THERAPY

Cell-type-specific approach to TNF inhibition

TNF inhibitors have proved highly successful in the treatment of numerous autoimmune diseases, but global inhibition of this pleiotropic cytokine may also nullify its protective functions. Emerging evidence that pathogenic TNF is derived from specific cell types in several disease models suggests a novel approach to anti-cytokine therapy that involves TNF blockade restricted to these cells, the feasibility of which is illustrated in a new study led by Sergei Nedospasov now published in *Proceedings of the National Academy of Sciences of the USA*.

The study builds on earlier work by the same group in which they examined the effects of cell-type-specific ablation of *Tnf* in a panel of conditional knockout mice. They found that TNF derived from myeloid cells was pathogenic in experimental models of septic shock, arthritis and autoimmune encephalomyelitis, whereas T-cell-derived TNF had protective functions in several other models, including experimental tuberculosis infection. “This gave us an idea to specifically target TNF produced by

myeloid cells and spare TNF produced by T cells,” recounts Nedospasov. The present work reports the design and evaluation *in vitro* and *in vivo* of bispecific reagents that specifically target TNF produced by F4/80⁺ myeloid cells.

Flow cytometry analysis demonstrated that these recombinant antibodies simultaneously bind human TNF (hTNF) and macrophage-associated F4/80. Termed myeloid-specific TNF inhibitor (MYSTI)-1 and MYSTI-2, the antibodies capture and retain TNF secreted by these cells, thus preventing its bioavailability. Notably, MYSTI antibodies did not interact with F4/80⁻ cells (mostly B cells and T cells) *in vitro*.

The investigators also designed control antibodies, STI-1 and STI-2, which bind hTNF but are unable to bind F4/80 and thus act as systemic TNF inhibitors. The MYSTI and

control antibodies had similar kinetics of interaction with recombinant hTNF (as measured by surface plasmon resonance) and similarly inhibited TNF-mediated cytotoxicity in the L929 cell line. In peritoneal cavity macrophages and in bone-marrow-derived macrophages from human TNF knock-in

mice (TNF-humanized mice), preincubation with MYSTI antibodies significantly decreased the secretion of hTNF into the supernatant upon stimulation with lipopolysaccharide (LPS), in comparison with preincubation with STI antibodies or a neutralizing single-domain anti-TNF antibody.

The next step was to compare the effects of targeted versus systemic TNF inhibition *in vivo*, for which the investigators used an experimental model of acute hepatotoxicity in which macrophages are known to be the main source of pathogenic TNF. At similar doses, MYSTI antibodies were more effective than STI antibodies in protecting TNF-humanized mice from *D*-galactosamine/LPS-induced hepatotoxicity.

The findings provide proof-of-concept that TNF produced by a particular type of cell can be selectively blocked with a pharmacological reagent. “We have yet to demonstrate the advantage of myeloid-cell-restricted versus systemic TNF neutralization in a chronic autoimmune disease,” acknowledges Nedospasov. “Many other surface molecules on myeloid and other cells have to be evaluated for this purpose in the future.”

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ORIGINAL ARTICLE Efimov, G. A. et al. Cell-type-restricted anti-cytokine therapy: TNF inhibition from one pathogenic source. *Proc. Natl Acad. Sci. USA* <http://dx.doi.org/10.1073/pnas.1520175113>