alpha-synuclein peptides presented on chimeric MHC class Ib molecules prevent loss of substantia nigra neurons in an animal model for Parkinson's disease

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Abstract

Aim: An accumulating body of evidence suggests the involvement of alpha-synuclein (aSyn) specific autoreactive T cells in Parkinson's disease (PD). Here, we investigate whether novel antigen-specific tolerance-inducing biomolecules, that present peptide antigens on MHC class Ib-related molecules, can inhibit neurodegeneration and prevent PD in vivo.

Methods: The alpha3 domain of the human MHC class Ib molecule HLA-G inhibits T cells via the human ILT–2 or the murine PIRB receptor. We fused this HLA-G alpha3 domain to MHC class I alpha1-alpha2 antigen presenting domains, antigenic peptides and beta2-microglobulin. These single-chain AutoImmunity Modifying Biologicals (AIM Bios) induce antigen-specific tolerogenic CD8⁺ T reg cells in human cells in vitro and in mice in vivo. We used this platform to prevent autoimmune disease symptoms in mouse models for autoimmune diseases, including an aSynA53T-AAV driven model for PD.

Results: Single-chain model AIM Bios with MHC-derived antigen-presenting domains and antigenic peptides polarize cognate CD8⁺ T cells towards a CD8⁺CD122⁺ IL-10 secreting T reg phenotype. Such antigen-specific T reg can also be induced in mice in vivo. Disease-associated aSyn CD8⁺ epitopes were identified in aSynA53T-AAV PD mice. Corresponding AIM Bios prevented mobility impairments. Post mortem histopathological assessment confirmed the induction a favorable in-situ immune cell composition and the rescue of substantia nigra neurons. The translational potential of this approach deserves further exploration.

Introduction I: Neuroinflammation in Parkinson's Disease

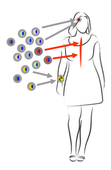
Parkinson's disease (PD) is the most common neurodegenerative movement disorder with over 7 million people affected worldwide and still no cure existing. Patients suffer from severe motor and non-motor symptoms such as tremor, bradykinesia, rigidity, postural instability, and depression. Pathologic disease hallmarks are degeneration of dopaminergic neurons in the *substantia nigra* and formation of a-synuclein-containing protein aggregates called Lewy-bodies. While the physiological role of a-synuclein (aSyn) points towards a function in synaptic transmitter release, the pathogenic role of insoluble a-synuclein remains enigmatic. The observation that multiplications and mutations of the aSyn encoding SNCA gene can elicit genetically inherited forms of PD has robustly linked aSyn to PD pathogenesis.

Neuroinflammation in PD: Autopsies of human PD brains are also characterized by microglia activation and T cell infiltration in the *substantia nigra* (1,2). Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-a, interferon (IFN)-γ and interleukins IL-1b and IL-6 in the nigrostriatal system further confirm that PD goes along with significant neuroinflammation (3). An increased ratio of IFN-γ to IL-4 producing T cells in peripheral blood of PD patients indicates a systemic shift to a pro-inflammatory environment (4). Recently, aSyn-specific T cell responses were detected in peripheral blood cells of PD patients and found to precede motor symptoms, indicating an active and specific involvement of the immune system in disease progression. Two aSyn-derived peptides were shown to elicit MHC-restricted cytokine release from peripheral blood mononuclear cells (5).

Introduction II: Autoimmune Diseases & Immunosuppressive Therapeutics

Autoimmune Diseases are driven by rare self-antigen-specific effector T cells (T_{eff}) which target healthy organs, initiate inflammation and cause tissue destruction.

