Metabolism of Tac (IL2Rα): Physiology of Cell Surface Shedding and Renal Catabolism, and Suppression of Catabolism by Antibody Binding

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Summary

The interleukin 2 receptor α (IL2Rα; CD25; Tac) is the prototypic model for soluble receptor studies. It exists in vivo as a transmembrane complete molecule (TM-Tac) on cell surfaces and as a truncated soluble form (sTac; sIL2Rα). sTac has been used as a serum marker of T cell activation in immune disorders and of tumor burden in Tac-expressing malignancies. In vivo, serum levels of all soluble proteins depend on the balance between production and catabolism, but little is known about the metabolic features of this class of molecules. We have developed a model for Tac metabolism that incorporates new insights in its production and catabolism. Tac was shed from the surface of malignant and activated human T cells with a modal half-life (t1/2) of 2–6 h, but which was prolonged under certain circumstances. The rate of shedding is first order overall and nonsaturable over a two order of magnitude range of substrate (TM-Tac) expression. Once shed from cells, sTac is subject to catabolic activities in the host. In vivo studies in mice showed that 90% of sTac was catabolized by the kidney with a t1/2 of 1 h and a filtration fraction of 0.11 relative to creatinine. The remaining 10% of catabolism was mediated by other tissues with a t1/2 of 10 h. Approximately 1–3% of sTac is excreted intact as proteinuria with the remaining 97–99% catabolized to amino acids. Antibody to the receptor induced a marked delay in sTac catabolism by preventing filtration of the smaller protein through the renal glomerulus and additionally suppressing other nonrenal catabolic mechanisms. A discrepancy between the catabolic rates for Tac and anti-Tac in the same complex was interpreted as a previously unrecognized differential catabolic mechanism, suggesting features of the Brambell hypothesis and immunoglobulin G transport and catabolism, in which the antigen-in-complex in intracellular vesicles is relatively less protected from catabolism than the associated antibody. In light of the pivotal role played by the kidney in sTac catabolism and the impact of administered antibody, the serum concentration of Tac in the settings of renal dysfunction or antibody therapy is not a suitable surrogate of activated T cells or of the body burden of tumor. These results provide parameters for assessing soluble receptor–ligand interactions generally.

Cell surface receptors, adhesion molecules, and their shed soluble forms are increasingly the subject of biological and clinical investigations (1, 2). They are widely evaluated for diagnosis and prognosis in human disease (3), and as treatment targets or as therapeutic agents themselves (4, 5). The array of surface structures generating soluble forms is very large, tallied at 43 in a 1991 review (6), with further molecules described since that time. Yet there is little data on the generation, distribution, and pharmacology of soluble receptors in general.

The prototypic model for these receptors is the IL2Rα chain. It was among the first to be recognized as having a soluble form, and has additionally been the subject of diverse laboratory and clinical investigations. The full IL2Rα plays a central role in lymphocyte activation and proliferation. It is comprised of at least three chains (α, β, γ), of which the α chain (IL2Rα; CD25), termed “Tac” for T

Abbreviations used in this paper: ATL, adult T cell leukemia; CEA, carcinoembryonic antigen; GFR, glomerular filtration rate; S, substrate surface TM-Tac; ss, steady state; sTac, soluble Tac; TM-Tac, transmembrane Tac; constants: k, Tac shedding rate constant; kmet, Tac metabolic rate constant; kren, renal component of kmet; knr, nonrenal component of kmet; krev, rate constants for vascular-to-extravascular and extravascular-to-vascular transport, respectively; q, Tac production rate constant; chemical species: A, anti-Tac; B, sTac; AB, monosaturated anti-Tac { Tac complex; AB2, bisaturated anti-Tac { Tac2 complex; A*, B*, AB*, A*B, A*B*, radioactive components and their complexes.
activation antigen, is the most subject to regulation. Tac is not expressed on resting lymphocytes but is markedly up-regulated with activation of T, B, and NK cells, and macrophages. As such, it has been used as a target for antibodies and other immunodirected therapies that selectively attack activated or malignant Tac-expressing cells while sparing the normal resting T cell repertoire (7). The expression of this molecule has been extensively investigated from the perspective of signaling events, promoter regulation, mRNA transcription, and protein synthesis (8).

The efferent part of Tac regulation—its catabolism—has been less well characterized. Similar to other shed molecules, little is known except that the soluble form of Tac (sTac) is generated in an active proteolytic process against the membrane-bound form (9). sTac levels have been suggested as surrogates of T cell activation in autoimmune and allograft settings and as a measure of tumor burden in Tac-expressing malignancies (10–15). sTac can accumulate to present a significant antigen load that binds administered antibody or IL2 toxin in anti-IL2R therapies, paralleling reports in antibody therapies directed against lymphomas expressing B cell idiomorphs. As such, it has been used as a target for antibodies and other immunodirected therapies that selectively attack activated or malignant Tac-expressing cells while sparing the normal resting T cell repertoire (7). The expression of this molecule has been extensively investigated from the perspective of signaling events, promoter regulation, mRNA transcription, and protein synthesis (8).

The serum level of sTac, as with all proteins, reflects a balance between the rate of synthesis and release from cells and the fractional rate of catabolism. To interpret soluble receptor levels in disease as a measure of cellular synthesis, one must equally understand the pathophysiological factors that regulate synthesis and that alter the catabolism of the protein. Similarly, the interaction with antibodies or other antireceptor molecules has the potential to perturb this in vivo balance. Accordingly, several aspects of the regulation of soluble receptor were examined. sTac production was measured from Tac-expressing human cells in culture to infer mechanisms of soluble receptor generation. Metabolic evaluations were performed in mice to derive pharmacokinetic parameters and to assess the role of the kidney in catabolism. Finally, reciprocal studies examined the impact of soluble receptor–antibody interactions on catabolism to anticipate the iatrogenic impact of such therapeutic interventions.

Materials and Methods

Cells and Cell Lines

Malignant cells from patients were harvested from blood on Ficoll-Hypaque. 2 × 10⁶ cells were dispensed in 24-well plates in 2 ml RPMI 1640 + 5% human AB serum. Tac-expressing T cell line HuT102 was maintained in RPMI + 10% FCS.

Surface Tac (Transmembrane-Tac) and s Tac

Viability was monitored by Trypan blue exclusion. Transmembrane (TM)-Tac was quantitated by 125I-anti-Tac antibody (gift of R. Kozak, NIH, Bethesda, MD) binding modified from earlier methods (20). s Tac was determined by ELISA (21) by a commercial laboratory (Hazleton Labs, Vienna, VA). The unit definition for s Tac was ~3 pg or 0.13 fmol. One evaluation employed T cell activation data from Jacques et al. (22) in an assay closely parallel to our own.

Derivation of Tac Production Rate and Surface t 1/2

s Tac (B) production is modeled as a first order process that is proportional to “substrate” surface TM-Tac expression (S):

\[ \frac{dB}{dt} = kS \]  

(Eq. 1)

For fixed cell numbers and changing TM-Tac (primary malignant cells), solution yields:

\[ \Delta B = \frac{1}{2} k \Delta t (S + S_0) \]  

(Eq. 2)

Where TM-Tac is constant and cell number is changing (cell line HuT102), then

\[ \Delta B = \frac{k}{c} S_0 \frac{N_1 - N_0}{\Delta t} \]  

(Eq. 3)

in which c = ln (N1/N0)/Δt. Where cell number and Tac/cell were varying (activated T cells), these methods were combined in a numerical solution (not shown).

