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# The metalloprotease ADAM10 generates soluble interleukin-2 receptor alpha (sCD25) *in vivo*

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**The cytokine interleukin-2 (IL-2) plays a critical role in controlling the immune homeostasis by regulating the proliferation and differentiation of immune cells, especially T cells. IL-2 signaling is mediated *via* the IL-2 receptor (IL-2R) complex, which consists of the IL-2Rα (CD25), the IL-2Rβ, and the IL-2Rγ. While the latter are required for signal transduction, IL-2Rα controls the ligand-binding affinity of the receptor complex. A soluble form of the IL-2Rα (sIL-2Rα) is found constitutively in human serum, though its levels are increased under various pathophysiological conditions. The sIL-2Rα originates partly from activated T cells through proteolytic cleavage, but neither the responsible proteases nor stimuli that lead to IL-2Rα cleavage are known. Here, we show that the metalloproteases ADAM10 and ADAM17 can cleave the IL-2Rα and generate a soluble ectodomain, which functions as a decoy receptor that inhibits IL-2 signaling in T cells. We demonstrate that ADAM10 is mainly responsible for constitutive shedding of the IL-2Rα, while ADAM17 is involved in IL-2Rα cleavage upon T cell activation. *In vivo*, we found that mice with a CD4-specific deletion of ADAM10, but not ADAM17, show reduced steady-state sIL-2Rα serum levels. We propose that the identification of proteases involved in sIL-2Rα generation will allow for manipulation of IL-2Rα cleavage, especially as constitutive and induced cleavage of IL-2Rα are executed by different proteases, and thus offer a novel opportunity to alter IL-2 function.**

Interleukin-2 (IL-2) is not only one of the earliest discovered cytokines, it is also one of the most important regulators of immune responses. After its initial description as a T cell proliferation factor (1), a variety of other functions of IL-2 have been described, including differentiation of $CD4^+$ T cell subsets, antibody production by B cells, and cytotoxic activity of NK cells (2). However, the most important function is probably the maintenance of the homeostasis of regulatory T cells and, consequently, self-tolerance (3, 4).

Signal transduction of IL-2 is mediated *via* different combinations of the receptor subunits IL-2 receptor α (IL-2Rα/CD25/Tac), IL-2Rβ (CD122), and IL-2Rγ (common γ chain/$\gamma_c$/CD132). A heterodimer of IL-2Rβ and $\gamma_c$ is required for the induction of intracellular signals *via* activation of the Janus kinases JAK1 and JAK3. The IL-2Rα is the only one of these receptors that is specific for IL-2. Its extracellular part consists of two sushi domains and a flexible, 54 amino-acid residues long stalk region that connects the extracellular domains with the transmembrane helix. The intracellular region is rather short and lacks interaction sites for kinases. Consequently, the IL-2Rα is not directly involved in signal transduction (5). It does, however, bind IL-2 and facilitate binding of the cytokine to the other two receptor subunits, giving the trimeric receptor complex a higher affinity than the dimeric complex. Thus, the expression of the IL-2Rα dictates sensitivity of a cell toward IL-2. Notably, high expression of IL-2Rα is especially found on regulatory T cells and transiently also on $CD4^+$ and $CD8^+$ T cells upon T cell receptor (TCR) stimulation (6, 7).

In addition to the membrane-bound receptor, also a soluble form of the IL-2Ra has been described. This soluble (s)IL-2Rα is present in the blood of healthy humans (8) and is considered to, at least in part, originate from activated T cells (9). Notably, the amount of soluble IL-2Rα (sIL-2Rα) is increased in a variety of diseases including autoimmune diseases, different cancer types, or inflammatory diseases like rheumatoid arthritis or atopic dermatitis, which has led to several studies analyzing its potential as a diagnostic marker (8, 10, 11). However, due to the high number of diseases that present with increased sIL-2Rα levels and the fact that it is increased upon T cell activation, the actual clinical use is limited (11).

While the sIL-2Rα retains the ability to efficiently bind IL-2 (12), the biological function of this binding is not completely clear. Different mechanisms how the sIL-2Rα affects IL-2 signaling have been proposed: (i) the complex of IL-2 and sIL-2Rα can bind to membrane-bound IL-2Rβ/γ receptors, thereby forming a high affinity receptor complex and increasing IL-2 signaling (13), (ii) sIL-2Rα binds IL-2, thereby preventing binding of the cytokine to the membrane-bound receptors, and thus inhibiting signaling (14–17), and (iii) the sIL-2Rα prevents degradation of IL-2, thereby increasing its

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half-life (18). Which of these proposed mechanisms is true, or whether all occur depending on the cellular context, is unclear (11).

Furthermore, it is also not completely understood how the sIL-2Rα is generated. Soluble cytokine receptors can originate from two different mechanisms: alternative splicing of mRNA or ectodomain shedding of membrane-bound receptors (19). So far, no alternatively spliced isoform of the *IL2RA* gene that could give rise to a sIL-2Rα has been described (20, 21). Consequently, the sIL-2Rα is considered to be derived from proteolytic processing of the membrane-bound receptor. This was supported by the observation that an increase in sIL-2Rα upon stimulation of T cell lines is associated with a decreased amount of the receptor on the cell surface (13). Indeed, several proteases have been described to be able to cleave the IL-2Rα, namely matrix metalloproteinase-9 (MMP-9), Der p 1, and the neutrophil-derived serine proteases elastase and proteinase 3 (22–24). Whether any of those are involved in sIL-2Rα generation *in vivo* is not yet clear. Notably, sIL-2Rα shedding from phytohemagglutinin-stimulated peripheral blood mononuclear cells could be almost completely abrogated with the metalloprotease inhibitor TAPI-0 (22), indicating that one (or more) metalloprotease(s) are responsible for IL-2Rα shedding. However, MMP-9 is not responsible for the constitutive shedding of IL-2Rα, as MMP-9–deficient mice showed no difference in sIL-2Rα serum concentration compared to WT mice (22).

Other metalloproteases that have emerged as important sheddases are members of a disintegrin and metalloprotease (ADAM) family. Within this family, especially ADAM10 and ADAM17 have gained a lot of attention cleaving a variety of substrates, including different cytokine receptors, and affecting the function of immune cells (25, 26). Furthermore, ADAM10 and ADAM17 have been reported to be upregulated in $CD4^+$ T cells upon *in vitro* activation (27). Therefore, we here analyze the role of the metalloproteases ADAM10 and ADAM17 in the generation of sIL-2Rα as well as the effect of the sIL-2Rα on IL-2 signaling in T cells.

## Results

### *IL-2Rα is constitutively cleaved by metalloproteases*

Cytokine receptors can be cleaved upon stimulation, but soluble receptors are often also constitutively released from cells. To analyze constitutive release of sIL-2Rα, we first transiently transfected HEK293 cells with an expression plasmid encoding IL-2Rα and treated them with broad spectrum inhibitors targeting different classes of proteases. We used marimastat (MM) to inhibit metalloproteases, AEBSF to block serine proteases, and E64 and pepstatin A to inhibit cysteine and aspartate proteases, respectively. Furthermore, we applied the more specific inhibitors GI (selective for ADAM10) and GW (selective for ADAM10 and ADAM17) (28). We monitored the production of sIL-2Rα by ELISA 24 h after the cells were treated with the protease inhibitors. As shown in Figure 1*A*, transfected HEK293 cells released sIL-2Rα into their supernatant, and this release was significantly decreased when the cells were treated with the ADAM-specific inhibitors GI and GW or the broad-spectrum metalloprotease inhibitor MM. In contrast, all other protease inhibitors had no measurable effect on sIL-2Rα generation. These results indicate that constitutive shedding is depending on metalloprotease activity and make ADAM10 the most likely candidate. To verify these results, we employed HEK293 cells which are deficient for either ADAM10 ($A10^{-/-}$), ADAM17 ($A17^{-/-}$), or both proteases ($A10^{-/-}/A17^{-/-}$). We again transiently transfected these cell lines and monitored constitutive production of sIL-2Rα. As shown in Figure 1*B*, cells deficient for ADAM10 or ADAM17 released similar amounts of sIL-2Rα as WT cells. In contrast, cells lacking both proteases produce significantly less sIL-2Rα than WT cells, verifying that these proteases are indeed involved in constitutive shedding of IL-2Rα. To exclude that different expression of IL-2Rα within the different cell lines accounts for the reduction in sIL-2Rα release, we further analyzed the amount of IL-2Rα within the cell lysates (Fig. 1*B*, right panel) and found no differences in IL-2Rα expression. This underlines that the reduced sIL-2Rα production is caused by lack of protease activity. Of note, deficiency of ADAM10 and ADAM17 decreased the amount of sIL-2Rα to approximately 20% compared to that of WT cells, but did not completely abrogate it. This indicates that ADAM-mediated proteolysis accounts for the vast majority of sIL-2Rα but that also at least one other mechanism contributes to sIL-2Rα production.

