Biology of TACE inhibition

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Abstract
Studies conducted over the past decade have demonstrated a central role for tumour necrosis factor α (TNFα) in inflammatory diseases. As a result of this work, a number of biological agents that neutralise the activity of this cytokine have entered the clinic. The recent clinical data obtained with etanercept and infliximab highlight the relevance of this strategy. TNFα converting enzyme (TACE) is the metalloproteinase that processes the 26 kDa membrane bound precursor of TNFα (proTNFα) to the 17 kDa soluble component. Although a number of proteases have been shown to process proTNFα, none do so with the efficiency of TACE. A series of orally bioavailable, selective, and potent TACE inhibitors are currently in clinical development. These inhibitors effectively block TACE mediated processing of proTNFα and can reduce TNFα production by lipopolysaccharide stimulated whole blood by >95%. Through a series of studies it is shown here that >80% of the unprocessed proTNFα is degraded intracellularly. The remainder appears to be transiently expressed on the cell surface. Although, in vitro, TACE inhibition has also been implicated in shedding of p55 and p75 surface TNFα receptors, the in vivo data cast doubt on the consequences of this finding. In a mouse model of collagen-induced arthritis, the inhibitors are efficacious both prophylactically and therapeutically. The efficacy seen is equivalent to strategies that neutralise TNFα. In many studies greater efficacy is observed with the TACE inhibitors, presumably owing to greater penetration to the site of TNFα production.

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The cytokine tumour necrosis factor α (TNFα), produced primarily by activated monocytes, macrophages, and T lymphocytes, has been shown to control a wide range of biological responses.1 These include activation of fibroblasts to produce prostaglandins, induction of adhesion molecules and chemokines to up regulate cell trafficking, induction of matrix destructive enzymes, and activation of the immune system. The stimuli that trigger TNFα production and the nature of the biological responses to this cytokine have led to the postulate that TNFα is a central mediator in human inflammatory diseases such as rheumatoid arthritis. In support of this hypothesis, the administration of neutralising anti-TNFα antibody, or of compounds shown to suppress TNFα production, has been efficacious in a wide range of preclinical inflammatory disease animal models, including experimental allergic encephalomyelitis, inflammatory bowel disease, adjuvant arthritis, and collagen-induced arthritis (CIA).2

If TNFα is considered as a therapeutic target, a range of methods can be postulated to regulate TNFα biology.3 These can be broadly categorised into either those that antagonise TNFα activities or those that suppress TNFα production. The former strategies include the generation and administration of soluble TNFα receptor constructs, anti-TNFα antibodies, or small molecule antagonists of ligand/receptor interaction. Indeed, clinical studies in man, with both neutralising anti-TNFα antibodies and a fusion protein, consisting of the extracellular portions of two p75 TNF receptors linked to the Fc region of human IgG1, have been strikingly efficacious in rheumatoid arthritis3,4 and inflammatory bowel disease.5 For example, in a significant fraction of patients with rheumatoid arthritis, administration of the TNFα receptor fusion protein etanercept promptly reduces the number of swollen and painful joints by >70%, a response which has been well maintained by chronic treatment for over three years.

Approaches directed at suppressing TNFα production can be focused on the suppression of activation signals, on the modulation of gene transcription or translation, or on the processing and release of mature TNFα.3 TNFα is released by activated cells as a soluble 17 kDa molecule through the constitutive secretory pathway. However, the molecule is initially synthesised as a 26 kDa membrane bound precursor form (proTNFα) which must be enzymatically processed by the cell to generate the soluble form. Through the use of broad based protease inhibitors, it has been shown that this processing occurs through a cell associated metalloproteinase.6 This enzyme has been isolated and cloned separately by Black