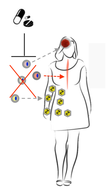
Meanwhile, protective effector T cells fight bacteria, viruses and tumors



Immunosuppressive Drugs

→ slow down autoimmune disease progression

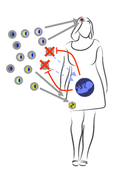
→ also inhibit protective effector T cells, which can lead to tumor growth or opportunistic infections



Selective Immunosuppression

Embryos must selectively inhibit maternal T cells directed against paternal embryonic antigens

This immunosuppression occurs without clinically relevant side effects on protective effector T cells

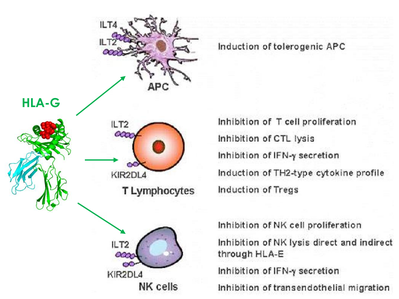


MHC class Ib molecules selectively inhibit defined immune responses

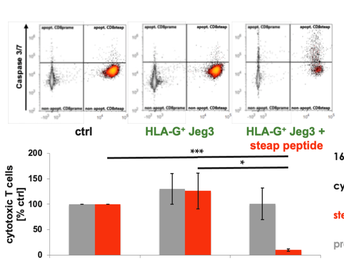
MHC class Ib molecules inhibit various immune cells

It has long been known that embryonic MHC Ib molecules such as HLA-G inhibit various immune cell populations and functions. HLA-G induced tolerogenic antigen presenting cells (APC) and inhibits target cell lysis, proliferation and secretion of proinflammatory cytokines in T cells and NK cells (5). Especially ILT2 and ILT4 receptors play a key role here.

It has also long been known that HLA-G can present peptide antigens (6). However, the role of the presented peptides has not been properly investigated.



(Modified from Menier et al., Biology 2011)



16h assay

cytotoxic T cells:

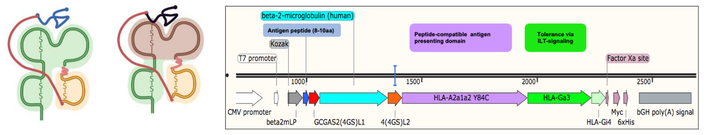
steap specific

prame specific

HLA-G expressing cells loaded with peptide antigens can induce apoptosis in congnate T cells.

HLA-A2-restricted CD8⁺ T cell clones specific for STEAP1 or PRAME peptides were mixed and left alone (ctrl), pretreated with control Jeg3 cells or STEAP1-peptide loaded JEG-3 cells. Within 16h, 90% of the targeted STEAP1 specific CD8⁺ T cells went into apoptosis.

Design of single-chain peptide-MHC Ib constructs (AIM Bios)



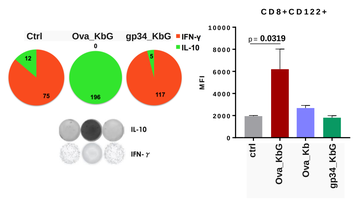
Design of immunosuppressive single-chain peptide-MHC Ib molecules (AIM Bios)

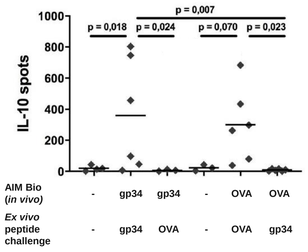
To improve stability and purification efficiency, linkers were added in between of the presented antigenic peptide, the beta 2-microglobulin and HLA-G-5. If a desired peptide cannot be presented on HLA-G, adequate human or murine MHC class I antigen presenting domains were used.

AIM Bios induce antigen-specific regulatory T cells

Surrogate AIM Bios in combination with DCs induce murine Treg cells specific for the presented peptide.

Bone marrow derived DCs were loaded with 5µg/ml murine surrogate AIM Bios containing H-2K^b alpha1-3 domains and Ovalbumin peptide (Ova_Kb), H-2K^b alpha1+2 domain, HLA-G alpha 3 domain and either an Ovalbumin (Ova_KbG) or a viral control peptide (gp34) and combined with Ova-specific OT-I CD8⁺ T cells. Cytokine secretion was determined in ELISpot after 14 days, CD122 quantified by flow cytometry afer 3 days.