Proteins and Radiolabeling

Recombinant human s Tac (gift of J. Hakimi, Hoffmann-La Roche, Nutley, NJ) was >95% pure by HPLC and SDS-PAGE (not shown). Nonspecific murine antibody UPC (IgG2a, κ) was obtained from Cappell Laboratories (Coeburnville, PA). Proteins were labeled with 131I or 125I to ~1–3 μCi/μg as described (20). Antibody bindability was assayed as described (20).

Mice

Nu/nu mice (Charles River Laboratories, Wilmington, MA) at 12–16 wk of age maintained on thyroid-blocking doses of KI were injected with radioproteins by tail vein or intraperitoneally in 0.2 ml of 1% BSA in PBS. Other animals were administered radioactivity in continuous infusions with 7-d microosmotic pumps (Alzet, Palo Alto, CA) implanted subcutaneously.

Assay of Protein Survival

For rapidly catabolized proteins, or with any protein in nephrectomized or ureter-ligated animals that cannot eliminate catabolic products, it is essential to assay protein-bound radioactivity instead of total radioactivity for pharmacokinetics. Methods were modified from Wochner et al. (23). Blood samples were collected in heparinized glass capillaries via tail vein, counted, and corrected for protein-bound counts by TCA precipitation or by exclusion chromatography of representative samples. For whole body catabolism, carcasses were individually homogenized and aliquots TCA-precipitated and processed.

Pharmacokinetic Modeling

Blood die-away curves of protein-associated counts were analyzed as two compartment models (Scheme 1) using numerical integration by pcNONLIN 4.0 (SCI Software, Lexington, KY), except as noted. For nephrectomized or ureter-ligated animals, the gradual impact of uremia made it possible only to do α-phase modeling, approximated as a first order system over the first 4–8 h
of the assay. Nonstandard nomenclature is used to avoid confusion with other constants in the exposition.

Schema 1.

vascular
inject \( \rightarrow \) \( k_{\text{met}} \) \( \rightarrow \) metabolism
\( k_m \) \( \rightarrow \) \( k_{\text{rev}} \)
extravascular

Relation of Renal Function to Tac Levels

\( \text{sTac} \) (B) concentration in vivo is modeled by zero order production \((q)\) and first order loss \((k_{\text{met}})\) constants:

\[
\frac{dB}{dt} = q - k_{\text{met}}B \quad \text{(Eq. 4)}
\]

\( k_{\text{met}} \) is composed of renal \((k_r)\) and nonrenal \((k_{\text{nr}})\) terms:

\[
k_{\text{met}} = k_r + k_{\text{nr}} \quad \text{(Eq. 5)}
\]

At steady state \((ss)\), \( \frac{dB}{dt} = 0 \), and

\[
B_{ss} = \frac{q}{k_{\text{met}}} = \frac{q}{(k_r + k_{\text{nr}})} \quad \text{(Eq. 6)}
\]

When renal filtration of \( \text{sTac} \) is changed to \( \alpha \), the fraction of normal glomerular filtration rate \((\text{GFR})\), then the new steady state Tac level, \( B_{ss}' \), is increased by the fraction:

\[
\frac{B_{ss}'}{B_{ss}} = 1 + \left( \frac{1 - \alpha}{1 + \beta} \right) \quad \text{(Eq. 7)}
\]

\[
= 1 + \left( \frac{1 - \alpha}{1 + \beta} \right) \quad \text{(Eq. 8)}
\]

in which \( \beta \), the serum creatinine, is inversely related to \( \text{GFR} \). The filtration ratio is the ratio of Tac relative to creatinine filtered through the glomerulus:

\[
\text{FR} = k_r \times \frac{V_d}{\text{GFR}} \quad \text{(Eq. 9)}
\]

GFR for mice is \( \sim 0.1 \text{ ml/min} \) and the plasma volume is \( \sim 1 \text{ ml} \), which is taken as the volume of distribution, \( V_d \), during the early time \((\beta\)-reduced) extrapolation (24).

Experimental Model for Effect of Antibody on Catabolism

Intravenous Bolus. Radiolabeled Tac \((B^*)\) or anti-Tac \((A^*)\) was administered alone or with excess unlabeled antibody or antigen. Radiolabeled UPC, an isotype-matched, irrelevant mAb, was included as an internal control. Five mice were used in each group. The design of Expt. A2 ensures that antibody will saturate labeled Tac throughout the experiment, but the excess of Tac in Expt. A4 saturates labeled anti-Tac only to 60 h by a two-compartment simulation (not shown). Accordingly, no statistics are reported for A4, which are derived in a more rigorous design in which unlabeled Tac was repeatedly injected intraperitoneally to maintain continuous excess of antigen over antibody. I.p. injection of Tac regenerates the 4–5-h \( t_{1/2} \) of the serum and whole body \( \beta \)-phase kinetics after i.v. injection, while avoiding the large \( \alpha \)-phase loss that accompanies i.v. dosing. This strategy follows that pursued by Chang et al. (25) to prolong IL2 survival. Tests of plasma confirmed >90% saturation of anti-Tac through the experiment, whereas anti-Tac before administration was >90% bioactive (bindable). Five mice were used in each group.

| Expt. Form | Labeled protein | Labeled protein | Unlabeled protein, i.p. |
|------------|----------------|----------------|------------------------|
| A3a        | A* \( ^{131}\text{I-anti-Tac} \) \( ^{125}\text{I-UPC} \) |                |                        |
| A4a        | A*B \( ^{131}\text{I-anti-Tac} \) \( ^{125}\text{I-UPC} \) Tac \( ^{50}\mu\text{g; 2,000 pmol} \) \( ^{2} \) |                |                        |
|            | \( (0.15 \mu\text{g; 1 pmol}) \) |                |                        |

\(^{2}\)Administered every 12–24 h

Continuous infusion. Animals were implanted subcutaneously with pumps secreting \( ^{131}\text{I-Tac} \) \((B^*)\), then injected with \( ^{125}\text{I-anti-Tac} \) \((A)\) or nonspecific IgG. Two or three mice were used in each group.

| Expt. Form | Continuously infused | Bolus injected |
|------------|----------------------|---------------|
|            | Labeled protein      | Labeled protein |
| B1         | \( ^{131}\text{I-Tac} \) \( ^{2.6}\mu\text{g; 80 pmol} \) \( ^{2} \) | \( ^{125}\text{I-UPC} \) \( ^{200}\mu\text{g; 670 pmol} \) |
| B2         | \( ^{131}\text{I-Tac} \) \( ^{2.6}\mu\text{g; 80 pmol} \) \( ^{2} \) | \( ^{125}\text{I-anti-Tac} \) \( ^{200}\mu\text{g; 670 pmol} \) |

