

Institute of Cell Biology
and Immunology

ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one specific antibody

University of Stuttgart
Germany

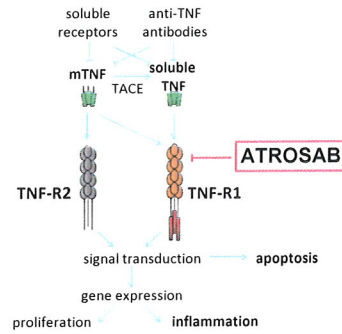
Kirstin A. Zettlitz¹, Verena Lorenz², Peter Scheurich¹, Klaus Pfizenmaier¹, Andreas Herrmann³ & Roland E. Kontermann¹

1.) Institute of Cell Biology and Immunology, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany, 2.) Celonic GmbH, Karl-Heinz-Beckurts-Str. 13, 52428 Jülich, Germany, and 3.) Celonic AG, Eulerstr. 55, 4051 Basel, Switzerland

INTRODUCTION

Tumor necrosis factor (TNF) is a central mediator of inflammation and key target for intervention in inflammatory diseases such as rheumatoid arthritis, psoriasis and Cohn's Disease. The currently approved protein therapeutics directly target TNF and inhibit binding to its two receptors. We have recently humanized a mouse anti-human TNFR1 monoclonal antibody exhibiting TNFR1-neutralizing activity. This humanized antibody (IZI06.1) has been converted into an IgG1 molecule (ATROSAB). Here we describe epitope mapping and functional characterization of ATROSAB. ATROSAB binds with sub-nanomolar affinity to human and rhesus TNFR1, but not to mouse TNFR1. Furthermore, ATROSAB completely blocks TNFR1-dependent apoptosis of Kym-1 cells and interleukin-6/8 release from HeLa and HT1080 cells, respectively. Importantly, TNFR2-mediated signaling is not affected, confirmed by TNF and interleukin-2-mediated costimulation of interferon- γ production by T cells. The epitope was mapped applying chimeric human/mouse TNFR1 molecules and several residues involved in antigen binding were identified by site-directed mutagenesis. ATROSAB could be a useful therapeutic alternative in diseases already known to clinically respond to anti-TNF treatment and particularly in those diseases where specific blockage of TNFR1 and maintenance of TNFR2 function seems as a promising therapeutic approach.

CONCEPT



SUMMARY

We have generated a humanized IgG1 version (ATROSAB) of a murine TNFR1-selective antagonistic antibody (H398), which shows identical binding characteristics and sub-nanomolar affinity to both human and rhesus TNFR1,

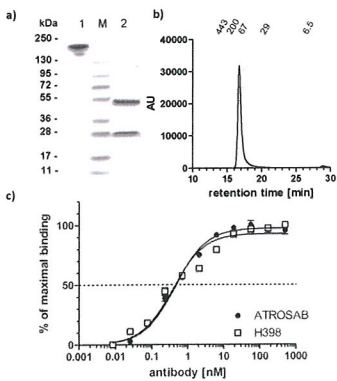
the epitope was mapped to aa 1-70 in the N-terminal region of TNFR1,

ATROSAB completely blocks TNFR1-dependent apoptosis of Kym-1 cells and interleukin-6/8 release from HeLa and HT1080 cells, respectively,

cross-reactivity to rhesus TNFR1 opens the way to in vivo evaluation of ATROSAB in relevant disease models in non-human primates.

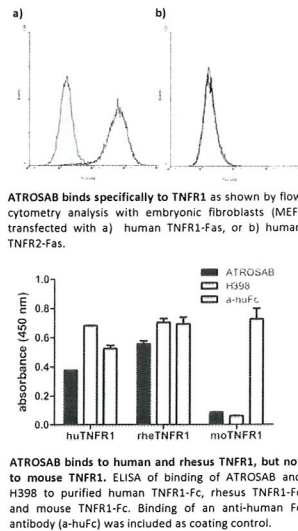
RESULTS

Characterization



Characterization of ATROSAB
(a) humanized IgG version of H398)
a) SDS-PAGE analysis of purified ATROSAB (4 µg/lane, Coomassie staining) analyzed under non-reducing (1) or reducing (2) conditions. b) Size exclusion chromatography of ATROSAB (the position of standard proteins is indicated). c) ELISA of ATROSAB and H398 for binding to human TNFR1-Fc.

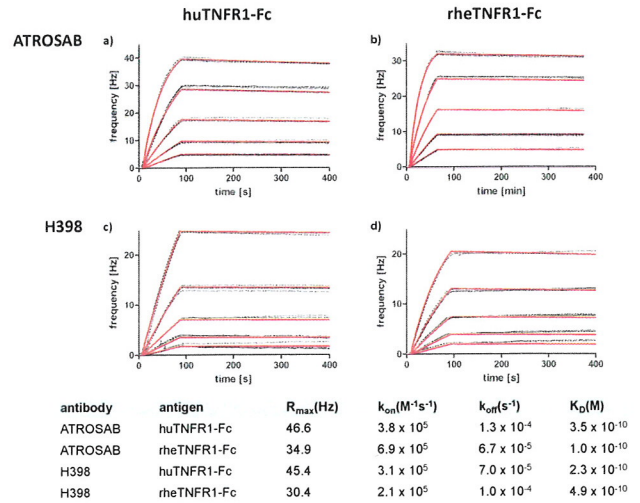
Specificity



ATROSAB binds specifically to TNFR1 as shown by flow cytometry analysis with embryonic fibroblasts (MEF) transfected with a) human TNFR1-Fas, or b) human TNFR2-Fas.