To verify the results obtained by heterologous expression of IL-2Rα, we next employed the Hodgkin lymphoma cell line HDLM-2, which expresses the IL-2Rα endogenously (29). We repeated the treatment with protease inhibitors as described for HEK293 cells and monitored sIL-2Rα by ELISA and Western blot. Our results confirm the involvement of metalloproteases and rule out a significant contribution of other protease classes (Fig. 1*C*). Importantly, inhibition of ADAM10 with the selective inhibitor GI inhibited sIL-2Rα shedding to the same degree as the broad-spectrum metalloprotease inhibitor MM. Furthermore, we detected an IL-2Rα signal at approximately 50 kDa in the supernatant, which corresponds to the sIL-2Rα (Fig. 1*D*). This band is missing when cells were treated with one of the three metalloprotease inhibitors, further corroborating their role in sIL-2Rα generation. Again, treatment with the ADAM10-specific inhibitor GI had the same effect as the inhibition of all metalloproteases. We concluded from these experiments that ADAM10 is the major protease responsible for the constitutive shedding of the IL-2Rα.

### *Activation of ADAM10 or ADAM17 increases soluble IL-2Rα release*

After analyzing constitutive shedding, we next sought to investigate whether activation of ADAM10 or ADAM17 would increase the amount of sIL-2Rα. We again transiently transfected the three protease-deficient HEK293 cell lines with an expression plasmid encoding IL-2Rα and stimulated them with the ionophore ionomycin for 1 h, which is a well-established

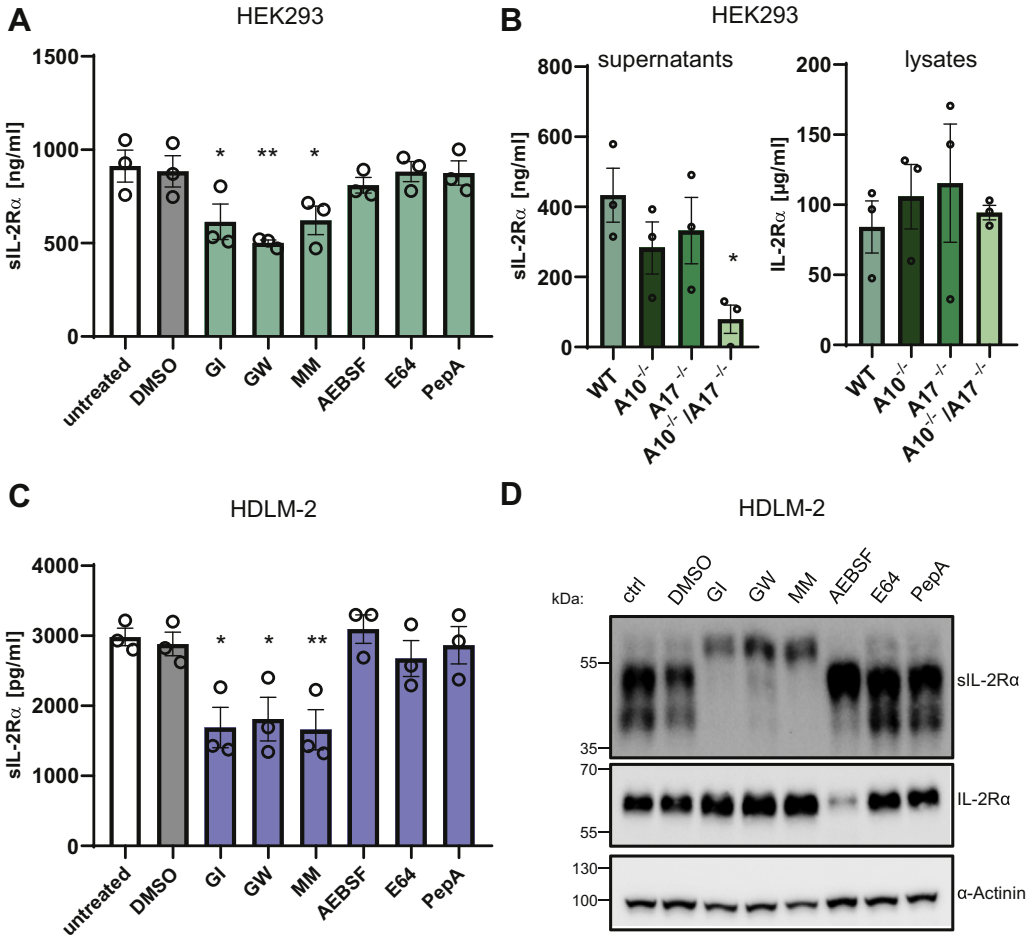


**Figure 1. ADAM10 is responsible for the constitutive shedding of the IL-2Rα.** *A*, HEK293 cells were transiently transfected with an expression plasmid encoding the IL-2Rα and treated with the protease inhibitors GI (selective for ADAM10, 3 μM), GW (selective for ADAM10 and ADAM17, 3 μM), MM (broad-spectrum metalloprotease inhibitor, 10 μM), AEBSF (serine protease inhibitor, 1 mM), E64 (cysteine protease inhibitor, 10 μM), pepstatin A (aspartate protease inhibitor, 1 μM), or DMSO as solvent control for 24 h. sIL-2Rα in the supernatant was detected by ELISA. Shown are the mean ± SEM from three independent experiments. *B*, HEK293 WT cells or HEK293 cells deficient for ADAM10 ($A10^{-/-}$), ADAM17 ($A17^{-/-}$), or both proteases ($A10^{-/-}$/$A17^{-/-}$) were transiently transfected with an expression plasmid encoding the IL-2Rα. IL-2 Rα in the supernatants (*left panel*) and the cell lysates (*right panel*) were analyzed by ELISA. Shown are the mean ± SEM from three independent experiments. *C*, HDLM-2 cells were treated with the different protease inhibitors as described under (*A*) for 24 h. sIL-2Rα in the supernatant was detected by ELISA. Shown are the mean ± SEM from three independent experiments. *D*, HDLM-2 cells were treated as described under (*C*) and the IL-2Rα in the cell lysates and supernatants was detected by Western blot. α-Actinin served as loading control. Shown is one example out of three independent experiments with similar outcome. Statistical analysis was performed using one-way ANOVA with Dunnet's multiple comparison test (A, C: samples treated with protease inhibitors compared to untreated samples, B: protease-deficient HEK293 cells compared to WT cells); *$p < 0.05$, **$p < 0.01$. ADAM, a disintegrin and metalloprotease; IL-2R, interleukin-2 receptor; sIL-2Rα, soluble IL-2Rα.

activator of ADAM10 (30). We found that in WT cells, ionomycin induced the release of sIL-2Rα (Fig. 2, *A* and *B*), further strengthening the notion that ADAM10 is involved in shedding of the IL-2Rα. Interestingly, the amount of sIL-2Rα upon ionomycin stimulation was not different in cells lacking either ADAM10 or ADAM17. The ionomycin-induced shedding in the $ADAM10^{-/-}$ cells is most likely caused by ADAM17, as it has been reported for other ADAM10 substrates that ADAM17 can compensatory cleave in the absence of ADAM10 (31, 32). This is further supported by the fact that cells deficient of both proteases did not release any sIL-2Rα with or without ionomycin stimulation (Fig. 2, *A* and *B*).

Next, we treated the cells with the phorbol ester phorbol-12-myristate-13-acetate (PMA) for 2 h, which activates protein kinase C and subsequently ADAM17 (33). Like with ADAM10, we found that activation of ADAM17 in WT cells resulted in a significant increase in sIL-2Rα (Fig. 2, *C* and *D*). This was unaltered in $A10^{-/-}$ cells. Interestingly, $A17^{-/-}$ cells showed an increase in sIL-2Rα release upon stimulation with PMA, which was, however, not significantly different from the dimethyl sulfoxide (DMSO) control, probably due to increased compensatory shedding by ADAM10 (Fig. 2*C*). Importantly, cells devoid of both proteases produced no sIL-2Rα upon PMA stimulation (Fig. 2, *C* and *D*). These data indicate that activation of either ADAM10 or ADAM17 results in increased shedding of the IL-2Rα and point toward a compensatory cleavage by ADAM17 upon ionomycin stimulation in the absence of ADAM10.