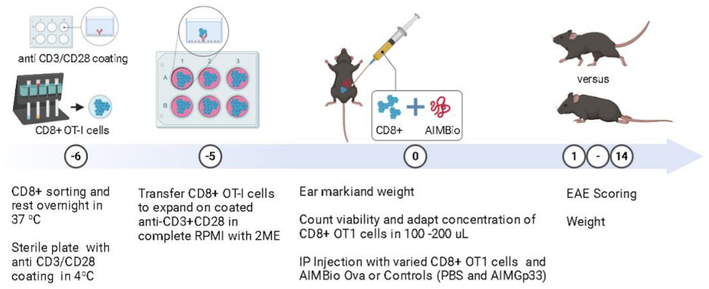


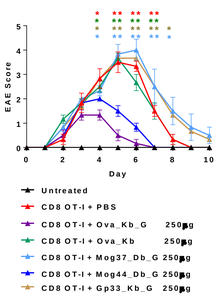


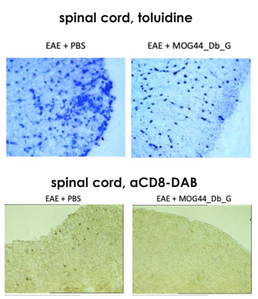
Surrogate AIM Bios selectively induce peptide-specific Treg that secrete IL10 in ex vivo recall assays.

500µg AIM Bio surrogate molecules with gp34 or Ova257 were injected i.p. into WT C57BL/6 mice. After 2 weeks, mice were sacrificed and splenocytes rechallenged in an IL-10 ELISpot assay either with 5µg/ml gp34 or Ova257 peptide. Most mice had generated regulatory T cells that could be activated to secrete IL-10 upon recall with a matching peptide.

Surrogate AIM Bios induce specific & bystander protection in EAE







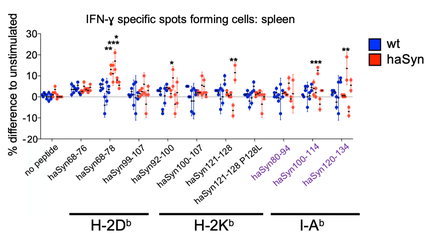
Surrogate AIM Bios presenting model Ova peptide or a Mog44 peptide selectively suppress EAE symptoms.

250µg AIM Bio surrogate molecules were injected i.p. into ODC-Ova mice, which express ovalbumin in oligodendrocytes. In parallel, activated OT-I T cells were injected. Surrogate AIM Bios inducing tolerance towards Ova257 or towards Mog44 selectively suppressed EAE symptoms and spinal cord inflammation.

CD8⁺ T cells in AAV-aSynA53T PD mouse model are activated by aSyn

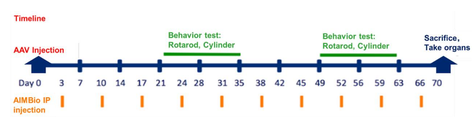
aSyn68-78 is a CD8⁺ T cell epitope targeted in aSynA53T mice

Unilateral injection of 2µl AAV (5.16 x 10^{12} gp/ml) coding for haSyn with the A53T mutant induces Lewy body formation, SN neuron loss and motoric deficits that highly resemble PD in humans (7). To test if this model is driven by specific aSyn T cell epitopes, mice were sacrificed and splenocytes were incubated with A53T-aSyn peptides predicted *in silico*. The H-2D^b peptide aSyn68-78 induced a strong IFN-γ response in ELISpot (8).