\(^{2}\)Administered every 12–24 h

Derivation of Equations for Survival Kinetics and Anti-Tac | Tac Interaction

Bolus Intravenous Dosing. The net clearance of anti-Tac \((A)\) is modeled as the sum of the separate first order clearances of the three States of A \((1–3)\) in chemical equilibrium (Schema 2). Clearance of \( \text{sTac} \) \((B)\) is similarly modeled by three States \((2–4)\), in which two are shared with anti-Tac as complexes and the last is occupied by free \( \text{sTac} \). \( \text{AB} \) is similarly related to B as shown for \( \text{AB}_2 \), as indicated by dashed lines.) The interacting kinetics and equilibria were solved numerically (not shown) for the time-dependent concentrations of A, B, AB, and \( \text{AB}_2 \).
Continuous Infusion and Antigen Steady State. Where antigen is continuously produced, the formulation must include the production rate, \( q \). In the absence of antibody:

\[
\frac{dB}{dt} = q - k_4 B
\]  
(Eq. 10)

\[
B_{ss} = \frac{q}{k_4}
\]  
(Eq. 11)

In the presence of antibody,

\[
\frac{dB_{tot}}{dt} = q - k_4 B - k_3 AB - 2 k_3 AB_2
\]  
(Eq. 12)

This is solved numerically as above, from which \( B_{tot,ss} \) is derived.

Model Simplifications. When antigen binding does not affect antibody survival, the kinetic model may be reduced from four to two states: State 1: Tac bound and all anti-Tac forms, with a prolonged clearance; State 2: Tac free, with a rapid clearance (Schema 3). This permits simplification of the rate equations

\[
\frac{dA_{tot}}{dt} = -k_1 \{ A + AB + AB_2 \} = (-k_1 A_{tot})
\]  
(Eq. 13)

\[
\frac{dB_{tot}}{dt} = -k_2 AB - 2 k_3 AB_2
\]  
(Eq. 14)

in which \( \gamma = B/B_{tot} \) (the fraction of free Tac) and \( k' \) is a number-average rate constant that lies between that of antibody \( (k_1) \) and free Tac \( (k_2) \), biased by the fraction of bound versus free Tac; for large \( A, k' = k_1 \). Eq. 14 retains the form of a first order differential equation with log-linear plots for \( \gamma \) constant over the experiment. The assumption of equal kinetic constants for Tac (B) and anti-Tac (A) in the same complex (AB or AB2) is a hypothesis tested in these studies.

The simplified equations for antigen steady state are similarly:

\[
\frac{dB_{tot}}{dt} = q - k' B_{tot}
\]  
(Eq. 15)

\[
B_{tot,ss} = \frac{q}{k'} = \left( \frac{qt_{1/2}}{ln2} \right)
\]  
(Eq. 16)

For large \( A, k' = k_1 \), and the new steady state is increased by \( k_4/k_1 \), the theoretical maximum. Anticipating results in text, this maximum is \( k_4/k_3 \) where catabolism of antigen-in-complex is faster than antibody-in-complex.

Results

Shedding of the IL2R \( \alpha \) Subunit, Tac

There is no independent secretory mRNA for Tac that could undergo independent regulation (27–29); hence, all sTac is derived from proteolytic cleavage of TM-Tac (9). We therefore wished to know the relation of Tac shedding to TM-Tac expression.

Patients with adult T cell leukemia (ATL) have elevated TM-Tac on their malignant cells, but this expression may increase substantially during culture in vitro that is not seen with normal T cells (30). For the patient of Fig. 1, TM-Tac expression increased progressively over the 4 d period of the assay. Cell number was stable and viability remained \( >90\% \). Therefore, increased expression in the culture reflected increased expression per cell, which was corroborated on flow cytometry (not shown). The cumulative level of sTac in the supernatant also increased during the period, such that by day 4 it exceeded TM-Tac on cells by a factor of 10 (Fig. 1 B), although TM-Tac was itself rapidly increasing over the same period.

Shedding of Tac Is First Order with Respect to Membrane Tac. We consider two general mechanisms of shedding, saturable and nonsaturable. If mechanisms of shedding are saturable—as in a typical Michaelis-Menten enzymic process—an increase in TM-Tac will yield a less-than-proportionate increase in sTac shedding as saturation is approached, yielding finally a zero order process with a fixed, “maximal” rate, although TM-Tac was increasing. If shedding mechanisms are not saturable—as in a spontaneous or autocatalytic process—the rate of shedding should be first order and proportional to TM-Tac. Stated alternately, the fractional shedding rate in a first order process would be constant for all levels of TM-Tac, with an invariant \( t_{1/2} \); in a zero order process (or Michaelis-Menten approaching saturation), fractional shedding would decrease with higher TM-Tac with a progressively increasing \( t_{1/2} \). This situation, in which the same cells express different levels of TM-Tac over time, presents a special opportunity to distinguish between these models of cellular shedding.

sTac levels for each 24-h period were fit to a model in which sTac production was first order relative to TM-Tac expression (see Materials and Methods). TM-Tac increased almost 40-fold in the example of Fig. 1, from 5,000 molecules/cell to 180,000/cell over 4 d, yet the \( t_{1/2} \) was virtually constant for all levels of TM-Tac (Table 1; ATL1/1). Similarly, a survey of donor cell assays in Table 1, including the activated T cells of a normal donor, shows no instance in which increasing TM-Tac to high levels correlates with a prolonged \( t_{1/2} \). The relative constancy of \( t_{1/2} \) with different TM-Tac expression levels in individual Tac-expressing malignancies and activated T cells is compatible with a first
order model. In addition, the same rapid $t_{1/2}$ for Tac shedding is seen with a malignant T cell line of activated phenotype (HuT102) which constitutively expresses high levels of TM-Tac. A zero order or saturable model would have shown proportionately longer $t_{1/2}$ with higher TM-Tac expression, which was not observed. We may therefore exclude any simple zero order model to explain soluble Tac production.

The determinations in Table 1 show a modal $t_{1/2}$ of 2–6 h for Tac shedding that encompasses the malignant T cells from two of six ATL patients, one malignant T cell line, and normal activated T cells. However, individual exceptions were noted, with outliers at the high end where shedding was almost inapparent ($t_{1/2}$ 40–180 h), despite measurable TM-Tac levels, and during late phases of normal T cell activation (Table 1).

When shed in vitro, Tac accumulates as a stable component in the medium. In vivo, however, Tac is subject to the normal catabolic activities of the host, which occupy the remainder of this investigation.

