Regulatory T cells have anti-inflammatory effects by diluting TNF

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Evaluation of: van Mierlo GJ, Scherer HU, Hameetman M *et al.*: Cutting edge: TNFR-shedding by $CD4^+CD25^+$ regulatory T cells inhibits the induction of inflammatory mediators. *J. Immunol.* 180(5), 2747–2751 (2008). Regulatory T cells (Tregs) of both human and murine origin were shown to express and shed significant amounts of TNF receptor 2. This is important new information as the shed soluble TNF receptor 2 binds TNF, which may explain the direct anti-inflammatory effects of Tregs. This adds to our current understanding of the various effects that Tregs may exert in order to dampen inflammation and autoimmunity. However, the interplay of these various Treg effects will need to be elucidated, particularly when considering their potential therapeutic use.

While it has become clear in recent years that regulatory T cells (Tregs) play a major role in controlling inflammation, the mechanisms by which Tregs exert such control are not yet sufficiently understood [1]. Among the established mechanisms are the production of anti-inflammatory cytokines, TGF-β and IL-10 in particular, as well as cell contact-mediated events. However, there are arguments for a more direct anti-inflammatory effect of these cells that have yet to be explained.

In the Cutting Edge section of the *Journal of Immunology*, a group of scientists from the Department of Rheumatology at the University of Leiden, The Netherlands, have published findings that may explain such an effect [2]. For both murine and human Tregs, they were able to show that large quantities of soluble TNF receptor 2 (sTNF-R2) are produced and shed upon stimulation of their T-cell receptors. Indeed, the authors demonstrated that Tregs were much more efficient in producing sTNF-R2 than were effector T cells.

TNF & its receptors

TNF is a strong proinflammatory mediator that is implicated in almost all inflammatory diseases. TNF is produced as a transmembrane trimeric cytokine that can be shed by the enzyme TACE and then acts as a soluble trimer [3]. Both transmembrane TNF and sTNF can bind to either of two other trimeric receptors, TNF-R1 and TNF-R2, leading to activation of the NF-κB and MAPK pathways. Among a variety of other effects, these pathways induce the translation of IL-1, IL-6 and IL-18, fostering an inflammatory response.

Like TNF, both of the TNF-Rs can be enzymatically shed from the cell surface. However, in contrast to the cytokine, where the shed cytokine receptors break into monomers and therefore lose their signaling properties, sTNF-Rs can bind TNF and block it from binding membrane-bound TNF-Rs, thus acting as negative regulators.

Findings from the study

The Dutch group noted that Tregs express high amounts of TNF-R2 on their surfaces [2]. Investigating this in more detail, they also found increased amounts of sTNF-R2 in their supernatants, reaching more than 5000 pg/ml over 6 days when cells were cultured in the presence of IL-2 on plates providing T-cell activation (anti-CD3 antibody) and costimulation (anti-CD28 antibody). Supernatants of Tregs, but not of TNF-R2-deficient *bona fide* Tregs, protected cells against TNF-induced cell death. In a more complex *in vivo* experiment, TNF blockade with etanercept, or the transfer of Tregs, but not the transfer of TNF-R2-deficient Tregs, reduced lipopolysaccharide-induced IL-6 production.

The authors then went on to investigate the subset of human $CD4^+CD25$ bright cells, which mostly consists of Tregs. As for the murine Tregs, these cells showed high surface expression of TNF-R2 and shed large amounts of sTNF-R2. Again, this was much less pronounced for effector T cells. When marimastat, a metalloproteinase inhibitor, was added, TNF-R2 shedding was prevented, as expected, and surface TNF-R2 increased considerably. Taken together, these results show convincing evidence that both

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murine and human Tregs produce and shed large amounts of TNF-R2, which are actually able to reduce TNF-induced effects, and TNF-induced inflammation in particular.

Impact on understanding of regulatory T-cell function

While sTNF-Rs bind TNF, and thus prevent the cytokine binding transmembrane TNF-Rs, it is important to stress that this will neither block TNF activity completely, nor remove TNF from the body in significant amounts. In fact, the binding affinity of the sTNF-R monomers is quite low compared with the trimeric receptors, and much lower still than that of the hybrid molecule etanercept, which targets TNF with two monomers and thus achieves a much higher binding affinity.

Therefore, TNF blockade by sTNF-Rs is a temporary event that does not eliminate TNF. Indeed, it can be shown that TNF is still bioactive despite the presence of huge amounts of sTNF-Rs. For example, in sera of patients with systemic lupus erythematosus (SLE), the increase in sTNF-Rs by far exceeds the increase in TNF, but TNF still exhibits essentially undiminished activity [4]. Accordingly, sTNF-Rs, dilute rather than eliminate, TNF, and this perfectly fits our understanding of Tregs.

After all, Tregs act locally, in the inflamed tissue and the regional lymph nodes, rather than exerting downmodulatory effects throughout the body. The high amounts of sTNF-R2 shed by Tregs will be able to dilute very high concentrations of TNF in local areas and thus protect against tissue damage and uncontrolled inflammation. In this high-concentration area, there will be some bystander benefit. However, the bound TNF will not be entirely blocked, but will be set free later, and may thus exert somewhat different effects slightly away from the focus of TNF production.