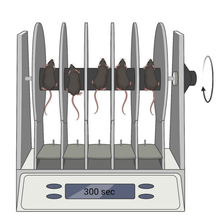
Predicted haSyn peptide sequence & corresponding MHC molecules

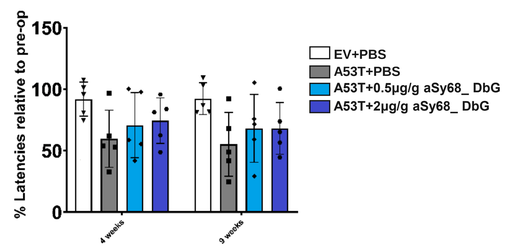
aSyn surrogate AIM Bios induce Treg and inhibit microglia



aSy68_DbG mouse surrogate AIM Bio treatment scheme

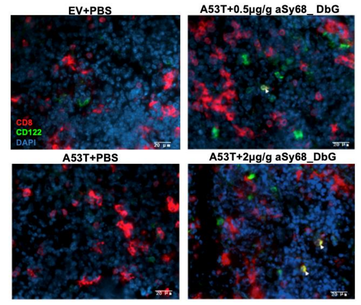
A surrogate AIM Bio surrogate comprising the aSyn68-78 epitope, H-2Db alpha1+2 domains, HLA-G alpha 3 domain and human beta2microglobulin (aSy68_DbG) was produced in Expi Hek293 cells. 0.5 or 2 µg/g body weight were injected i.p. into PD mice as indicated.

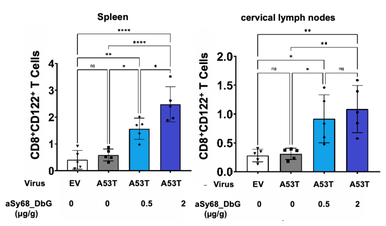




aSy68_DbG does not significantly improve rotarod performance

A rotarod test was used to assess motoric deficits at week 4 and 8 of the experiment. Deficits were comparatively mild, an aSy68_DbG did not significantly improve the performance (only 5 mice per group).

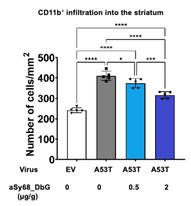


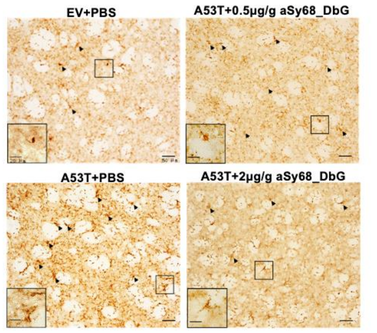


aSy68_DbG significantly induces regulatory CD8⁺CD122⁺ Treg both in the spleen (IF, left graph) **and in cervical lymph nodes** (right graph, IF not shown)

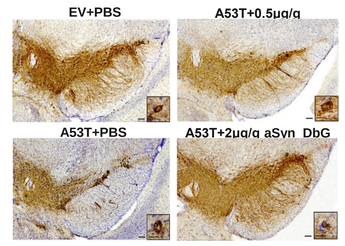
aSy68_DbG inhibits infiltration of CD11b⁺ microglia into the striatum

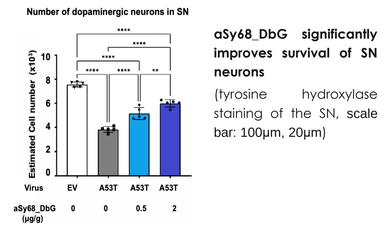
anti-CD11b DAB staining of the striatum





aSyn surrogate AIM Bios prevent SN neuron and striatal fiber loss



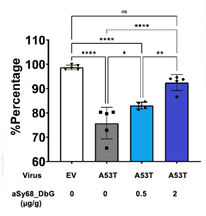


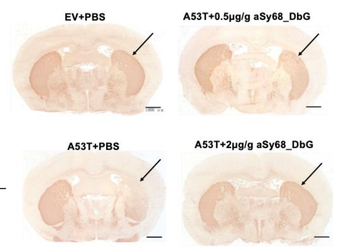
aSy68_DbG significantly improves survival of SN neurons

(tyrosine hydroxylase staining of the SN, scale bar: 100µm, 20µm)

aSy68_DbG significantly improves survival of terminal fibers in the striatum

(tyrosine hydroxylase staining)





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