**Metabolism of sTac**

To estimate the in vivo fate of sTac after it is released from cells, we investigated the pharmacokinetic behavior of a recombinant human sTac protein in mice. Our initial studies examined the serum die-away profile to derive rate and volume constants via compartmental analysis. Tac was rapidly cleared in a two-phase kinetic pattern (Fig. 2),

**Table 1. Shedding $t_{1/2}$ Values Are Not Prolonged by High TM-Tac Levels**

| Cell population studied | ATL1/1 | ATL2 | ATL3 | ATL4 | ATL5 | ATL6 | ProLL | Hut 102 | T cell |
|-------------------------|--------|------|------|------|------|------|-------|---------|--------|
| Shedding Tac/cell $t_{1/2}$ Day | $t_{1/2}$, h | $10^{-3}$ | ATL1/2 | ATL2 | ATL3 | ATL4 | ATL5 | ATL6 | ProLL | Hut 102 | T cell |
| 0 | 10.7 | 6.5 | 3.4 | 7.7 | 18.8 | 45.0 | 23.1 | 3.7 | 3.7 | 3.7 | 3.7 |
| 1 | 6.0 | 3.2 | 1.3 | 8.0 | 11.3 | 13.9 | 190.2 | 109.7 | 4.4 | 3.2 | 3.2 |
| 2 | 6.1 | 6.2 | 4.3 | 16.2 | 16.2 | * | 86.8 | 86.3 | 3.2 | 3.2 | * |
| 3 | 5.6 | 3.9 | 3.7 | 14.4 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 |
| 4 | 8.1 | 80.5 | 80.5 | 80.5 | 80.5 | 80.5 | 80.5 | 80.5 | 80.5 | 80.5 | 80.5 |
| 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |

Primary malignant T cells or T cell line HuT102 were cultured and assayed at various times for TM-Tac and released sTac. $t_{1/2}$ values in hours (bold) and TM-Tac $10^{-3}$/cell (in parentheses) are represented. $t_{1/2}$ values were derived as in Materials and Methods. The two instances for ATL1 represent evaluations before treatment and during a relapse 6 mo later. Too high culture cell number in ATL1/2 exhausted medium by day 3, slowing shedding and Tac upregulation, which both recovered with medium replenishment on day 3 (day 4 data). Activated T cell data were calculated from published data of Jacques et al. [22] using rested T cell blasts that were stimulated at time zero. *Rate constant could not be calculated.

Figure 1. TM-Tac upregulation and high level sTac production by primary ATL cells in culture. Cells were obtained from donor 1 (Table 1). (A) Number of TM-Tac per cell and concentration in U/ml of sTac in the culture medium. (B) Total moles of Tac which are cell associated or soluble. (■) TM-Tac; (bars) sTac accumulation between zero time and cell harvesting.
whereas a typical slow catabolism of coadministered IgG was observed (not shown, but see Fig. 4 B). The terminal, \( \beta \)-phase of the blood curve parallels the fractionated (protein-associated) whole body curve, as expected (31). The \( \alpha \)-phases are not comparable because of key differences in the formulations' treatment of the distribution constants: for blood, the rate of loss is accelerated by extravascular distribution; for whole body, the rate of loss is delayed by extravascular distribution. The derived constants from the venous sampling of two experiments are listed in Table 2.

Table 2. Kinetic Constants Demonstrating Pattern of Normal Tac Distribution and Catabolism

| Kinetic constants | Derived \( t_{1/2} \) values |
|------------------|---------------------------|
|                  | Expt. 1 | Expt. 2 | Expt. 1 | Expt. 2 |
| \( \alpha \)      | (Macroscopic rate constant) | 1.12 (0.17) | 1.10 (0.06) | 0.62 | 0.63 |
| \( \beta \)       | (Macroscopic rate constant) | 0.14 (0.04) | 0.14 (0.01) | 4.95 | 4.95 |
| \( k_{\text{met}} \) | (Metabolic rate constant) | 0.63 (0.05) | 0.49 (0.02) | 1.10 | 1.41 |
| \( k_{\text{ve}} \) | (Vascular to extravascular rate) | 0.38 (0.10) | 0.45 (0.03) | 1.82 | 1.54 |
| \( k_{\text{ev}} \) | (Extravascular to vascular rate) | 0.26 (0.09) | 0.32 (0.03) | 2.67 | 2.17 |
| \( V_s/V \)       | (Normalized distribution volume) | 2.6 (0.48) | 2.4 (0.10) |

Kinetic constants were derived by two-compartment modeling (Schema 1) as in Materials and Methods. Expt. 1 is represented in Fig. 2; expt. 2 involving nine mice is not shown. Fitting errors are in parentheses. \( \alpha \) and \( \beta \) are the macroscopic rate constants. The microscopic constants are \( k_{\text{met}} \), the overall metabolic rate constant, and \( k_{\text{ve}} \) and \( k_{\text{ev}} \), the rate constants for transfer from blood to tissue and from tissue to blood. \( V_s/V \) is the distribution volume at steady state as a multiple of the plasma volume (the initial volume of distribution).
mimic this in vivo situation more directly, we investigated the peritoneum as an extravascular site for Tac administration.

Fig. 3 shows a comparison of blood and whole body kinetics of animals administered Tac by i.v. or i.p. routes. The blood kinetics after i.v. injection show an α-phase $t_{1/2}$ of 0.6 h, comparable with that of Fig. 2, representing the sum of catabolism and distribution. In contrast, the i.p. injection shows a delayed transfer of radioactivity from the peritoneum to the blood over a period of 2 h and then displays loss kinetics with a $t_{1/2}$ of 4 h that is paralleled in the whole body curves (Fig. 3 B). The 4-h $t_{1/2}$ of intraperitoneally injected material is comparable to the 5-h terminal $t_{1/2}$ of the i.v. data of Fig. 2, which was interpreted as the delayed return to blood of sTac that transferred extravascularly. The control IgG transports from peritoneum to blood and then remains stable over the remainder of the experiment. A further comparison of i.p. and s.c. administrations of Tac suggested comparable transfer rates to blood from either of these two extravascular sites (not shown). We conclude that Tac shed into tissues will be transported to the vascular space and catabolized with a combined $t_{1/2}$ for the transport and catabolic processes of about 4–5 h, adding 3–4 h of net delay to the catabolic $t_{1/2}$ of Tac in blood.

Renal Catabolism of Tac. Small proteins such as Bence Jones proteins (Ig L chain monomer [22 kD] and dimer [44 kD]) are cleared primarily by renal mechanisms with a serum $t_{1/2}$ of ~1 h in humans and in mice, which typically parallel humans in the handling of proteins by the kidney (23, 24, 31, 33). Our studies demonstrating rapid clearance of Tac led to our hypothesis of renal catabolism for this ~30kD (33a) glycoprotein as well.

The role of the kidney in catabolism of Tac was assessed by nephrectomy in mice. The ratio of catabolic rates between control and nephrectomized animals indicates the fraction of catabolism attributable to kidney. Ureter ligation inhibits filtration by 11–67% over the first 8 h (34, 35) and provides a setting of moderately reduced GFR. Because whole body sampling represents the sum of vascular and extravascular compartments, the distribution of protein to tissue delays the loss kinetics in contrast to venous sampling in which distribution accelerates loss kinetics. In this setting, the catabolic rate constant is obtained from the initial time kinetics when the system approximates a single, vascular compartment.

As for the venous sampling studies, $^{131}$I-Tac was co-injected with $^{125}$I-irrelevant IgG as a large protein not normally catabolized in the kidney (Fig. 4). The catabolic rate constants ($k_{\text{met}}$) and time to catabolism of 50% of injected Tac were 0.72 h$^{-1}$ for control mice ($t_{1/2}$ 1.0 h), 0.28 h$^{-1}$ for ureter-ligated mice ($t_{1/2}$ 2.4 h), and 0.07 h$^{-1}$ for nephrectomized mice ($t_{1/2}$ 10 h) (Table 3). These rates indicate that 90% of catabolism is renal with the remaining 10% due to other tissues. In contrast, the concentration of IgG remained high over the study period for normal, ureter-ligated and nephrectomized mice in accord with prior results (23). The catabolic rate constant, $k_{\text{met}}$, represents the sum of renal ($k_r$) and nonrenal ($k_{ur}$) terms (Eq. 5). Assigning 0.07 h$^{-1}$ to $k_{ur}$ from the nephrectomy data, we infer a rate constant of 0.72–0.07 = 0.65 h$^{-1}$ for $k_r$ in normal mice. For the ureter-ligated animals, $k_r$ is 0.28–0.07 = 0.21 h$^{-1}$, indicating a 68% reduction in GFR, which is compatible with expected GFR loss (34, 35). From Eq. 9, we derive a renal filtration ratio of 0.11 for Tac relative to creatinine.