Abbreviations
ADAM = A disintegrin and a metalloproteinase-containing enzyme; CIA = collagen-induced arthritis; IL1, 6 = interleukin 1, 6; IP = intraperitoneal; LPS = bacterial lipopolysaccharide; MMP = matrix metalloproteinase; proTNFα = precursor form for TNFα; TACE = TNFα converting enzyme; TNFα = tumour necrosis factor α; TNFR = tumour necrosis factor receptor; TSST-1 = toxic shock syndrome toxin-1.
and colleagues,10 and Moss and colleagues,11 and termed TACE or ADAM-17. The enzyme is a member of the adamalysin family of metalloproteinases and appears to be responsible for the processing of pro-TNFα in all cell backgrounds examined. The enzyme is expressed constitutively,7 though it appears to require activation to become a pro-TNFα processing protease.8 Blockade of the processing of the precursor to the soluble form of TNFα should result in the elimination of soluble TNFα.

The finding that TACE was a member of the ADAM family of metalloproteinases is important in separating this enzyme from the matrix metalloproteinases (MMPs). Although MMPs and ADAMs both belong to the metzincin family of metalloproteinases,10 they have structural dissimilarities in the amino acids surrounding the active site, as well as considerable overall structural differences. Although MMPs are clearly associated with degradation of the extracellular matrix, the ADAMs, in general, are cell associated and the substrates remain ill defined. Among the best linkages of an enzyme/substrate interaction with biology within the ADAM family is the association of TACE/ADAM-17 with cleavage of pro-TNFα. The structural differences between branches of the metzincin family offer the potential to develop selective inhibitors of TACE/ADAM-17 that do not inhibit MMPs and therefore avoid the toxicities that have been associated with clinical studies using MMP inhibitors.11

We show here that inhibition of TACE, and therefore blockade of the processing of the precursor to the soluble form of TNFα, eliminates soluble TNFα and achieves the same or greater efficacy in an animal model of inflammatory disease as that seen with the available anti-TNFα biological agents. Despite the marked activity of the biological agents that neutralise TNFα from the standpoint of ease of administration, reduced cost of treatment, patient compliance, and the potential for more precise control of TNFα levels, an orally administered, selective small molecule inhibitor of TNFα would be desirable. Small molecule inhibitors of this type may therefore represent a significant step forward in the treatment of inflammatory diseases that are characterised by TNFα overproduction, most notably rheumatoid arthritis and inflammatory bowel disease.

Materials and methods

Materials

Medical grade TNFRI:IgG fusion protein (etanercept, Immunex Corporation, Seattle, WA) was obtained from pharmaceutical supplies. Collagen monoclonal antibodies and incomplete Freund’s adjuvant were obtained from Chondrex LLC (Seattle, WA). Lipopolysaccharide (derived from E.coli) was obtained from Sigma Chemical Co (St Louis, MO). Enzyme linked immunosorbent assay (ELISA) kits to measure TNFα in rodent plasma samples were obtained from Genzyme Corporation (Cambridge, MA). ELISA kits to measure IL6, interferon gamma, JE, or KC in rodent plasma samples were obtained from R&D Systems (Minneapolis, MN). Alzet mini-osmotic pumps were obtained from the Durect Corporation (Cupertino, CA). The superantigen toxic shock syndrome toxin-1 (TSST-1) was obtained from Bachem (Torrance, CA). The Department of Chemical and Physical Sciences of the DuPont Pharmaceuticals Company synthesised the MMP inhibitor and TACE inhibitors. TACE inhibitor compounds were classified into four categories for these studies. Category 1 compounds are potent but non-selective for TACE relative to other metalloproteinases. Category 2 compounds are selective (>1000-fold) against most MMPs, whereas category 3 compounds are selective against all MMPs tested (>10 enzymes). Category 4 compounds are orally bioavailable selective inhibitors.