In order to verify the results obtained with heterologous IL-2Rα expression, we performed the same experiments in HDLM-2 cells. We treated the cells with ionomycin or PMA in order to activate ADAM10 or ADAM17, respectively, and

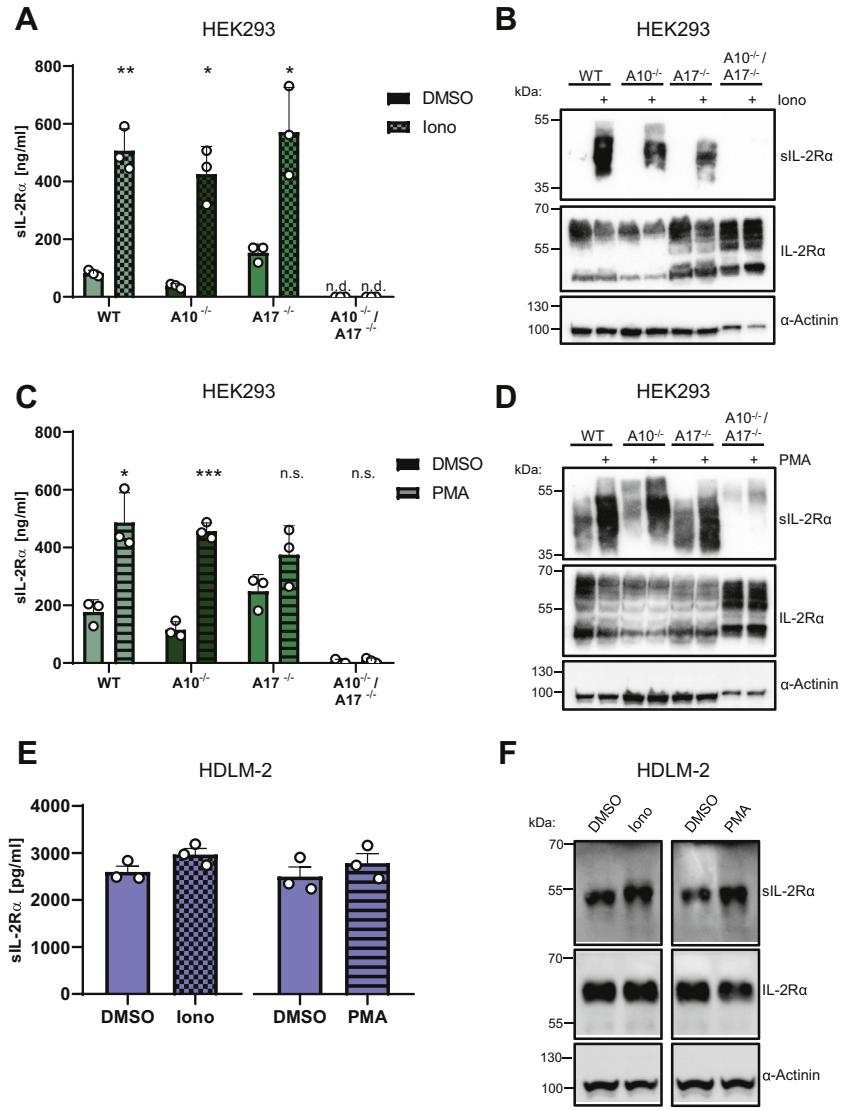


**Figure 2. Activation of ADAM10 or ADAM17 increases sIL-2Rα release.** *A*, HEK293 WT cells or HEK293 cells deficient for ADAM10 ($A10^{-/-}$), ADAM17 ($A17^{-/-}$), or both proteases ($A10^{-/-}/A17^{-/-}$) were transiently transfected with an expression plasmid encoding the IL-2Rα. The cells were treated with 1 μM ionomycin (Iono) for 1 h in order to induce ADAM10 activity or DMSO as solvent control. sIL-2Rα in the supernatants was detected by ELISA. Shown are the mean ± SEM from three independent experiments. *B*, the different HEK293 cell lines were treated as described under (*A*) and the IL-2Rα in the cell lysates and supernatants was detected by Western blot. α-Actinin served as loading control. Shown is one example out of three independent experiments with similar outcome. *C*, the experiment was performed as described under (*A*), but the cells were stimulated with 100 nM PMA in order to activate ADAM17. Shown are the mean ± SEM from three independent experiments. *D*, the different HEK293 cell lines were treated as described under (*C*) and the IL-2Rα in the cell lysates and supernatants was detected by Western blot. α-Actinin served as loading control. Shown is one example out of three independent experiments with similar outcome. *E*, HDLM-2 cells were treated with 1 μM ionomycin (Iono) for 1 h (*left panel*) or 100 nM PMA for 2 h (*right panel*) in order to activate ADAM10 or ADAM17, respectively. sIL-2Rα in the supernatants was detected by ELISA. Shown are the mean ± SEM from three independent experiments. *F*, HDLM-2 cells were treated as described under (*E*) and the IL-2Rα in the cell lysates and supernatants was detected by Western blot. α-Actinin served as loading control. Shown is one example out of three independent experiments with similar outcome. Statistical analysis was performed using unpaired t tests with Welch's correction in order to compare between DMSO-treated and ionomycin- or PMA-treated cells; $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. ADAM, a disintegrin and metalloprotease; IL-2R, interleukin-2 receptor; PMA, phorbol-12-myristate-13-acetate; sIL-2Rα, soluble IL-2Rα.

monitored sIL-2Rα release. However, no statistically significant differences were observed between stimulated and unstimulated cells, which is most likely due to the high constitutive sIL-2Rα release of the HDLM-2 cells.

### *IL-2Rα shedding is dependent on the stalk region*

It has been previously described that ADAM10 and ADAM17 substrates are cleaved within the stalk region, often in close proximity to the plasma membrane (25). Therefore, we first deleted the entire stalk region of the IL-2Rα (E187_Q240, Fig. 3*A*) and analyzed whether this variant can still be cleaved by ADAM10 and ADAM17. While IL-2RαΔE187_Q240 was expressed and transported to the cell surface (Fig. 3*B*), no sIL-2Rα was detected in the supernatant of transfected HEK293 cells although the protein was readily detectable in the cell lysate (Fig. 3, *C* and *D*). These results indicate that the cleavage site is indeed located within the stalk region, as deletion of this region prevented constitutive shedding.

In a next step, we generated three deletion variants where we deleted stretches of 10 amino acids each in the