All whole body sTac curves in Fig. 4 A show progressive prolongation of survival with time. For the normal animals, this may be explained (above) by the portion of sTac penetrating extravascular tissues during the first 4 h that then diffused back into the blood to gain access to the renal catabolic compartment, as shown previously for L chain dimers.
Table 3.  Kinetic Constants Demonstrating Role of Kidney in Tac Metabolism

|        | \(k_{\text{met}}\) | Protein \(t_1/2\) | Percent metabolism due to catabolism | Percent metabolism due to proteinuria |
|--------|------------------|------------------|-------------------------------------|--------------------------------------|
| Control | 0.72 (0.06)      | 1.0              | 97-99%                              | 1-3%                                 |
| Ureter ligated | 0.28 (0.03) | 2.4              | N/A                                 | N/A                                  |
| Nephrectomy | 0.07 (0.01) | 10.2             | N/A                                 | N/A                                  |

The data of Fig. 4 were analyzed by curve stripping to define the \(\alpha\)-phase of the control animals, and derived graphically for the two operated sets of animals. In this experimental design, the derived macroscopic \(\alpha\)-phase rate constant equals the microscopic metabolic rate constant \((k_{\text{met}})\) (Materials and Methods). N/A, not applicable.

(23). These considerations should apply equally to the ureter-ligated animals that retain 30% of GFR, but these intercompartmental rates are less important in the \(\beta\) phase in rate of uraemia-induced suppression of catabolism in these animals, as previously shown (23).

Renal Excretion of Tac. The excretion products after \(^{125}\)I-Tac administration were examined to quantitate the degree of proteinuria. Inasmuch as the tissue-

stable Tac will release iodide and iodotyrosine to the kidneys, urinary collection represents the sum of all catabolic products rather than just those generated by the kidney. The spot urine of three normal mice 6 h after injection and the cumulative 48-h collections on a further three animals showed comparable fractions of protein-associated radioactivity (0.7-2.3 and 1.2-3.3%, respectively). The remaining 97-99% was degraded to low molecular weight radioiodine products (Fig. 5), interpreted principally as free iodide (36).

Thus, of the \(s\) Tac entering the blood, 10% is catabolized at external sites and the remaining 90% is filtered through the glomerulus. Of this 90% renal metabolism, 1-3% is due to proteinuria; the remaining portion (87-89%) of Tac passing the glomerular filter is catabolized in the renal tubules (24, 33).

Fig. 6 summarizes the data on the generation and normal catabolism of soluble Tac.

Antibody Inhibits Tac Catabolism

During antibody therapies, the binding to soluble antigen has the potential to disturb the receptor's normal catabolism. To define the impact of this interaction, two experimental approaches were employed: (a) an i.v. bolus injection model to derive catabolic constants, and (b) a continuous production model to measure changes in the steady state soluble receptor levels.

Bolus Injection: Anti-Tac Binding Prolongs In Vivo Survival of Tac. The survivals of radiolabeled Tac (\(B^*\)), radiolabeled anti-Tac (\(A^*\)), and their complexes were examined. Inspection of Schema 4 reveals that only four species can be studied directly: the free forms, \(B^*\) and \(A^*\), and their saturated complexes, \(AB^*\) and \(A^*B_2\); the crossed forms can only be created in the presence of additional labeled species.

Schema 4.

![Diagram of Schema 4](image)

Figure 5. Minor loss of Tac by proteinuria. The urinary products of Tac metabolism were examined by Sephadex G25 chromatography of the cumulative 48-h urine output of two mice after \(^{125}\)I-Tac administration (---■---, ---▲---). As control, the profile of a \(^{125}\)I-sTac preparation with 5% free iodide is shown (---×---).
Antigen excess was maintained via recirculation to maintain excess B over A* beyond 60 h (Expt. A4). Anti-Tac antibody increased Tac* (AB*) survival, with a 13 tt/2 of 82 h, with a catabolic t1/2 measured for unbound Tac (B*). Tac and a catabolic response for radiolabeled bound Tac (AB*) is significantly different from radiolabeled anti-Tac (A*) (p < 0.001).

Because of concerns over the design of this experiment to maintain excess B over A* beyond 60 h (Expt. A4, Materials and Methods), a more rigorous test was performed, in which antigen excess was maintained via recurrent i.p. Tac (B) dosing (Fig. 8). The new rate of anti-Tac* decline after Tac dosing was not different from that in mice with no antigen administration (p = NS for all comparisons) (Table 5). We conclude that saturation binding with Tac antigen does not alter antibody survival, that is, A*B 2 survival equals A*.

An unexpected feature of the data of Fig. 8 was the positive increment of 131I-anti-Tac* radioactivity in the blood after the first Tac dose, which was not seen in the control group. This was observed in each of the five mice in this test, and it was not observed in the nonspecific 125I-IgG control antibody that was coadministered in the same mice (not shown). Different physiologic phenomena may be posited for this observation, but this was not a focus of our study and we did not investigate it further.

These survival data (Tables 4 and 5) present us with a further surprising result. Paradoxically, anti-Tac (A*) and bisaturated anti-Tac complex (A*B2) were equal, suggesting no effect of Tac binding on antibody survival, yet the clearance of the monosaturated anti-Tac complex with Tac-labeled (AB*) was significantly faster than either, although it is intermediate structurally between them (Schema 4). Accordingly, the equivalence of the clearance rates of A*, A*B (presumed, supra) and A*B2 versus the apparent faster clearance of AB* suggests a compromise between (a) the accelerated clearance of dissociated Tac, and (b) the prolonged survival of Tac complexed with antibody. To examine this hypothesis, we modeled the setting in which k1 = k2 = k3 and the rate constants k2 and k3 apply to both members of each complex (Schema 2). We then tested whether the predicted fraction of free Tac (B*) could explain the accelerated clearance of Tac* in the AB* complex versus anti-Tac (A) in the same complex. The equation for this setting is (Schema 3):

\[
dB_{tot}/dt = - \{k_1 \gamma + k_1 (1 - \gamma)\} B_{tot} = k' B_{tot} \quad \text{(Eq. 14)}
\]

