cells has an important role in the CSR process and deficiency of Flt3L leads to a skewed antibody response towards the more potent IgG subclasses IgG2b and IgG2c. Together, these results suggest that Flt3L might play a protective role during arthritis by reduction of bone destruction, induction of regulatory T-cells and regulation of antibody effector functions. The conclusion of this thesis is that signaling through the tyrosine kinase Flt3 plays an important role in modulating immune responses during experimental arthritis.

Keywords: Flt3, Flt3L, dendritic cells, regulatory T-cells, B-cells, osteoclasts, rheumatoid arthritis

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