The novel findings are also in keeping with other features of Tregs. TNF-R2 expression and shedding took several days in the presented experiments. Tregs, like other T cells, need to be activated via their T-cell receptors, and require costimulation and survival factors. Whereas the sTNF-R2 production of effector T cells reached a maximum after 3–4 days of stimulation, Treg-derived sTNF-R2 further increased over at least 6 days. This will lead to a pronounced local increase in sTNF-R2 several days after onset, thus setting a timeline for the inflammatory reaction.

It is also interesting that Tregs almost exclusively produced TNF-R2 and shed sTNF-R2, whereas no sTNF-R1 could be found in supernatants. Moreover, the protective effects were completely prevented in TNF-R2-deficient cells, further supporting such a notion [2]. At the present time, any answers to why only TNF-R2 is produced are speculative at best. However, there is some circumstantial evidence that may be relevant.

On one hand, it is clear that any cell that is able to shed sTNF-Rs will have the same receptors as functioning surface receptors first, if only for a very short time. TNF-R1 mediates proapoptotic signals, particularly under circumstances of high TNF concentrations and on cells not protected by previous activation. Such TNF-R1 signaling may be dangerous for Tregs migrating into an inflamed area to dampen inflammation in that they might not live long enough to act, and may therefore be better off being devoid of TNF-R1.

On the other hand, while TNF-R1 binds TNF only, TNF-R2 (but not TNF-R1) is bound by lymphotoxin-α, another cytokine that could theoretically play more of a role in local inflammation than we can prove at present. One hint in this direction is the effect of the TNF-R2–immunoglobulin molecule etanercept on TNF-R-associated periodic syndrome, an autosomal-dominant autoinflammatory disease caused by mutations in TNF-R1 [5].

The Dutch findings may also shed a somewhat different light on the high levels of sTNF-Rs found in some systemic autoimmune diseases, and in SLE in particular. These levels are highly correlated with TNF and with SLE disease activity, and may even be a sensible biomarker for SLE activity, since measuring sTNF-R2 is relatively simple. These new findings may now actually indicate that, rather than being a direct consequence of the ongoing inflammation, increased sTNF-R2 may be an expression of counter-regulation, insufficient as it may be.

Finally, the apparent fact that Tregs produce soluble receptors that may, at least locally, block TNF, also renders our understanding of immunology more complex. Whereas anti-TNF measures are of obvious clinical benefit in dampening inflammation, they are not usually helpful against autoimmunity. Rather, therapeutic TNF blockade in autoimmune diseases apparently fosters antinuclear and antiphospholipid autoantibody production [6], and increases

such autoantibodies in SLE [7]. Moreover, as has been known for some time, there are impressive murine data on the autoimmunogenic effects of TNF deficiency [8].

There are several explanations for this phenomenon, including apoptosis of cells by survival-factor withdrawal, removal of the functional blockade of T-cell receptors effected by chronic TNF exposure, and increased IFN-α production when its counterplayer, TNF, is removed [9]. Some of these mechanisms may also be relevant, alone or in combination, for the newly found anti-TNF effects of Tregs.

Under some circumstances, this could theoretically increase the risk of autoimmunity, if not counterbalanced by other functions of Tregs. In the much more common case of an adequate response against a pathogen, this effect could be important for enabling the adaptive immune system to mount a protective response. However, the same mechanisms may also facilitate the activation of further Tregs, and protect them against the harmful effects of high TNF concentrations.

Conclusion

We now know that, in addition to other key functions, Tregs exert short-term blockade and thus spatial dilution of TNF, and do so by shedding sTNF-R2 following activation. This has important implications for their function both in immunity and autoimmunity, many of which have yet to be understood in detail. In this sense, like most good research papers, the manuscript by René Toes' group answers one question, but leaves us with many more open ones.

Future perspective

While several important mechanisms of Treg function have now been elucidated, it is unclear whether there is more to come, and some of the mechanisms implied have to be understood in more detail. It may be even more important, however, to understand how these mechanisms come together. Do all Tregs have all the aforementioned anti-inflammatory effects, or are there different subtypes of regulatory cells that have different roles – some that make TGF, some that shed TNF-R2, and so on? Does the mode of action depend on the situation, or are all down-modulating mechanisms employed at the same time? This is even more relevant when considering the potential therapeutic use of Tregs in human disease. After all, the discussed article does imply that Tregs could be helpful in any disease with pronounced local inflammation.

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Executive summary

Findings from the study

- Regulatory T cells (Tregs) shed soluble TNF receptor 2.
- Both murine and human Tregs produced large amounts of TNF-blocking soluble TNF receptor.
- Specificity was shown in TNF receptor 2-deficient cells.

Impact on understanding of regulatory T-cell function

- So far, cell contact-mediated effects and anti-inflammatory cytokines (TGFβ, IL-10) are known.
- The new findings now show local TNF blockade in addition to this.

Conclusion

- Tregs directly block local TNF, thus effecting anti-inflammatory effects.
- This may have distinct implications for inflammation and autoimmunity.

Future perspective

- Interplay between various effects of Tregs has to be analyzed in detail.
- Local TNF blockade may make Tregs attractive for dampening local inflammation.

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