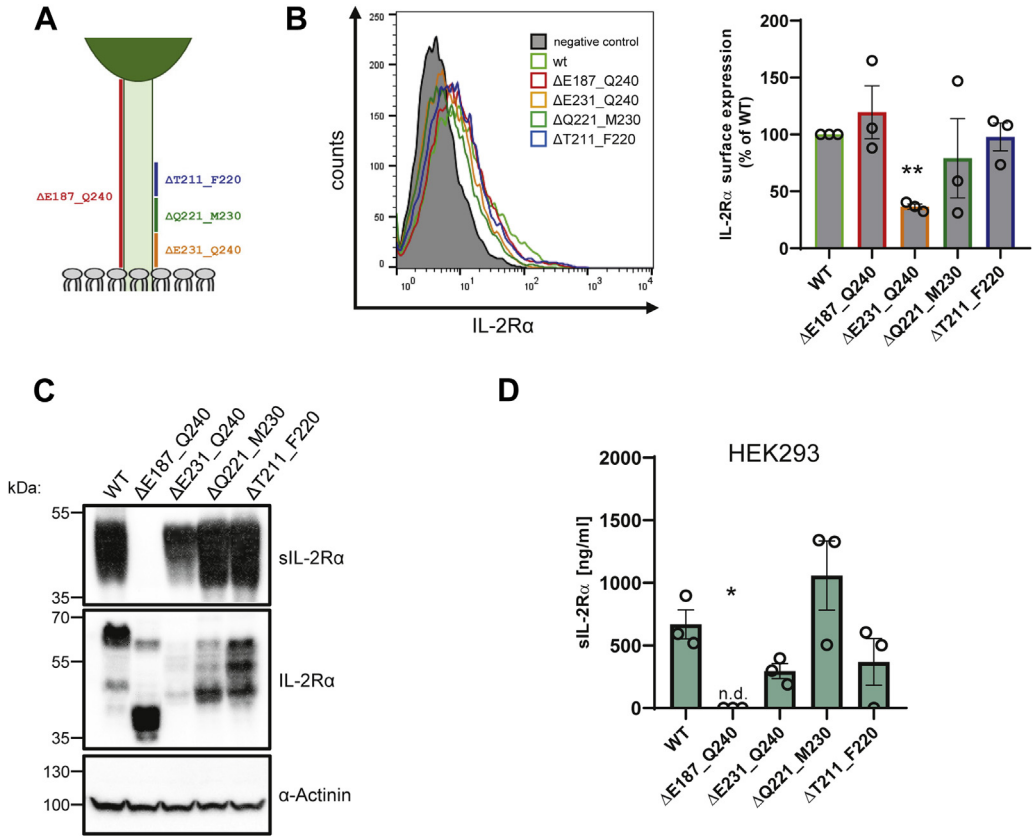


**Figure 3. The stalk region of the IL-2Rα is required for constitutive shedding.** *A*, schematic depiction of the IL-2Rα stalk region (E187_Q240). Deletions within the stalk region are indicated by color. *B*, IL-2Rα and deletion variants thereof were transiently transfected into HEK293 cells and the cell surface amount of the proteins was determined by flow cytometry. Depicted are one exemplary histogram (*left*) and the normalized relative expression of the variants of three independent experiments as mean ± SEM (*right*). *C*, HEK293 cells were transiently transfected with the IL-2Rα variants and the amount of IL-2Rα in the cell lysates and supernatants was determined by Western blot. α-Actinin served as loading control. Shown is one example out of three independent experiments with similar outcome. *D*, the experiment was performed as described under (*C*), but sIL-2Rα in the supernatants of the HEK293 cells was analyzed by ELISA. Shown are the mean ± SEM from three independent experiments. Statistical analysis was performed using one sample *t* test with bonferroni correction (*B*) and one-way ANOVA with Dunnet's multiple comparison test (*D*); $*p < 0.05$, $**p < 0.01$. IL-2R, interleukin-2 receptor; sIL-2Rα, soluble IL-2Rα.

juxtamembrane part of the IL-2Rα stalk region, namely ΔE231_Q240, ΔQ221_M230, and ΔT211_F220 (Fig. 3*A*), and analyzed the shedding of these variants. Like the deletion of the entire stalk region, also the deletion of the different 10 amino acid sequences did not prevent cell surface expression of the IL-2Rα, although it has to be noted that the IL-2RαΔE231_Q240 showed a decreased expression compared to WT IL-2Rα (Fig. 3*B*). Notably, soluble forms of all of these deletion variants could be detected in the cell culture supernatant by ELISA and Western blot (Fig. 3, *C* and *D*). It has to be noted, however, that the IL-2RαΔE231_Q240 was barely detectable in the cell lysate, which is in agreement with the flow cytometry data (Fig. 3*B*) and points toward a reduced expression of this variant compared to the WT IL-2Rα. However, these results show that, in contrast to the deletion of the entire stalk, neither of the 10 amino acid deletions prevented constitutive shedding of these variants from transfected HEK293 cells (Fig. 3, *C* and *D*). This indicates that either the cleavage site used by ADAM10 and ADAM17 is not located between amino acids T211 and Q240 or that multiple cleavage sites exist within the IL-2Rα stalk region. It is also possible that the deletions in the stalk region resulted in a novel cleavage site that is then used in absence of the original cleavage site. Further investigations are necessary to find a definitive answer.

### *IL-2Rα release from activated T cells is mediated by ADAM10 and ADAM17*

As described above, sIL-2Rα is increased in various pathological conditions. Considering the fundamental role of T cells in human pathophysiology as well as the impact of IL-2 on T cell biology, we next focused on IL-2Rα shedding from T cells. Therefore, we first used the Jurkat T cell line and treated the cells with αCD3 and αCD28 antibodies for 2 days in order to activate the cells *via* the TCR. We found that *in vitro* activation increased the amount of IL-2Rα on the cell surface, which has been previously reported for human T cells and Jurkat cells (34, 35) (Fig. 4*A*). We then performed the same experiment in the presence of either one of the metalloprotease inhibitors GI, GW, or MM, or DMSO as control, and analyzed sIL-2Rα generation. We found that *in vitro* TCR activation increased the release of sIL-2Rα from Jurkat cells (Fig. 4*B*), which is in agreement with previously reported data