Whole blood assay

Blood, obtained from normal, human, drug free volunteers was drawn into heparinised tubes (Becton Dickinson, Franklin Lakes, NJ). A 225 µl aliquot of blood was pipetted directly into Biorad (Hercules, CA) polypropylene tubes. Lipopolysaccharide (LPS; S typhimu- rium, Calbiochem, La Jolla, CA) was added to a final concentration of 100 ng/ml and the blood incubated for five hours at 37°C. A 750 µl aliquot of serum-free medium (Gibco-BRL, Frederick MD) was added and cells pelleted at 1500 rpm for 15 minutes. A 500 µl aliquot of supernate was collected and used in assays for TNFα, interleukin 1 (IL1), and IL6 production using standard sandwich ELISA assays (developed in house or obtained from R&D Systems).

Pulse chase studies

The procedures for these studies using purified human peripheral blood monocytes have been previously described in detail.12

Membrane TNF expression

The methods for detection and measurement of TNFα expression on membranes of isolated human peripheral blood monocytes have been previously described in detail.12 Similar methods were applied to the analysis of activated human T lymphocytes.

TNFRI and TNFRII assays

Human peripheral blood monocytes were purified by centrifugal elutriation to >95%.12 Cells were plated in RPMI 1640 (Gibco BRL, Frederick MD) with 5% fetal bovine serum (Hyclone, Utah). Compound was added at time 0 and cells incubated at 37°C, 5% CO2, for up to 48 hours. Supernatants were collected and inhibition of TNFRI and TNFRII measured by standard ELISAs (R&D Systems, Minn, MN). Human T lymphocytes were activated using the superantigen TSST-1 and cultured for eight days in the presence of the human T lymphocyte growth factor IL2 (R&D Systems). After this culture period, cells were enriched for T lymphocytes. These cells were placed into culture with autologous peripheral blood adherent cells and rechallenged with...
antigen. After overnight culture, supernates were collected and evaluated for TNFα or TNFRⅡ levels by ELISA.

ANIMALS
Balb/c mice (18–20 g) were obtained from Taconic (Germantown, NY). The Institutional Animal Care and Use Committee (IACUC) of the DuPont Pharmaceuticals Co, Wilmington, DE, approved all animal procedures.

MOUSE MODEL OF ENDOTOXAEAMIA
Male Balb/c mice (18–20 g) were randomly placed in groups of 5–10 animals/group. All groups except the negative control group received E. coli derived LPS IP (10 µg/mouse). At different time points after receiving the dose, mice were killed, with whole blood collected by cardiac puncture and used for the measurement of plasma cytokine and chemokine concentrations. Results were expressed as mean concentration for all the animals in the group.

COLLAGEN ANTIBODY-INDUCED ARTHRITIS IN MICE
Male Balb/c mice (18–20 g) were randomly placed in groups of 5–10 mice each. Treatment groups included vehicle controls, compound at different doses given by IP administration (bolus injections or continuous infusion using mini-osmotic pumps), or etanercept at 250 µg/mouse twice a week by IP administration. All groups were injected IV with a cocktail of four monoclonal antibodies against the CB11 region of chick type II collagen on day 0 and E. coli LPS at 10 µg IP on day 2. Clinical scores and paw volumes of mice from all groups were recorded three times a week starting on day 0. Clinical scores were assigned using the following scale: 0 = no signs of swelling or redness, normal flexion; 1 = redness and swelling of paws; 2 = marked swelling of whole joint, ankylosis, and loss of function.

Results are expressed as average clinical scores for each paw for the entire group of mice.

Paw volumes were measured by immersion plethysmometry as described previously.15

Results
PROTNFα PROCESSING IS ASSOCIATED WITH TACE ACTIVITY
ProTNFα is synthesised as a 26 kDa precursor protein. This protein is then processed rapidly to the 17 kDa mature TNFα protein. Pulse chase analysis of this processing event demonstrates rapid cleavage that occurs within 15 minutes of the protein being synthesised.12 No intermediate forms are noted, implying a single clip of the precursor. The processing is closely associated with the cellular release of the 17 kDa mature TNFα protein.