At the concentrations of anti-Tac present, γ < 0.001 (the fraction of free Tac, B/Btot), and the net survival of Tac (k') should have been indistinguishable from that of the antibody (kj). Yet the survival of Tac-in-complex (Table 4) is only 55% of that predicted. Analysis of the terminal decay phase and steady state levels of Fig. 9 below and other tests not shown likewise demonstrate significantly low fractions (35–45%) of predicted survival and concentrations of Tac by this model. Therefore, we conclude that this hypothesis—that the fraction of Tac that exists dissociated anti-Tac antibody increased Tac* (AB*) survival, with a β t1/2 of 38 h for Tac in the presence of excess anti-Tac and a catabolic t1/2 of 24 h, more than 15-fold above the 1.4-h catabolic t1/2 measured for unbound Tac (B*). Anti-Tac antibody (A*) showed a β t1/2 of 82 h, with a corresponding catabolic t1/2 of 43 h, typical of mAbs in mice. The t1/2 for radiolabeled bound Tac (AB*) is significantly different from radiolabeled anti-Tac (A*) (p < 0.001).
Figure 7. Prolongation of Tac survival in vivo by anti-Tac binding. Samples were prepared and administered intravenously to four groups of animals as in Materials and Methods. (A) Blood die-away curves for 125I-Tac* alone, B* (--Δ--); Tac* plus excess unlabeled anti-Tac, AB* (--■--); and 125I-anti-Tac*, A* (-- ○--); anti-Tac* plus excess unlabeled Tac, A*B2 (--□--). (B) Blood die-away curves for 125I irrelevant IgG (UPC) coadministered in the experiments of A with corresponding symbols; that is, 125I-UPC in the experiment with B* (--Δ--); in the experiment with A* (--■--); and in the experiment with A*B2 (--□--). SE not shown, equals 0-3% for all points.

A wide array of cell surface proteins are present in soluble form in blood that have been assessed for clinical and biological significance. Some, such as B cell IgG, derive from alternately spliced mRNAs that yield the membrane and secreted forms. For all others, the TM protein is shed to generate the soluble form. Phospholipases may be responsible in some instances, but most are thought to be cleaved by membrane-associated proteases (6, 37-40), including at least one phosphoinositol-linked protein (41). Despite the importance of this feature (shedding) and the number of relevant molecules, a recent review concluded that "no convincing candidate for a [shedding protease] has been unequivocally identified and isolated" (6). To our knowledge, this statement remains true. It is notable that the only evidence for shedding of cell surface proteins comes from in vitro studies of cultured cells (42-44), where it has been shown that proteolytic cleavage of cell surface molecules is increased in the presence of membrane-bound proteases. In vivo, the presence of these proteases is less clear.

To mimic the therapeutic setting, we then examined the impact of specific antibody. 20 h after pump placement, mice were injected intraperitoneally with a large excess of 125I-anti-Tac (A) (Fig. 9). In contrast to the control Tac (B) infusion, which was already stable at the 20-h time point, the concentration of Tac with anti-Tac injection (AB) was just approaching plateau at the time of pump expiration after day 7. The blood-soluble Tac level increased 11-fold with anti-Tac antibody during the experiment, and was projected by graphical methods to plateau finally at a 13-fold increase. At no time, however, did Tac reach a concentration that could saturate antibody, which was always at least 100-fold in molar excess. After pump expiration, AB complex declined with a terminal β t1/2 of 41 h that compares with the 38-h β t1/2 after bolus injection (Table 4).

These studies indicate that the blood concentration of Tac was increased more than 10-fold by the presence of anti-Tac. This change in concentration occurred in the absence of any alteration in the rate of Tac delivery into the system ("production") and was therefore due solely to suppression of catabolism and prolongation of Tac survival.

### Table 4. Anti-Tac Prolongs Tac Survival

| Form | Description | β t1/2 | Catabolic rate constant | Catabolic t1/2 | Difference versus A* |
|------|-------------|--------|-------------------------|----------------|---------------------|
| B*   | Tac*        | 4.8 (0.4) | 0.485 (0.016) (k4) | 1.43 (0.05) | p < 0.001 |
| AB*  | Tac* + excess anti-Tac | 38 (1) | 0.0294 (0.0008) (k4) | 23.6 (0.6) | p < 0.001 |
| A*   | Anti-Tac*   | 82 (2)  | 0.0160 (0.0002) (k4) | 43.1 (0.6) | ——— |

Constants (and fitting errors) were derived from two-compartment analysis of the data of Fig. 7A. A*B2, radiolabeled anti-Tac saturated with excess cold Tac, is not represented in this table but is separately compared with A* in Table 5. The kinetic constants (k4, k5, k4), are defined in Schema 2. The catabolic constants were compared with anti-Tac (A*) by Student's t test.
Table 5. sTac Does Not Alter Anti-Tac Survival

| Form | Description | \( t_{1/2} \) full range | \( t_{1/2} \) before injection | \( t_{1/2} \) after injection |
|------|-------------|--------------------------|-----------------------------|---------------------------|
| A*   | Anti-Tac*   | 5.4 (0.3)                | 4.5 (1.0)                   | 6.2 (0.8)                 |
| A*B₂ | Anti-Tac* + excess sTac | 5.5 (0.4)                | 5.1 (0.4)                   | 5.8 (0.3)                 |

\( p \) value NS NS NS

Constants were derived from regression analysis of the data of Fig. 8. The macroscopic \( t_{1/2} \) values (and fitting errors) are expressed in units of days. The full range applies to the approximately linear phase between 1.5 and 6.5 d on the control anti-Tac* (A*) injection. Because of the positive displacement of the A*B₂ curve, a full range \( t \) was derived as a weighted average of the before injection and after injection averages. The time points before injection are 1.5-3.5 d, and after injection are 3.5-6.5 d. There are no significant differences between the control and experimental slopes by Student’s \( t \) test.

Our two key experimental observations pertinent to mechanism are: (a) shedding is nonsaturable over a two-log range of substrate TM-Tac expression; and (b) the fractional rate is not uniform across all settings. Accordingly, all models are constrained to appear first order over the range of TM-Tac here, yet must incorporate a potential for inter-individual variation in absolute fractional rates of shedding. Both true first order (autocatalytic) and Michaelis-Menton (enzymic) mechanisms can be accommodated, but with significant restrictions to each.

Figure 8. Undisturbed anti-Tac survival in vivo with soluble Tac binding. The experiment was performed as in Materials and Methods, with the first dose of 50 μg of Tac i.p. at 75 h (arrow) in the experimental series with repeat dosing every 12–24 h until the end of the experiment. anti-Tac*, A* (■); anti-Tac* plus excess unlabeled Tac, A*B₂ (□). (Bars) ±1 SE.

Figure 9. Increased Tac levels with anti-Tac administration in model of constant in vivo antigen production. Pumps (7 d) containing \(^{131}\text{I}-\text{Tac} \) were inserted subcutaneously (Materials and Methods). A large excess of \(^{125}\text{I}\text{-anti-Tac} \) or \(^{125}\text{I}\text{-irrelevant IgG (UPC)} \) was given by i.p. bolus at 20 h (arrow) as Tac levels approached steady state. Results from two or three mice are pooled in each experiment for graphing. \(^{131}\text{I}-\text{Tac} \), B* (□); \(^{131}\text{I}-\text{Tac} \) plus excess anti-Tac, AB* (○); \(^{131}\text{I}\text{-anti-Tac} \), A (○), to estimate the anti-Tac excess: the lowest anti-Tac (A) levels were 80 μg/ml plasma or ~500 nM; the highest soluble Tac levels in the AB* mice were ~5 nM.
lignant T cells show variation by gel mobilities of extracted proteins (28, 47). Whether this pattern also changes during T cell activation and downregulation of Tac is, to our knowledge, unknown.