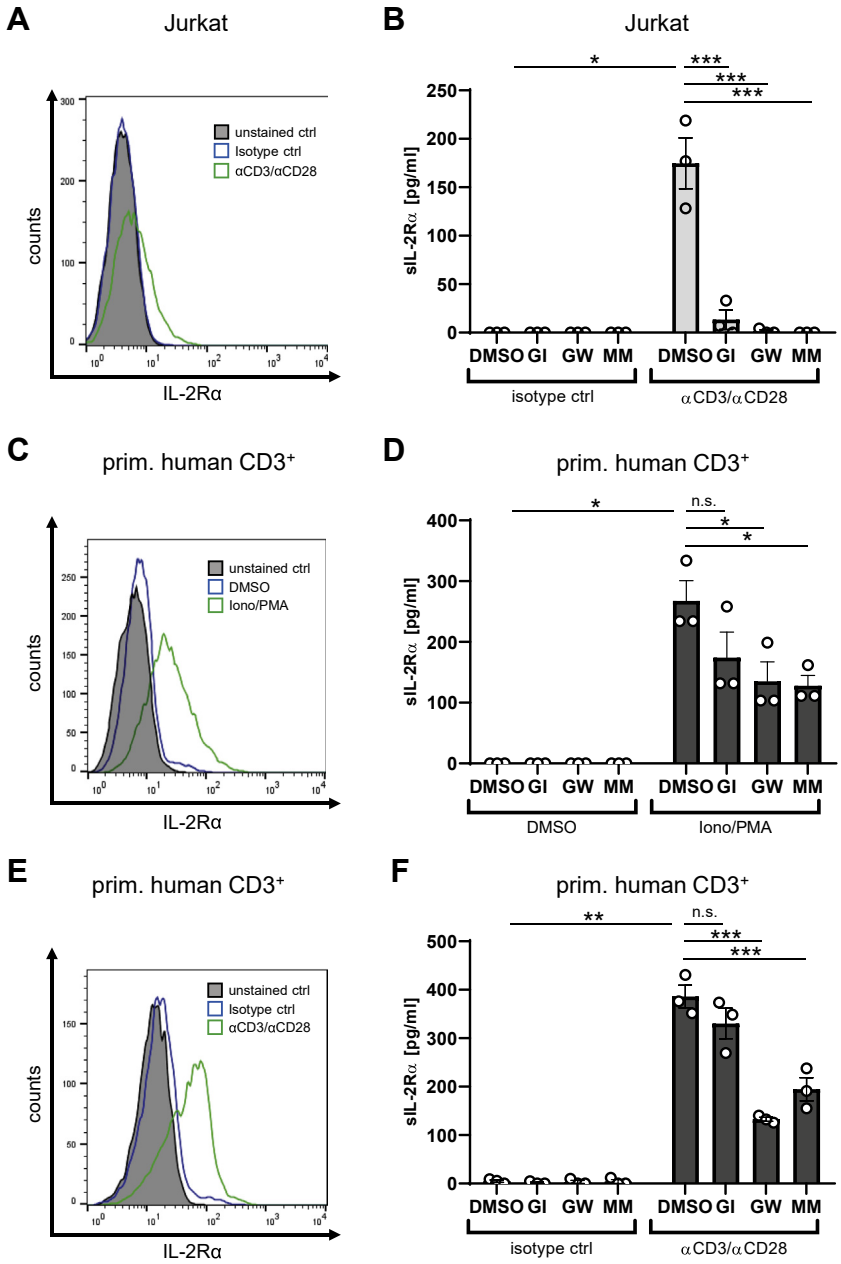


**Figure 4. IL-2Rα shedding from activated T cells is mediated by ADAM10 and ADAM17.** *A*, cell surface expression of the IL-2Rα on Jurkat cells without (*blue*) or with (*green*) activation of the cells using αCD3/αCD28. Unstained cells are shown in *gray*. Depicted is one experiment out of three with similar outcome. *B*, Jurkat cells were treated either with isotype control antibodies or with αCD3/αCD28 for 48 h in the presence or absence of 3 μM GI, 3 μM GW, or 10 μM MM as indicated. sIL-2Rα in the supernatants was analyzed by ELISA. Shown are the mean ± SEM from three independent experiments. *C* and *E*, cell surface expression of the IL-2Rα on primary human $CD3^+$ cells without (*blue*) or with (*green*) activation of the cells using 1 μM ionomycin and 100 nM PMA (*C*) or αCD3/αCD28 (*E*). Unstained cells are shown in *gray*. Depicted is one experiment out of three with similar outcome. *D* and *F*, primary $CD3^+$ cells were treated either with DMSO as solvent control or with 1 μM ionomycin and 100 nM PMA (*D*) or with either isotype control antibodies or αCD3/αCD28 (*F*) for 48 h in the presence or absence of 3 μM GI, 3 μM GW, or 10 μM MM as indicated. sIL-2Rα in the supernatants was analyzed by ELISA. Shown are the mean ± SEM from three independent experiments. Statistical analysis was performed using unpaired t tests with Welch's correction in order to compare between DMSO-treated samples with or without activation, and one-way ANOVA with Dunnet's multiple comparison test was performed in order to compare between the samples treated with the protease inhibitors compared to DMSO within the activated groups (αCD3/αCD28 or Iono/PMA); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n.s.: not significant. ADAM, a disintegrin and metalloprotease; IL-2R, interleukin-2 receptor; PMA, phorbol-12-myristate-13-acetate; sIL-2Rα, soluble IL-2Rα.

(9). sIL-2Rα release was almost completely abrogated when the cells were treated with the different metalloprotease inhibitors (Fig. 4*B*). Notably, inhibition of ADAM10 alone had the same effect as inhibition of all metalloproteases, indicating that ADAM10 is also the main protease responsible for IL-2Rα shedding from activated Jurkat cells.

In order to not only rely on a T cell line, we extended our analysis to human $CD3^+$ T cells isolated from peripheral blood. We used two different stimuli to activate the $CD3^+$ cells *in vitro*: we stimulated the cells either with ionomycin and PMA (Fig. 4, *C* and *D*) or αCD3 and αCD28 (Fig. 4, *E* and *F*). Analysis of the cell surface expression of the IL-2Rα confirmed

that both stimuli resulted in increased IL-2Rα surface amount (Fig. 4, *C* and *E*). Furthermore, also the primary T cells produced significant amounts of sIL-2Rα upon activation (Fig. 4, *D* and *F*). In agreement with the data obtained using Jurkat cells, inhibition of metalloproteases did significantly decrease sIL-2Rα release, although it did not completely abolish it. Of note, in contrast to Jurkat cells, inhibition of ADAM10 activity had no significant effect on sIL-2Rα release from activated primary CD3$^+$ cells. A significant reduction was only observed when ADAM10 and ADAM17 were both inhibited with GW or with the broad-spectrum metalloprotease inhibitor MM. These results show that on activated T cells, ADAM17 is mainly responsible for the cleavage of the IL-2Rα from the cell surface. Furthermore, it appears likely that other mechanisms contribute to sIL-2Rα release from human T cells.

### *sIL-2Rα inhibits IL-2 signaling in T cells*

As the biological function of the sIL-2Rα is not yet completely understood, we next analyzed the effect of sIL-2Rα on IL-2 signaling in T cells. To this end, we stimulated resting or *in vitro*–activated human CD3$^+$ T cells with IL-2 and increasing concentrations of recombinant sIL-2Rα. As shown in Figure 5*A*, stimulation of resting CD3$^+$ cells with IL-2 resulted in an increase in phosphorylated (p)STAT5. This effect was significantly decreased when IL-2 was preincubated with 1 μg/ml sIL-2Rα and completely abrogated with 10 μg/ml sIL-2Rα. Notably, the basal pSTAT5 levels in activated CD3$^+$ cells was considerably higher than in resting cells, and stimulation with IL-2 showed no significant increase in pSTAT5 (Fig. 5*B*). This observation is most likely caused by the fact that TCR activation leads to secretion of cytokines from T cells, which includes IL-2 but also other STAT5-activating cytokines. In summary, these results clearly show that the sIL-2Rα acts as an antagonist for IL-2 signaling in CD3$^+$ cells.

### *ADAM10 is involved in the production of sIL-2Rα* in vivo

Finally, we aimed to analyze whether ADAM10 or ADAM17-mediated shedding is also involved in the steady-state production of sIL-2Rα *in vivo*. We first analyzed mice deficient for the metalloprotease inhibitor TIMP-3, which is a known physiological inhibitor of ADAM10 and ADAM17, but also a variety of other metalloproteases (36–38). However, the sIL-2Rα concentrations in the serum were not significantly different between TIMP-3 deficient and WT mice (Fig. 6*A*), indicating that the constitutive sIL-2Rα production in mice is not exclusively mediated by a TIMP-3 sensitive mechanism.

As a next step, we aimed to specifically analyze the contributions of ADAM10 and ADAM17 for the steady-state sIL-2Rα serum levels in mice. As T cells are considered the main source of sIL-2Rα, we analyzed the serum of mice which lacked either ADAM10 or ADAM17 on CD4$^+$ cells. We found that lack of ADAM10 indeed decreased the sIL-2Rα concentration in the serum compared to WT littermates (Fig. 6*B*), showing that ADAM10 is also responsible for IL-2Rα shedding *in vivo*. In contrast, lack of ADAM17 in CD4$^+$ cells resulted in an increased sIL-2Rα concentration in the serum of these mice (Fig. 6*B*). The lack of reduction in sIL-2Rα clearly rules out a contribution of this protease to constitutive IL-2Rα cleavage in mice under normal conditions. The increase is probably due to a compensatory upregulation of ADAM10 when ADAM17 is lacking. Notably, a compensatory increase of ADAM17 activity in the absence of ADAM10 has indeed been reported in B cells

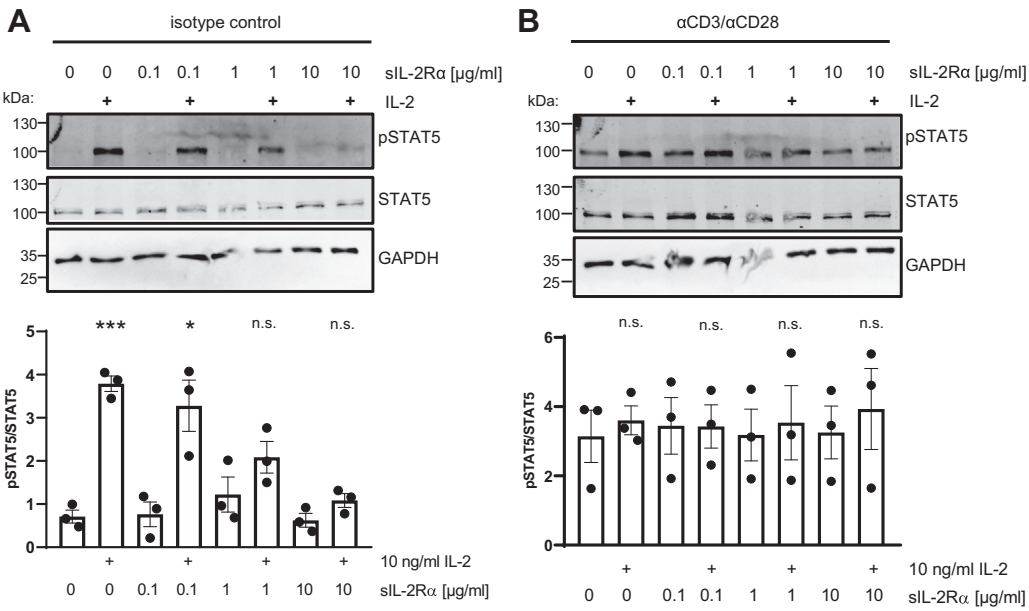


**Figure 5. The soluble IL-2Rα inhibits IL-2 signaling in T cells.** *A* and *B*, human CD3$^+$ cells were isolated from peripheral blood and treated either with (*A*) isotype control antibodies or (*B*) with αCD3/αCD28 for 48 h. Then, the cells were stimulated with 5 ng/ml IL-2 and increasing amounts of recombinant sIL-2Rα as indicated for 30 min. Phosphorylated (p) and total STAT5 were detected by Western blot. GAPDH served as loading control. Shown is one example out of three independent experiments with similar outcome and quantification of the three experiments. Statistical analysis was performed using unpaired t tests with Welch's correction in order to compare between unstimulated and IL-2 treated samples; *$p < 0.05$, *** $p < 0.001$, n.s.: not significant. IL-2, interleukin-2; IL-2R, IL-2 receptor; sIL-2Rα, soluble IL-2Rα.