The addition of a broad spectrum metalloproteinase inhibitor has been shown to block processing and release of the mature TNFα protein in cells.6 We confirmed this finding using LPS stimulated whole human blood and consistently found inhibition >95%, implying strong dependency on a metalloproteinase mediated cleavage. LPS activation of monocytes also leads to the direct expression of a number of other cytokines. However, no inhibition of the production of the cytokines IL1 or IL6 was seen in these same samples, indicating cytokine specificity and lack of cellular toxicity. No variation in these findings was found between individual subjects (n>50).

Studies published to date have been conducted with non-selective metalloproteinase inhibitors and the question has remained as to whether TACE is the sole mediator of the pro-TNFα cleavage. This is particularly relevant with the discovery that MMPs, such as MMP-7, MMP-14, and MMP-17, together with ADAM proteins, such as ADAM-10, can cleave proTNFα.14–17 We have evaluated a series of inhibitors with increasing selectivity for TACE over other metalloproteinases and have shown that processing in cells consistently tracks with inhibitor specificity for ADAM-17. The inhibitor profiles of the compounds evaluated ruled out the other metalloproteinases cited in the literature from being central to the production of TNFα by human monocytes.

Besides monocytes, an alternative source of TNFα secretion exists in the activated T lymphocyte.18 Activation of T lymphocytes leads to rapid expression and release of soluble TNFα. Therefore, we compared TACE dependency of TNFα secretion from human monocytes with human activated T lymphocytes (fig 1). Although there are considerable differences in the quantity of TNFα produced by the two cell types, both cell sources showed similar TACE dependency based on these inhibitor studies.

REGULATION OF MEMBRANE PROTNFα EXPRESSION
Because unprocessed 26 kDa proTNFα is membrane bound, TACE inhibition could lead to an increase in cell surface associated proTNFα, which has been shown to have potent biological activity in vitro.19 Indeed, activation of human monocytes with LPS,
The cell is rapidly degraded. In addition, the (>85%) of proTNF inhibition and showed that the majority 3–10-fold. Based on the normal levels of TNF inhibition. This increase is in the range of 3–10-fold. Based on the normal levels of TNF secreted, a calculation of the expected levels of expression after blocking TNF processing indicates that the level should be much higher (>10-fold). We conducted pulse chase analysis of proTNF in the presence of TACE inhibition and showed that the majority (>85%) of proTNF that is not processed by the cell is rapidly degraded. In addition, the proTNF that is expressed on the membrane is transient, with a calculated half life of four to six hours. Finally, removal of the TACE inhibitor resulted in a very rapid loss (half life 7.5 minutes) of surface proTNF. Taken together the data indicate a modest, but transient, increase of membrane TNF after LPS stimulation in the presence of continual TACE inhibition. However, the vast majority of the proTNF that is not processed when the TACE enzyme is inhibited appears to be rapidly degraded by the cell.

**TNF RECEPTOR SHEDDING**

After cell activation, a number of cell surface proteins are released into the surrounding media by a metalloproteinase dependent mechanism. Again, with such studies, non-selective inhibitors have generally been evaluated. Because there is evidence that TACE inhibition can affect receptor shedding, the question arises as to whether selective TACE inhibitors would have similar effects. The whole blood assay could not be used to evaluate the impact of selective TACE inhibitors on receptor shedding owing to the relatively high level of endogenous shed receptors present in normal plasma (for example, TNFRII and L-selectin) or owing to low induction after stimulation (for example, CD40 and Fas ligand). Therefore isolated human monocytes were first examined for shedding of TNFRI and TNFRII.

**Figure 2** Time course of the release of soluble TNFRII by human monocytes without stimulation or after LPS stimulation in the absence or presence of 1 µM TACE inhibitor, category 1.