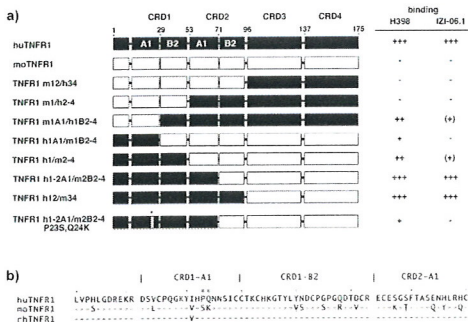
ATROSAB binds to human and rhesus TNFR1, but not to mouse TNFR1. ELISA of binding of ATROSAB and H398 to purified human TNFR1-Fc, rhesus TNFR1-Fc and mouse TNFR1-Fc. Binding of an anti-human Fc antibody (a-huFc) was included as coating control.

Affinity



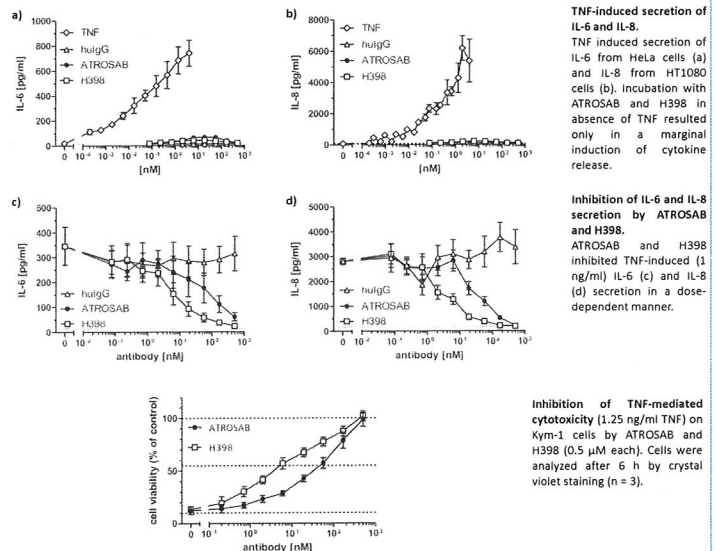
Affinity determination by quartz crystal microbalance (QCM) measurements using an Attana A-100 C-Fast system. The affinities of ATROSAB (a,b) and H398 (c,d) for human (a,c) and rhesus (b,d) TNFR1 were determined using immobilized TNFR1-Fc. ATROSAB bound with sub-nanomolar affinity to human and rhesus TNFR1-Fc, similar to H398.

Epitope mapping



ATROSAB binds to the N-terminal region of TNFR1. a) Epitope mapping of ATROSAB and H398 using wild-type and chimeric human/mouse TNFR1-Fc fusion proteins. b) Sequence comparison of the identified epitope region (aa 1-70) of human (huTNFR1), mouse (moTNFR1), and rhesus (rheTNFR1) TNFR1. Cysteine residues are marked with grey boxes and the 2 positions (P23, Q24) analyzed by site-directed mutagenesis are marked by asterisks.

Inhibition of TNF-action



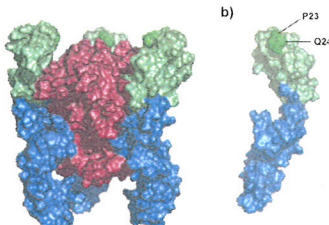
TNF-induced secretion of IL-6 and IL-8.

TNF induced secretion of IL-6 from HeLa cells (a) and IL-8 from HT1080 cells (b). Incubation with ATROSAB and H398 in absence of TNF resulted only in a marginal induction of cytokine release.

Inhibition of IL-6 and IL-8 secretion by ATROSAB and H398.

ATROSAB and H398 inhibited TNF-induced (1 ng/ml) IL-6 (c) and IL-8 (d) secretion in a dose-dependent manner.

Inhibition of TNF-mediated cytotoxicity (1.25 ng/ml TNF) on Kym-1 cells by ATROSAB and H398 (0.5 µM each). Cells were analyzed after 6 h by crystal violet staining (n = 3).



Structure of TNFR1. a) structure of TNF (red) bound to TNFR1 (blue). The identified epitope region is marked in green. b) a single TNFR1 chain. The 2 positions (P23, Q24) identified by mutagenesis to contribute to binding of ATROSAB and H398 are highlighted in dark green.