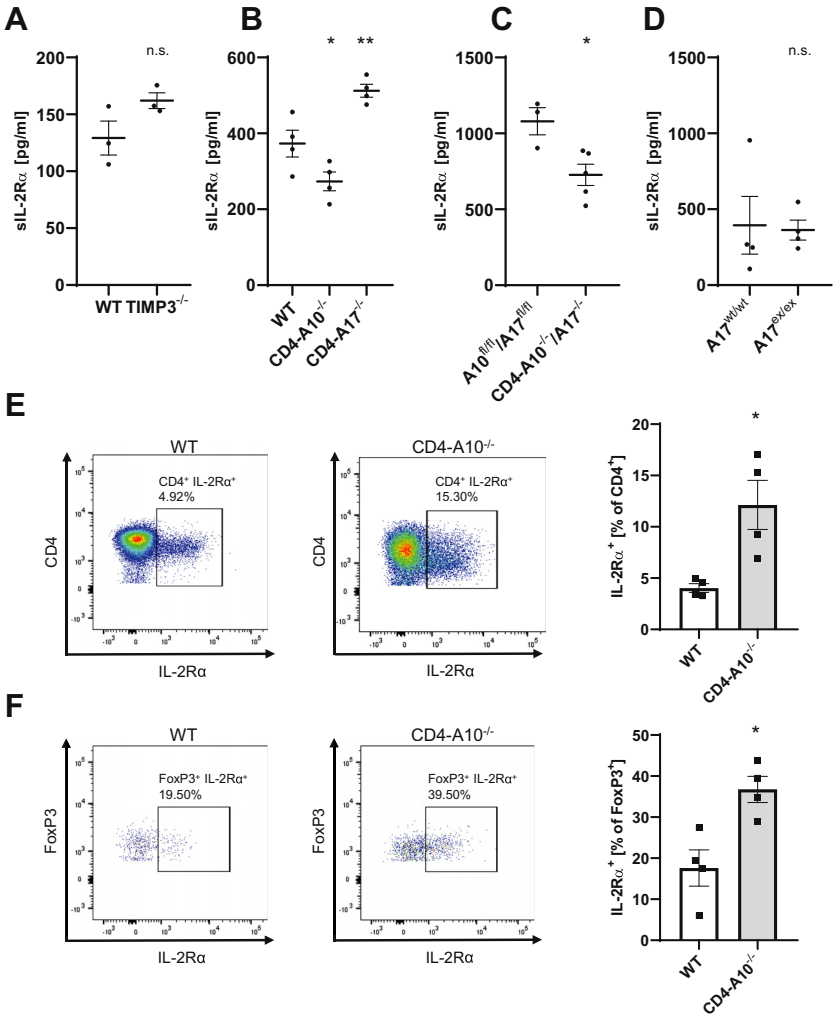


**Figure 6. ADAM10 is responsible for constitutive shedding of IL-2Rα *in vivo*.** Concentration of sIL-2Rα in serum samples of (*A*) three TIMP3$^{-/-}$ mice and three WT littermates, (*B*) four WT, CD4-A10$^{-/-}$, and CD4-A17$^{-/-}$ mice, (*C*) three A10$^{fl/fl}$/A17$^{fl/fl}$ and five CD4-A10$^{-/-}$/A17$^{-/-}$ mice, or (*D*) three A17$^{ex/ex}$ mice and three A17$^{wt/wt}$ mice were determined by ELISA. *E* and *F*, surface expression of IL-2Rα on (*E*) all CD4$^+$ cells or (*F*) FoxP3$^+$ regulatory T cells isolated from the intestine of WT or CD4-A10$^{-/-}$ mice was analyzed by flow cytometry. Shown are one exemplary dot plot per genotype and quantification of 4 mice per group. Statistical analysis was performed using Mann-Whitney test to compare between two groups of mice (*A*, *C*–*F*), and one-way ANOVA with Dunnet's multiple comparison test was performed in order to compare between three groups of mice (*B*); *$p < 0.05$, **$p < 0.01$, n.s.: not significant. ADAM, a disintegrin and metalloprotease; IL-2R, interleukin-2 receptor; sIL-2Rα, soluble IL-2Rα.

(39). Importantly, this is in agreement with our above mentioned findings in the protease-deficient HEK293 cell lines, where A17$^{-/-}$ cells also showed an increase in constitutive IL-2Rα shedding (Fig. 2*A*), indicating that this is conserved beyond species and cell types.

In order to further dissect the individual roles of ADAM10 and ADAM17 in constitutive IL-2Rα shedding, we then analyzed mice, which were lacking both proteases on CD4$^+$ T cells. Here, we again detected a decrease in sIL-2Rα serum concentration in the protease deficient compared to the proficient mice (Fig. 6*C*). However, this decrease was similar to the one observed in mice lacking only ADAM10 (Fig. 6*B*), suggesting that ADAM17 does not act synergistically but rather that ADAM17 only contributes to cleavage of the IL-2Rα in the absence of ADAM10. Furthermore, the increase in sIL-2Rα, that was observed in the single ADAM17 knockout, is no longer detectable in the ADAM10/ADAM17 double knockout, further strengthening the notion that this increase is caused by an increase in ADAM10 activity. To further confirm this, we finally looked into hypomorphic ADAM17$^{ex/ex}$ mice, which only possess approximately 5% of the ADAM17 amount of WT mice (40). These mice showed normal sIL-2Rα serum levels compared to their WT littermates (Fig. 6*D*), further confirming that ADAM17 does not contribute to the constitutive generation of the sIL-2Rα in mice when ADAM10 is present.

To exclude that the reduced sIL-2Rα amount in the serum of CD4-ADAM10$^{-/-}$ mice is caused by a decreased expression of the protein, we analyzed the amount of IL-2Rα on the cell surface of T cells. To this end, we stained IL-2Rα on intestinal CD4$^+$ cells isolated either from WT or CD4-ADAM10$^{-/-}$ mice and determined the amount of IL-2Rα positive cells. As shown in Figure 6*E*, deficiency of ADAM10 did not reduce the amount of IL-2Rα positive cells. Strikingly, it was even higher in ADAM10-deficient cells than WT cells. As regulatory T cells are the main T cell population that constitutively expresses IL-2Rα (6, 7), we also stained surface IL-2Rα on

FoxP3$^+$ regulatory T cells from these mice. Again, the amount of IL-2Rα positive cells was higher in the absence of ADAM10 (Fig. 6*F*). Thus, the absence of constitutive shedding of the IL-2Rα by ADAM10 does not only lead to reduced sIL-2Rα serum concentration but also concomitantly enhances the amount of membrane-bound IL-2Rα on CD4$^+$ cells.

Taken together, our data show that ADAM10 is responsible for the steady-state sIL-2Rα levels in mice and consequently also controls the surface expression of IL-2Rα. However, the CD4-specific KO of ADAM10 does not result in a complete loss of sIL-2Rα, which is most probably caused by the fact that CD4$^+$ T cells are not the only cell type from which the constitutive circulating sIL-2Rα originates.

## Discussion

IL-2 is one of the major regulators of the immune response, which has pleiotropic functions on various immune cells and is especially important for the function of regulatory T cells (2, 3). While a sIL-2Rα that originates from activated T cells has already been described in 1985 (9), until today neither its function in IL-2 biology nor the mechanism(s) of its generation are understood.

Here, we analyzed the role of the metalloproteases ADAM10 and ADAM17 in ectodomain shedding of the IL-2Rα. Ectodomain shedding is a unique form of post-translational protein modification not only because it is the only irreversible mechanism but also because it has dual effects. It creates a soluble ectodomain that has own biological functions and it regulates the cell surface amount of the substrate protein. ADAM10 and ADAM17 have emerged as important sheddases with a wide variety of substrates, some of them being shared by both proteases while others are only cleaved by one of them (25, 41). While the complete knockout of ADAM10 or ADAM17 in mice, which both results in embryonic lethality, point toward a main function in development due to cleavage of Notch or epidermal growth-factor receptor ligands, respectively (42, 43), further analysis also revealed other functions of the two proteases, namely in cytokine signaling and immune cell functions (26).