Without inhibitor addition, will lead to a 1.5–2-fold increase in membrane TNFα expression. We found that LPS further increases the level of membrane TNFα (both human monocytes and T lymphocytes) in the presence of TACE inhibition. This increase is in the range of 3–10-fold. Based on the normal levels of TNFα secreted, a calculation of the expected levels of expression after blocking TNFα processing indicates that the level should be much higher (>10-fold). We conducted pulse chase analysis of proTNFα in the presence of TACE inhibition and showed that the majority (>85%) of proTNFα that is not processed by the cell is rapidly degraded. In addition, the proTNFα that is expressed on the membrane is transient, with a calculated half life of four to six hours. Finally, removal of the TACE inhibitor resulted in a very rapid loss (half life 7.5 minutes) of surface proTNFα. Taken together the data indicate a modest, but transient, increase of membrane TNFα after LPS stimulation in the presence of continual TACE inhibition. However, the vast majority of the proTNFα that is not processed when the TACE enzyme is inhibited appears to be rapidly degraded by the cell.

**Human T lymphocytes represent another source of TNFα and TNF receptors.** Therefore, inhibition of TNFRII expression after T cell activation was evaluated and compared with inhibition of TNFα secretion. In these studies, human T lymphocytes were activated by the superantigen TSST-1. These cultures contain 5% autologous monocytes, and studies showed that the T lymphocyte population was responsible for >90% of the production of both proteins under these stimulation conditions.

After overnight culture, the release of both TNFα and TNFRII into the media was significantly suppressed by TACE inhibition. When a dose-response was run and cultures assayed at the same time point (16 hours), the shedding of both molecules was similarly inhibited. Unlike the case with monocytes, where a 10-fold separation was seen with selective TACE inhibitors, the shedding of both proteins was inhibited in T lymphocytes with comparable IC50 values. This implies no discrimination between TNFRII shedding and proTNFα processing in T lymphocytes. However, as with the monocytes, although >95% inhibition of TNFα release could be achieved, the inhibition of receptor shedding was limited to 65–80% of maximum with selective TACE inhibitors.

**Figure 3** Effect of TACE inhibitors with different degrees of selectivity on residual surface expression of TNFRII after antigen activation of human T lymphocytes. Cells were incubated in the absence or in the presence of TSST-I antigen stimulation to induce TNFRII shedding.

TNFRII did not shed or accumulate to any measurable extent in the supernate of LPS stimulated human monocytes and therefore studies focused on TNFRII. TNFRII is released by monocytes between 8.5 and 20 hours after LPS activation (fig 2). Addition of selective TACE inhibitors blocks this release.

In general, using human monocytes, we observed a difference in the potency of TACE inhibitors, with inhibitors being about 10-fold more potent at inhibiting TNFα secretion than the blockade of TNFRII shedding. In addition, although >95% inhibition of TNFα release could be readily achieved, inhibition of receptor shedding was limited to 65–80% of maximum with high concentrations (10 µmol/l) of selective TACE inhibitors (>90% was observed with non-selective inhibitors at this concentration). In these studies, when cell surface expression of TNFRII was examined, TACE inhibition led to no or only a small accumulation of TNFRII on the cell surface as measured by flow cytometry.

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An examination of the lymphocyte cell surface for TNFRII expression showed that the more selective TACE inhibitors showed less shedding of membrane TNFRII (fig 3). In fact, the evaluation of an MMP inhibitor that is without TACE activity showed shedding of about 50% of the surface receptor—comparable with control without any inhibitor present. However, as with monocytes, despite the continual presence of a high level of TACE inhibitor, the slight increase of TNFRII expression that did occur early after activation declined back to baseline with time. Overall, these data indicate that TACE is responsible for a large portion of TNFRII shedding after cell activation but that inhibition does not lead to a significant increase in surface receptor expression on either monocytes or T lymphocytes.

**IN VIVO ANTI-INFLAMMATORY ACTIVITY**

Owing to an increase, albeit transient, in membrane TNFα and inhibition of TNFRII shedding, the question has been raised as to whether TACE inhibition would be pro- or anti-inflammatory. Indeed, the studies of Brennan and coworkers indicated that there was a likelihood of a null effect of TACE inhibition owing to these two events.21 Therefore, we examined the effect of TACE inhibition in a model of CIA in mice. In this model, animals are passively immunised with a cocktail of anticytokine antibodies and the disease triggered by administering LPS.