On the other hand, this variation in shedding rates is what most suggests a separately regulated protease. If such a "shedding protease" is present, it appears to be membrane bound because (a) shedding proceeds in serum-free medium, and (b) early and late rates of shedding are constant—medium accumulation of a secreted protease would be expected to progressively accelerate shedding. A recently described matrix metalloenzyme is such a membrane protease (48). The unusual character of the Tac cleavage site and the resistance of Tac shedding to several protease inhibitors argue against a range of nonspecific proteases, but the activity must be widespread, including lymphoid and nonlymphoid cell lines of human and nonhuman origin, since all such cells shed efficiently when transfected with human TM-Tac (29; and R.P. Junghans, unpublished results). "Widespread expression" is expected for an autocatalytic model. Finally, it can be shown that the first order kinetic pattern imposes a high $K_m$ on such a protease, or it implies compensating substrate modification by altered glycosylation at high TM-Tac levels and/or coordinate regulation of the protease. Protein kinase C has been implicated as an activator for a putative shedding protease in one case (37).

Whichever mechanism underlies the shedding process, shedding could have as its normal role to increase receptor for a putative shedding protease in one case (37).

Once Tac is shed as a soluble protein, it is subject to the catabolic activities of the host. We studied exogenously administered human sTac in a mouse model, which typically parallels humans in metabolic handling of proteins. sTac was rapidly cleared after i.v. injection with a nominal $t_1/2$ of 1 h that increased to 10 h in nephrectomized mice. We conclude that Tac is 90% catabolized by the kidney, with a filtration fraction of 0.11, and the remaining 10% of catabolism is accounted for by nonrenal tissues (Fig. 6). After filtration, all protein is either absorbed in the tubules and catabolized to amino acids with reutilization, or excreted as proteinuria (24), documented as 1-3% in our experiments. Extrapolating to humans, with a normal mean serum sTac of 175 U/ml, a creatinine clearance of 180 liters per day, and an 11% of GFR filtration ratio for Tac from our result in mice, we estimate a daily filtration of $3.5 \times 10^8$ U of Tac and production (and total metabolism) of $4 \times 10^8$ U of Tac (12 $\mu$g). Urinary clearance (proteinuria) of sTac in humans was measured at $1.1 \times 10^8$ U per 24 h (13), from which we estimate a mean normal proteinuric rate of 3% relative to total catabolism, which corresponds closely with our result in mice (1-3%) and with L chain proteinuria in humans (~1%) (33). In all respects, these data with sTac parallel the metabolic fate of Bence-Jones proteins and isolated L chain in mice and in humans (23, 24, 33). This clearance pattern is likely to be shared by all shed molecules of a size small enough to pass the renal filter (<~50 kD).

With this picture of normal Tac catabolism, we considered the potential of two settings to perturb this balance. One is the pathologic changes associated with kidney disease; the other is the iatrogenic condition imposed during therapies with antibodies or other antireceptor molecules. The 68% reduction of renal catabolism of Tac with ureter ligation (Fig. 4) is compatible with the impact on GFR in the hours after ligation (34, 35). Reduced catabolism accompanying reduced GFR is predicted to increase plasma levels of endogenously generated sTac, as modeled in Fig. 10. Since the major part of catabolism is kidney dependent, sTac increases roughly with creatinine for low creatinines until nonrenal catabolism becomes a significant portion of the total. Ultimately, in anephric patients on hemodialysis, creatinine is filtered but molecules the size of Tac are not, and all catabolism is nonrenal, predicting plasma levels 11 times normal without any change in the rate of Tac synthesis and release.

In contrast to filtration, tubular dysfunction has no impact on blood levels but only increases the proteinuric fraction concomitant with the decreased tubular protein reabsorption. Urinary Tac has been used as a measure of Tac expression in malignancy and in allo- and autoimmune settings (10, 13). However, urinary Tac will be dramatically increased in rejection and other renal diseases with a tubular component, for which there is no valid normalization. Given the additional hazards of urinary Tac interpretation relative to serum, with six-fold interindividual variation in proteinuric fractions among human "normals" (13) (threefold in our mice), there is probably no justifiable role for urinary Tac measurements in any setting.

We then examined the effect of antibody binding on the catabolism of Tac, which shares many features with renal failure without the systemic physiologic consequences. The

![Figure 10](attachment:figure10.jpg)

**Figure 10.** Decreased GFR predicts increased plasma Tac with no change in sTac production. Relative increase in creatinine with normal defined as 1, and fraction of normal GFR represented as the inverse of the relative creatinine. NB: This relation is not applicable in hemodialysis, in which creatinine is filtered and Tac is not.
combination of molecules with different native catabolic rates will have different outcomes, depending upon the mechanisms of clearance. For example, when antidigitoxin Fab antibodies were administered to neutralize digitoxin, the abbreviated survival of the Fab fragment was imposed on the slow $t_{1/2}$ of free drug, leading to a marked acceleration in drug clearance (49). When complete IgG antibodies are themselves the target of antibody-mediated immune reactions, their clearance is usually also greatly accelerated by specific host mechanisms (16). Rapidly catabolized components may alternately have their catabolism delayed by antibody reaction, as first shown for anti-insulin antibodies by Berson and Yalow (50). Similarly, we show that a biologically and clinically important target antigen in soluble form can have its survival markedly prolonged by the interaction with antibody. In contrast, we noted that antibody survival was unaffected by antigen binding.

When antibody elicits antiglobulin responses in vivo, every epitope is repeated twice in IgG (10 times in IgM), and the polyclonality of the response ensures that many epitopes will be targeted, yielding large aggregates that present multivalent Fc to the FcR of the monocyte-macrophage system, and leading to rapid systemic clearance. That anti-Tac survival is unaffected by antigen binding is probably due to the fact that Tac is monomeric and presents a single binding epitope to antibody, which is itself monospecific, thereby avoiding formation of higher aggregates. This should also be generally true for other mAbs targeting monomeric soluble proteins. Furthermore, the size of the antibody was not greatly increased by reaction with antigen. The fully saturated anti-Tac | Tac$_2$ complex (AB$_2$) is ~220 versus 160 kD for antibody alone, far below that of other serum proteins such as IgM and complement component C1 (each 900–1,000 kD). The $t_{1/2}$ of IgM is humans is 5 d (31), and greater size alone is insufficient to accelerate clearance.

In contrast, Tac survival increased more than 15-fold with antibody binding, from a nominal 1.4-h catabolic $t_{1/2}$ to 24 h for i.v. injected material. The failure of Tac to pass the glomerular filter when bound to antibody is presumed to be the cause of prolonged Tac survival in the present studies, yielding the equivalent of total renal failure for Tac metabolism. Yet the survival of Tac-in-complex is also much longer than measured for nonrenal catabolism (catabolic $t_{1/2}$ ~10 h), and antibody evidently protects Tac from this catabolism as well.