We have identified ADAM10 as the major sheddase involved in the constitutive release of heterologous or endogenous sIL-2Rα. Additionally, ADAM10 is also involved in IL-2Rα shedding upon *in vitro* T cell activation, which has been reported to be a major source of sIL-2Rα. However, the impact of ADAM10 seems to be smaller in primary human T cells than cell lines, as specific inhibition of the protease had no significant effect on sIL-2Rα levels as opposed to combined inhibition of ADAM10 and ADAM17. Notably, ADAM10 has already been reported to be upregulated upon T cell activation (27). Likewise, the IL-2Rα is also upregulated upon activation of T cells, making it likely that the upregulation of both proteins is responsible for the increase in sIL-2Rα release. However, it is also possible that, in addition to the increased expression, ADAM10 is also activated upon T cell activation, although no data supporting this hypothesis have been reported yet.

Our data furthermore show that activation of ADAM17 also results in increased sIL-2Rα production from cultured cells, while this protease plays no major role in constitutive IL-2Rα shedding. Importantly, sIL-2Rα shedding from activated T cells is mainly dependent on ADAM17 activity. Of note, ADAM17-mediated shedding upon T cell activation has also been reported for other substrates like the IL-6R (27, 44). Interestingly, it has been shown for the IL-6R that ADAM17 is not involved in steady-state production of sIL-6R but cleaves the IL-6R during inflammatory responses (31, 45). Our results show that a similar, context-depending role for ADAM17 is also likely in IL-2Rα shedding.

Furthermore, the biological function of the sIL-2Rα is still under debate, with agonistic as well as antagonistic properties being discussed (11). We here show that the sIL-2Rα has antagonistic functions in IL-2 signaling in T cells. Our results indicate that an excess of sIL-2Rα might bind the majority of the available IL-2 and prevent binding of the cytokine to the target cells. This is in agreement with a previous observation, where it was reported that recombinant sIL-2Rα decreased proliferation of T cells (15). Of note, the concentrations used in our experiment are higher than what has been reported in serum samples. However, it should be kept in mind that in pathological situations, where the highest serum concentrations are described, sIL-2Rα is likely produced at the site of infection/inflammation, where massive activation of T cells occurs. Therefore, it is likely that the local concentration of sIL-2Rα is also significantly higher than the serum concentration. However, as others also reported agonistic functions of the sIL-2Rα (13), we cannot rule out that both functions are true. The specific effect of the sIL-2Rα might be cell type specific, possibly depending on the amount of membrane-bound IL-2Rα expressed in a given cell. Further research will be necessary in order to dissect the biological function of the sIL-2Rα.

Finally, we could also show that ADAM10 is responsible for the constitutive shedding of the IL-2Rα in mice. Whether ADAM10 also plays a role in constitutive sIL-2Rα release in humans is not yet clear. However, as our *in vitro* experiments, which show the role of ADAM10 in constitutive IL-2Rα shedding, were conducted with human cells, we think it likely that ADAM10 also controls the sIL-2Rα levels in human blood. This is supported by the fact that different diseases that show increased ADAM10 expression, for example, immune thrombocytopenia or dermatitis (46, 47), also display increased sIL-2Rα serum levels (48, 49).

The role of the sIL-2Rα is not yet understood and might be different in distinct diseases, reflecting the complex functions of IL-2 on various immune cell types (21). In addition to expanding our understanding of the role of this soluble receptor, it is important to understand how the sIL-2Rα is generated because this will allow for future targeted intervention in sIL-2Rα production. We have identified ADAM10 as a sheddase that not only regulates the amount of sIL-2Rα but likewise controls the amount of cell surface IL-2Rα, thereby regulating the sensitivity of a cell toward IL-2. This opens the possibility to manipulate membrane-bound as well as sIL-2Rα

levels through modulating ADAM10 activity. In contrast to constitutive shedding, IL-2Rα shedding from T cells, which is a major source of sIL-2Rα, appears to rely more on ADAM17 activity. Notably, ADAM10 as well as ADAM17 dysregulation have been reported in different diseases (41, 50), and it is tempting to speculate that altered levels of sIL-2Rα could be partly responsible for the disease mechanisms.

In summary, we could show that the sIL-2Rα acts as an antagonist for IL-2 signaling in T cells. Further, we identify ADAM10 and ADAM17 as two novel proteases that can cleave the IL-2Rα, thereby producing sIL-2Rα. When examining constitutive and induced shedding, it is striking that both proteases have different functions. Given the importance of the IL-2Rα chain for the sensitivity of cells toward IL-2, this adds a novel regulatory mechanism to the complex biology of IL-2. Importantly, we could identify ADAM10 as the first protease known to be responsible for the steady-state levels of sIL-2Rα *in vivo*.

## Experimental procedures

### Cells & reagents

HEK293 cells and HEK293 cells deficient for ADAM10 ($A10^{-/-}$), ADAM17 ($A17^{-/-}$), or both proteases ($A10^{-/-}/A17^{-/-}$) have been described previously (51) and were cultured in dulbecco's modified eagle's medium (DMEM, PAN-Biotech) supplemented with 10% fetal calf serum (FCS, Gibco by Thermo Fisher), 60 mg/l penicillin, and 100 mg/l streptomycin. Jurkat cells and HDLM-2 cells were cultured in RPMI1640 (PAN-Biotech) supplemented with 10% or 20% FCS, respectively, 60 mg/l penicillin, and 100 mg/l streptomycin. All cells were kept in a standard incubator at 37 °C, 5% $CO_2$, and a water saturated atmosphere.

Ionomycin was purchased from Thermo Fisher Scientific, and PMA as well as the protease inhibitors MM (broad-spectrum metalloprotease inhibitor), E64 (cysteine protease inhibitor), pepstatin A (aspartate protease inhibitor), and AEBSF (serine protease inhibitor) were from Sigma-Aldrich. The specific inhibitors GI254023X (GI, selective for ADAM10) and GW280264X (combined ADAM10/ADAM17 inhibitor) were synthesized by Iris Biotech (28). Recombinant sIL-2Rα-His was purchased from Bio-techne and IL-2 was from ImmunoTools.

### Ethics statement

Ethical approval for the isolation of primary T cells from human blood was obtained from the Ethics Committee of the Medical Faculty of the Otto-von-Guericke University Magdeburg (approval number 129/19). The study complies with the principles of the Declaration of Helsinki. All donors gave written informed consent.

### Mice

Hypomorphic ADAM17 mice ($Adam17^{ex/ex}$), floxed $CD4cre^{+}x\ Adam10^{fl/fl}$ ($CD4\text{-}A10^{-/-}$), and floxed $CD4cre^{+}x\ Adam17^{fl/fl}$ ($CD4\text{-}A17^{-/-}$) were previously described (40, 44, 45). $ADAM17^{fl/fl}$ mice were homozygous for the floxed ADAM17 allele and heterozygous for the CD4cre recombinase. $ADAM10^{fl/fl}$ mice were homozygous for the floxed ADAM10 allele (52) and heterozygous for the CD4cre recombinase. These mouse lines were crossed in order to obtain $CD4cre^{+}x\ Adam10/17^{fl/fl}$ ($CD4\text{-}A10^{-/-}/A17^{-/-}$). Mice were housed in a 12-h light–dark cycle under specific pathogen-free conditions. Blood was obtained from 8 to 12 week old mice, and serum was generated by centrifugation of whole blood at 2000*g* for 10 min at 4 °C.

Sera from $Timp3^{-/-}$ mice (53) and corresponding WT animals were kindly provided by Anne Joutel (INSERM, University of Paris).

### Plasmid construction

The myc-tagged sequence of the human IL-2Rα was ordered from Genscript Biotech and subcloned into pcDNA3.1 *via* NheI and NotI. Deletions within the stalk were cloned *via* splicing by overlapping extension PCR. All sequences were confirmed by Sanger sequencing (Eurofins Genomics).

### Shedding assay

Shedding of endogenous IL-2Rα was analyzed in HDLM-2 cells. For shedding of exogenous IL-2Rα and variants thereof, HEK293 cells were transiently transfected with 5 μg expression plasmid encoding the IL-2Rα using the Turbofect transfection reagent (Thermo Fisher Scientific) according to manufacturers' instructions. Experiments were performed 48 h after transfection. For constitutive shedding, cells were either left untreated, treated with DMSO as solvent control, or with the different protease inhibitors 3 μM GI, 3 μM GW, 10 μM MM, 1 mM AEBSF, 10 μM E64, or 1 μM PepA for 24 h in serum-free medium as indicated. For induced shedding, cells were either treated with 1 μM ionomycin for 1 h or 100 nM PMA for 2 h in order to activate ADAM10 or ADAM17, respectively. Cell lysates and cell culture supernatants were analyzed *via* ELISA or Western blotting (see below).