To examine the correlation between inhibition of TNFα production and efficacy in the CIA model, we first infused a selective TACE inhibitor at different blood concentrations and then challenged mice with LPS. After one hour, the blood level of TNFα was determined. TACE inhibitor concentrations that reflected three different levels of TNFα inhibition (15–20%, 40–60%, and 85–95%) as measured in this LPS mouse model of endotoxaemia were then infused into the CIA model. The data (fig 4) indicate that the levels of TNFα inhibition achieved, as measured in the systemic LPS challenge model, correlate well with clinical efficacy in the CIA model.

Studies with bolus dosing of the inhibitor parallel these findings (fig 5). In fact, the efficacy achieved with the TACE inhibitor consistently exceeded that obtained with administration of the anti-TNF biological agent, etanercept. The clinical observations were supported by histological evaluation of the joints. In control animals the lesions were characterised by pyogranulomatous inflammation, extensive influx of monocytoid and polymorphonuclear cells, extensive synovitis and capsulitis, and loss of normal joint structure. These histopathological findings were completely suppressed with a high dose of TACE inhibitor, but only partially ameliorated with etanercept. The doses of etanercept used were greater than that needed for maximal efficacy in this model and exceeded the human dose by approximately 20-fold based on body surface area.

**TNF RECEPTOR SHEDDING IN VIVO**

To examine receptor shedding in vivo, samples from the LPS-challenged mice were evaluated for increases in TNFRII. The plasma levels of TNFRII were increased 10-fold and this was attenuated by <20% when the TACE inhibitor was infused at levels that block TNFα release by >95%. Therefore, the inhibition of receptor shedding in vivo parallels the findings in vitro using human monocytes that showed no association between inhibition of TNFα secretion and TNFRII receptor shedding.

**THE CYTOKINE CASCADE**

Feldmann and colleagues have described, in in vitro studies with human rheumatoid synovium explants, a cascade of cytokines that follows spontaneous TNFα production.21 In contrast with direct activation of the cells as noted above with LPS stimulation of human blood monocytes, with this system inhibition of this
The issue of TACE dependency of shedding can be considered in a number of ways. For example, TIMP-1 has been shown to block MMP dependent cleavage but to be ineffective at blocking cell associated TACE activity. Likewise, TIMP-3 has been shown to be relatively ineffective against the MMPs but able to inhibit cell TACE activity. We chose to examine this issue by using a set of selective hydroxamate-based TACE inhibitors. The evidence indicates that inhibition of TNFα production is associated with TACE/ADAM-17 inhibitory activity.

Because unprocessed 26 kDa proTNFα remains membrane bound, TACE inhibition could lead to an increase in cell associated, biologically active proTNFα. Membrane associated TNFα has been shown to serve as a ligand for TNF receptors and to activate lymphocytes, affect cellular cytotoxicity, enhance prostaglandin release, and up regulate adhesion molecules. In vivo, Probert and coworkers created transgenic mice overexpressing a membrane form of TNFα (through construction of a transgenic mouse expressing a non-cleavable form of proTNFα) and showed that these mice develop spontaneous joint inflammation. On the other hand, Mueller et al more recently showed that expression of lower levels of non-cleavable TNFα does not lead to this phenomenon.

We found that only a minor amount of unprocessed proTNFα is actually expressed at the cell surface of activated human monocytes. Over 85% of the unprocessed protein appears to be degraded by the cell. Preliminary evidence indicates that this degradation depends on proteasomes. Although the membrane form that is expressed is biologically active, the expression is only transient. The surface TNFα is apparently internalised by the cell in the presence of continual TACE inhibition or may be rapidly shed by the cell after removal of the inhibitor. The observation appears to be quite similar on antigen activated human T lymphocytes. Therefore, the net effect of TACE inhibition is to reduce significantly the amount of soluble TNFα released by the cell, accompanied by a modest and transient increase in cell associated, active membrane proTNFα.

