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AUTOIMMUNE DISEASES

Inhibitor of adaptor protein shows self-antigen selectivity

> the therapeutic effect of AX-024 persisted even after the treatment was discontinued

Patients suffering from autoimmune diseases are usually treated with immunosuppressive drugs, leaving them prone to infection. Now, reporting in *Science Translational Medicine*, Alarcon and colleagues show that a small molecule inhibitor of the adaptor protein NCK, which acts downstream of the T cell receptor (TCR), is highly potent in different models of autoimmune conditions, without affecting immune responses to infectious agents.

NCK is a modular adaptor protein that connects different transmembrane receptors to multiple intracellular signalling pathways and is a crucial regulator of the actin cytoskeleton. In mature T cells, antigen stimulation triggers the recruitment of NCK to the TCR — an interaction that is particularly important for TCR signalling in response to weak antigens.

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Using virtual screening and combinatorial chemistry, the authors developed AX-024, an orally available low-molecular weight compound that binds to an uncommon amino acid pocket in the amino-terminal SH3 domain of NCK and specifically inhibits its interaction with the cytoplasmic tail of the TCR subunit CD3ε. *In vitro*, AX-024 potently inhibited the release of cytokines by T cells in response to anti-CD3-mediated TCR stimulation, but did not seem to disrupt other cellular functions of NCK.

In vivo experiments in mice and rats demonstrated that AX-024, administered orally or by intraperitoneal injection, was well tolerated, even at high doses. In the mouse model of imiquimod-induced psoriasis, daily oral treatment with AX-024 significantly reduced skin thickening and attenuated psoriasis-like symptoms of inflammation. In the ovalbumin-antigen-induced asthma model, it prevented allergic airway sensitization, and in the experimental autoimmune encephalomyelitis model of multiple sclerosis (MS), treatment with AX-024 was more effective than current MS drugs such as glatiramer acetate and cladribine. Even when treatment was delayed until the onset of neurological symptoms, AX-024 had therapeutic efficacy. This was comparable to treatment with the MS drug fingolimod (albeit with slightly slower kinetics). However, in contrast to fingolimod, the therapeutic effect of AX-024 persisted even after the treatment was discontinued.

Further *ex vivo* and *in vivo* experiments indicated that AX-024, by altering the quality and/or strength of the TCR signal, promotes differentiation of $CD4^+$ effector cells towards anti-inflammatory $CD4^+$ T cell subsets and $FOXP3^+CD4^+$ regulatory T cells — providing an explanation for the lasting effects of the drug. Surprisingly, treatment with AX-024 did not impair the generation of protective memory T cell responses after vaccination with a poxviral $CD8^+$ T cell epitope, and it did not affect survival rates after viral challenge with ectromelia virus, which causes mousepox.

The authors point out that the mechanism of selectivity for self-antigens versus pathogen-derived antigens is not completely understood but may lie in the differential requirement for NCK recruitment to the TCR in response to weak versus strong antigens. As AX-024 directly interferes with TCR signalling, it is an attractive candidate as a broad-spectrum agent for autoimmune and inflammatory conditions. It is first-in-class for both targeting an SH3 domain and for directly inhibiting TCR signals and has passed phase Ia/Ib clinical trials.

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ORIGINAL ARTICLE Borroto, A. *et al.* First-in-class inhibitor of the T cell receptor for the treatment of autoimmune disease. *Sci. Transl Med.* **8**, 370ra184 (2016)