### ELISA

For detection of sIL-2Rα within the supernatants of transiently transfected HEK293 cells, HDLM-2 cells, Jurkat cells, or isolated primary $CD3^{+}$ T cells, the supernatants were first cleared by centrifugation for 20 min at 18,000*g* and 4 °C after separation from the pelletized cells. Samples were analyzed using the human CD25/IL-2R alpha Duo Set ELISA (R&D Systems) according to manufacturer`s instructions. Serum samples of mice were analyzed using the mouse CD25/IL-2R alpha Duo Set ELISA (R&D Systems) according to manufacturer`s instructions. When necessary, samples were appropriately diluted in order to stay within the detection range of the ELISA Kit.

### Sample preparation & Western blot

Cells were lysed with lysis buffer (1% 1 M Tris–HCl, 140 mM NaCl, 1% 0,5 M EDTA, 1% Triton-X 100, pH 7,5) with protease inhibitor cocktail and, for detection of phosphorylated STAT5, phosphatase inhibitor (both from Sigma

Aldrich) at 4 °C. Cleared supernatants were mixed with same volume of 20% trichloroacetic acid and incubated on ice for 20 min before a centrifugation step (18,000*g*, 4 °C, 20 min) to collect protein precipitates. After discarding the supernatants, pellets were washed with 350 µl ice-cold acetone and incubated on ice for 20 min before a second centrifugation step. After removing the acetone, the pellets were allowed to dry at room temperature. Samples were boiled in Laemmli buffer before Western blot analysis.

Equal amounts of cell lysates or precipitated supernatants were separated by SDS-PAGE and transferred to PVDF or nitrocellulose membranes. Membranes were blocked with skimmed milk or bovine serum albumin for 1 h at room temperature and afterward treated with primary antibodies at 4 °C over night. After washing, membranes were incubated with either horseradish peroxidase-linked or IRDye-linked secondary antibodies for 1 h at room temperature. After another washing step, signals were detected using the Chem-iDoc MP Imaging System (Bio-Rad) or the Fluor Chem E System (Protein Simple). The following antibodies were used for Western blotting: α-IL-2Rα Rabbit mAb (D6K5F), α-pSTAT5 (Y694, D47E7), α-GAPDH (14C10) α-Actinin XP Rabbit mAb (D6F6), and horseradish peroxidase-linked α-rabbit IgG Ab (all from Cell Signaling Technology) α-STAT5 (ST5-8F7, from Thermo Fisher Scientific), IREDye 800CW donkey α-rabbit IgG and IREDye 680RD-goat α-mouse IgG (both from LI-COR Biosciences).

### *Isolation of primary T cells*

Peripheral blood from healthy volunteers was obtained by venipuncture and collected in heparin-tubes. $CD3^+$ cells were isolated *via* magnetic associated cell sorting using Straight-From Whole Blood CD3 MicroBeads and the Whole Blood Column Kit (all material from Miltenyi Biotech) according to manufacturer`s instructions. Isolated primary cells were cultured in RPMI supplemented with 10% FCS, 60 mg/l penicillin, and 100 mg/l streptomycin.

### In vitro *activation of T cells*

In order to activate primary human T cells or Jurkat cells, plates were first coated with 5 µg/ml αCD3 (clone Okt3, Thermo Fisher Scientific) antibody or the appropriate isotype control (clone eBM2a, Thermo Fisher Scientific) at 4 °C over night. Then, cells were seeded into the coated plates and supplemented with 2 µg/ml αCD28 (clone CD28.6, Thermo Fisher Scientific) antibody or isotype control for 48 h. Alternatively, cells were activated by addition of 100 nM PMA and 1 µM ionomycin for 48 h. Where indicated, cells were also treated with the metalloprotease inhibitors GI (3 µM), GW (3 µM), or MM (10 µM) for the duration of the activation.

### *IL-2 stimulation of $CD3^+$ cells*

To analyze the effects of sIL-2Rα on IL-2 signaling, primary human $CD3^+$ cells were isolated from human blood and *in vitro* activated as described above. Different amounts of sIL-2Rα were incubated with 5 ng/ml IL-2 in serum-free medium for 30 min at 37 °C to allow complex formation. Then, cells were stimulated with these mixtures for 30 min. Phosphorylated and total STAT5 were analyzed by Western blot. Quantification of STAT5 activation was performed by densitometric analysis using the ImageStudio Lite software (Li-COR Biosciences) and calculation of the pSTAT5/STAT5 ratio from three independent experiments.

### *Flow cytometric analysis of cell lines and human $CD3^+$ cells*

Flow cytometry was used to assess cell surface expression of IL-2Rα and variants thereof. IL-2Rα deletion variants were analyzed 48 h after transfection into HEK293 cells. Heterologous IL-2Rα in HEK293 cells was stained with the α-Myc-tag rabbit mAb (71D10, Cell Signaling Technology) and the AlexaFluor488-conjugated goat α-rabbit IgG (Thermo Fisher Scientific), and endogenous IL-2Rα on Jurkat cells and primary $CD3^+$ cells was stained with FITC-conjugated α-CD25 (clone BC96). Cells were analyzed using a BD LSR flow cytometer and FlowJo Software (FlowJo, LLC). Surface expression was calculated by subtraction of the geometric mean fluorescence intensity of the negative control and subsequent normalization to IL-2Rα WT.

### *Isolation and flow cytometric analysis of murine intestinal T cells*

Mouse colon and small intestine were opened longitudinally, cleaned, cut into fragments and dissociated ($Ca^{2+}$/$Mg^{2+}$-free HBSS (Gibco by Thermo Fisher), 5 mM EDTA, 10 mM Hepes) for 30 min at 37 °C. After washing ($Ca^{2+}$/$Mg^{2+}$-free HBSS with 4% FCS), the tissue was digested using 0.5 mg/ml Collagenase D (Roche), 0.5 mg/ml DNAse I (Applichem), and 0.5 mg/ml Dispase (Roche) in HBSS (with $Ca^{2+}$ and $Mg^{2+}$) for 30 min at 37 °C. The cells were dissociated through a 40 µm cell strainer and lymphocytes were isolated using a percoll gradient. Then, cells were stimulated with 20 ng/ml PMA and 1 µg/ml ionomycin for 2 h at 37 °C followed by addition of 1 µg/ml Brefeldin A for additional 4 h at 37 °C. Cells were then treated with FcR block prior to staining of surface proteins for 20 min at 4 °C. Cells were then washed, fixed, and permeabilized for 1 h at 4 °C. Afterward, the cells were again treated with FcR block followed by staining of the intracellular proteins for 1 h at 4 °C. After a final washing step, the cells were analyzed. The following antibodies were used: AlexaFluor700-conjugated α-CD4 (clone RM4-5), FITC-conjugated α-FoxP3 (clone FJK-16S, Thermo Fisher Scientific), BrilliantViolet510-conjugated α-TCRβ (clone H57–597), BrilliantViolet711-conjugated α-IL-2Rα (clone PC61), and Viability Dye eFluor780 (Thermo Fisher Scientific). Cells were analyzed using a BD LSRFortessa flow cytometer and FlowJo Software (FlowJo, LLC). We defined $CD4^+$ cells as viable, $TCR\beta^+$ $CD4^+$ cells and regulatory T cells as viable, $TCR\beta^+$ $CD4^+$ $FoxP3^+$ cells.

### *Data presentation & statistics*

ELISA data are presented as mean ± SEM of at least three different experiments. Flow cytometry data are shown as

mean ± SEM of at least three different experiments/mice and one exemplary histogram. Western blots are presented as one example out of at least three experiments with similar outcome.

Statistical analysis was performed using GraphPad Prism (GraphPad Software). One-way ANOVA with Dunnet's multiple comparison test was used to compare three or more sets of data. Differences between two different sets of cells were calculated with unpaired t tests with Welch's correction. Serum concentrations in two groups of mice were compared using the Mann-Whitney test. Normalized data with more than two groups were analyzed with one-sample *t* test and bonferroni correction.

## Data availability

All data are contained within the article.

## Conflict of interestCOI

The authors declare that they have no conflicts of interest with the contents of this article.

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*Abbreviations*—The abbreviations used are: ADAM, a disintegrin and metalloprotease; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; IL-2, interleukin 2; IL-2R, IL-2 receptor; MMP-9, matrix metalloproteinase-9; PMA, phorbol-12-myristate-13-acetate; sIL-2Rα, soluble IL-2Rα; TCR, T cell receptor.

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