After cell activation, a number of cell surface proteins are released into the surrounding media. Such proteins include the tumour necrosis factor receptors I and II (TNFRI, II), the IL6 receptor (IL6R), the adhesion protein L-selectin, the type II interleukin 1 receptor (IL1RII), transforming growth factor α, and the CD40 and Fas ligands. For all proteins, such “shedding” has been found to depend on metalloproteinases. Recently, cells isolated from a mouse knockout of TACE activity have been shown to be incapable of shedding surface molecules such as TNFRII, IL6R, and L-selectin. The authors conclude that TACE is necessary for shedding of these surface molecules. This issue as to whether TACE is the sole enzyme responsible for shedding remains uncertain owing to the use of an inactive TACE and not the generation of a true knockout.

Discussion
In this report we have examined a number of aspects of TACE inhibition in human cells. At least three potential issues can be raised with TACE inhibition as a means of inhibiting TNFα production. The first is the potential redundancy of enzymes that can process proTNFα, which may mean that inhibiting TACE alone would be ineffective in blocking TNFα secretion. The second is the potential to generate significantly increased levels of the biologically active membrane TNFα. The third is the ability to inhibit TNF receptor shedding. This might potentially lead to an increase in TNF receptor expression that would make the cells hypersensitive to TNFα stimulation.

Figure 6 Induction of cytokines and the effect of TACE inhibitor (category 4) or etanercept administration on LPS-induced cytokine production after LPS challenge in mice. IFNγ = interferon γ.
Because a similar inactive TACE construct also exhibits dominant negative activity, an alternative explanation is that TACE is one of a number of enzymes that can process cell surface proteins, and the dominant negative phenotype of the construct used in generating the mouse precludes processing by other enzymes through competitive association with the substrate. This issue of sheddase specificity also cannot be resolved using the metalloproteinase inhibitors published to date because none have been shown to discriminate among the members of the ADAM/replosyn family.

We found that selective TACE inhibition significantly inhibits surface TNFRII shedding as measured in cell culture supernates after activation. The potency for inhibition of TNFRII shedding by human monocytes is about 10-fold less than the potency for inhibition of TNFRII release. In contrast, human T lymphocytes inhibit TNFRII secretion and TNFRII release with similar potency. These results imply that monocytes may express an additional enzyme that can process TNFRII at the cell surface.

Despite the potent inhibition of shedding, as measured in the supernates, this inhibition does not lead to a significant build up of TNFRII on the cell surface of either monocytes or T lymphocytes. With T lymphocytes, despite the similar potency between inhibition of TNFRII and TNFRII, the induction of TNFRII upon cell activation and the inhibition of receptor shedding, selective inhibitors show no significant build up of membrane TNFRII (fig 3). It is quite probable that the activated cell has mechanisms for controlling the level of expression of receptor and internalises protein to prevent a build up of receptors on the surface.

Despite the hypothetical reasons why TACE inhibition may not be active in vivo, TACE inhibition is anti-inflammatory. This is clearly demonstrated in a mouse model of CIA. This model has served as a predictor for compounds that demonstrate clinical efficacy. We have evaluated compounds in a model of passive immunisation that exhibits a more rapid onset than the collagen immunisation model, but a similar degree of histological damage. We confirmed the efficacy of both the anti-TNF inhibitor antibodies and the receptor fusion protein etanercept in this model (fig 6).

The efficacy of selective TACE inhibition in models of inflammation has been predicted from the use of non-selective inhibitors. However, owing to the well documented role of MMPs in tissue remodelling, the role of TACE inhibition in this efficacy has been uncertain. Cell activation produces high levels of collagensases, gelatinases, and stromelysins, which have been shown to degrade a number of matrix components important for the integrity of the joint. Efficacy may be attributed, to various degrees, to inhibition of these other proteases. However, we have illustrated here that selective TACE inhibitors, with no activity against MMPs, can achieve full efficacy in models of inflammation. Such inhibitors are now being evaluated in human clinical trials and may represent a new and important treatment for inflammatory diseases.

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