To mimic the in vivo setting of human therapies, we applied a continuous production model in mice using implantable microosmotic pumps. As a concomitant of prolonged survival, this model showed increased steady state levels of Tac in the presence of antibody. The steady state concentration ($B_{ss}$) is a simple function of the rate of production times the catabolic $t_{1/2}$ (not the $B_{1/2}$; see Materials and Methods):

$$B_{ss} = q/k = q/t_{1/2}/\ln 2 \quad \text{(Eq. 10)}$$

If the $t_{1/2}$ is changed by antibody, then the new steady state for the sum of all B forms, $B'_{ss, tot}$, is related to the former steady state by the ratio of the net $t_{1/2}$ values:

$$\frac{B'_{ss, tot}}{B_{ss, tot}} = \frac{t_{1/2}'}{t_{1/2}} \quad \text{(Eq. 11)}$$

We measured the $t_{1/2}$ for Tac complexed with anti-Tac as 23.6 h versus a value of 1.4 h for Tac alone (Table 4), predicting a final steady state increase of 16.5-fold. This is comparable with the projected ratio of 13 from our data (Fig. 9), within the uncertainties of this system.

A further consequence of prolonged Tac survival due to any cause (renal failure or antibody binding) will be to alter the normal spatial distributions of Tac in the body. For sTac generated extravascularly, the rapidity of the renal clearance mechanisms in contact with the vascular compartment will generate a steep gradient in the unperturbed setting, with substantially higher levels in the extravascular compartment versus that in blood. With the longer survival, however, this gradient of Tac concentration in vivo is substantially flattened, yielding a marked increase in the normally low physiologic ratio of Tac in serum to Tac body burden.

An unexpected outcome of these studies of Tac metabolism was the apparent faster catabolism of Tac-in-complex versus antibody-in-complex. Our analysis (see Results) eliminated the hypothesis that the shorter $t_{1/2}$ was due to the rapid clearance of the dissociated, free portion of Tac (B$^*$) in the equilibrium. This left us with the more surprising second alternative: that catabolic activities may attack one part of the anti-Tac | Tac complex, Tac, while sparing the other part, anti-Tac. One speculation may be warranted on the nature of such a process which can selectively "extract" Tac from this high affinity complex. This phenomenon suggests features shared by normal IgG catabolism and intestinal and placental IgG transport (51–53). To explain the acceleration of IgG catabolism with higher serum IgG, Brambell et al. (51) proposed that IgG taken into pinocytic vacuoles is bound to specific IgG receptors (FcR) that redirect its transport to the circulation. Once the putative "protection receptors" are saturated, the remainder of the IgG is subject to unrestricted lysosomal catabolism for a net acceleration at high serum concentrations.

The Brambell hypothesis may be adapted to the present setting. We propose that anti-Tac | Tac complexes are internalized to acidified vesicles as normal IgG. Anti-Tac bound to the protection FcR is returned to circulation, whereas Tac released from the complex in the vesicle is catabolized, thus yielding faster net catabolic rates for circulating antigen-in-complex than antibody-in-complex. That vesicles may be thus topologically "divided" was demonstrated with transferrin and horseradish peroxidase (HRP); both colocalize in early endosomes, but transferrin returns to the surface bound to its receptor whereas HRP proceeds to the late endosomes and lysosomes (54). Endosomes are characterized by reduced pH that is thought to promote ligand-receptor dissociation (54), whereas the relevant Fc–FcR interactions are acid enhanced (53, 55), thereby plausibly enabling IgG recycling to the surface via bound FcR,
inconceivable concentration since the highest antibody vivo. On the other hand, other cytokines or cytokine re-
affinity for IL2 by Tac in isolation is so low (~ 10^8 M^-1) increased biologic activity in vivo if the active site is not 
will not increase its ability to capture ligand. Moreover, the 
by Tac, and higher Tac levels due to antibody complexing 
pressed by the therapy itselfs We know from other work 
prolongation of antigen survival will result in increased 
may be expected to experience similar fates. It is a necessary consequence of these kinetic studies that 
prolongation of antigen survival will result in increased steady state sTac levels during antibody therapy unless the 
mechanisms of Tac production are directly and rapidly suppressed by the therapy itself. We know from other work that 
Tac production can persist at high levels for at least several days into therapy, even in patients who ultimately undergo complete remission, during which serum Tac levels may increase 10-fold or more (our unpublished results).

In the case of anti-Tac, the antibody blocks IL-2 binding by Tac, and higher Tac levels due to antibody complexing will not increase its ability to capture ligand. Moreover, the affinity for IL2 by Tac in isolation is so low (K, 10^8 M^-1) that it would require Tac concentrations of the order of 10 ^4 M to compete 50% with high affinity receptor (μβγ), an inconceivable concentration since the highest antibody concentration in therapy would probably not exceed 0.3 ^4 M (50 μg/ml). (The highest serum sTac level we have observed is 0.05 μM; our unpublished results.) There is accordingly no evidence to suggest an independent regulatory role for the soluble form of this molecule in vitro or in vivo. On the other hand, other cytokines or cytokine receptors to which therapy might be directed could lead to increased biologic activity in vivo if the active site is not also blocked by the antibody, as recently most convincingly shown for anti-IL3, anti-IL4, and anti-IL7 antibodies that capture and stabilize these proteins in active configurations.

The role of sTac and other soluble molecules as a surrogate for body burden of tumor or activated T cells can be interpreted in the context of these results. Among ATL patients, there is a general correspondence between disease volume and sTac levels (our unpublished results), as also observed for hairy cell leukemia, another Tac-expressing malignancies (15). Higher sTac levels should correlate with more shedding, but where individuals differ in Tac expression or shedding rate, as noted here, this correlation weakens at the level of estimating numbers of Tac-expressing cells. Donor 1 in Table 1 underwent remission during her treatment with antibody directed at TM-Tac on the surface of her malignant cells. Subsequently, she experienced a relapse of her disease, at which time her cells exhibited a nearly identical pattern of Tac expression and shedding. For this individual, the in vitro results suggest that serum sTac could reasonably be used as a measure of her disease. sTac may thus be useful as a serum marker for many patients in the same sense that elevated CEA concentrations are a marker for recurrence in colon cancer and other malignancies, but in which individuals are known to differ in the relative expression of the marker (59).

Although the plasma level of the soluble form of Tac and other cell surface molecules is a reasonable correlate of physiologic condition or disease expression, its use must be tempered with knowledge of the biology of the protein. We showed that antibody binding markedly inhibits the metabolism of sTac with a corresponding increase in the serum concentration. In addition, as is true for other small molecules, Tac is catabolized chiefly by the kidney. Thus, it is concluded that serum concentrations of soluble antigen in renal failure or during antibody therapy cannot be used as a surrogate measure of tumor burden or T cell activation because receptor levels will both increase and differentially redistribute as a consequence of prolonged survival, in the absence of changes in soluble antigen production.

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Note added in proof: The receptors for IgG protection (FcR) [51,53] and intestinal transport (FcRn) [52,53,55], both described by Brambell, were recently shown to be identical using mice knocked-out for the receptor light chain (Junghans, R.P. and C.L. Anderson, manuscript submitted for publication). In such mice, IgG protection and differential catabolism were both lost, yielding clearance of antibody- and antigen-in-complex at the same accelerated rate as albumin. This confirms the central role of the FcR to the differential catabolism mechanism, as postulated in this article.

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