

Prospects to translate the biology of IL-33 and ST2 during organ transplantation into therapeutics to treat graft-versus-host disease

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Comment on: Zhang J, Ramadan AM, Griesenauer B, *et al.* ST2 blockade reduces sST2-producing T cells while maintaining protective mST2-expressing T cells during graft-versus-host disease. *Sci Transl Med* 2015;7:308ra160.

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Interleukin (IL)-33 (also known as IL1F11, NF-HEV) is an IL-1 family cytokine constitutively expressed at endothelial and epithelial barrier surfaces where it is rapidly released from cells during tissue injury (1). IL-33 signals via a receptor complex of ST2 (serum stimulation 2; also known as IL1RL1, DER4, FIT-1, T1, IL33R) and IL-1 receptor accessory protein to initiate and amplify inflammatory responses through activation of NF κ B and MAP Kinase signalling pathways (1,2). Due to its rapid action following injury or infection, IL-33 is often referred to as an “alarmin” or endogenous danger signal.

The transmembrane form of ST2 (mST2) is expressed constitutively by Th2 cells, mast cells and group 2 innate lymphoid cells (2). In addition, IL-33 has been shown to activate a number of other cell types including endothelial cells, NK cells, NKT cells, Th1 cells, CD8+ T cells, regulatory T cells (Treg), dendritic cells, basophils and eosinophils. Thus, depending on the background tissue environment, IL-33 can drive a variety of inflammatory, repair and resolution responses (2). Multiple mechanisms exist to regulate IL-33. Most notably a soluble form of ST2 (sST2) is proposed to act as a decoy receptor, sequestering IL-33 and limiting its activity (2). Other mechanisms controlling IL-33 bioactivity include mechanism of release, N-terminal processing, oxidation and regulation of mST2 expression (2-5).

Over activation of IL-33/ST2 signalling has been implicated in various pathological settings including asthma,

related allergic diseases, respiratory viral pathology and chronic obstructive pulmonary disease (5-7). Thus, blockade of IL-33/ST2 interaction is an attractive target for disease modulation. Conversely, high levels of serum sST2 are a biomarker of poor prognosis in cardiovascular disease (8) and, more recently, as a marker for risk of therapy resistant graft-versus-host disease (GVHD) and death (9).

GVHD is a life threatening medical complication of allogeneic hematopoietic cell transplantation (allo-HCT) that is used for treatment of haematological malignancies and autoimmune diseases (10). Disease is characterised by severe damage to the gastrointestinal tract, liver, skin, and other mucosal tissues and typically requires intense immunosuppression involving steroids as standard of care. Latest estimates suggest GVHD still impacts up to 50% of HCT patients and accounts for 15% of post-transplantation mortality (10). Thus, there remains a large unmet medical need for more effective and safer treatments and requirement for improved understanding of the disease mechanisms.

In the paper of Zhang *et al.*, published in *Science Translational Medicine* in 2015 (11), the authors discover that treatment with an ST2-neutralising monoclonal antibody protects from development of GVHD in several mouse models suggesting that, in addition to being a disease marker, the IL-33/ST2 axis might play a pivotal role in GVHD pathogenesis. Others have also confirmed an important role of the IL33/ST2 axis in models of GVHD.

Studies in which the IL-33/ST2 axis has been blocked through administration of sST2-Fc show decreased GVHD development and reduced mortality (12). Furthermore, when exogenous IL-33 is added following allo-HCT, GVHD is exacerbated and mortality is increased. This study suggests that IL-33/ST2 signalling plays a pathogenic role and that neutralisation of IL-33 by sST2 is protective (12).

Although the beneficial effects of blockade of endogenous IL-33 activity via anti-ST2 and sST2-Fc have been consistent between investigators, studies involving administration of exogenous IL-33 have been considerably more variable. In contrast to Reichenbach *et al.* (12), Zhang and colleagues found no effect of recombinant IL-33 treatment on GVHD when given during the peri-transplant period (11). Furthermore, in a third study where exogenous IL-33 was dosed prior and subsequent to allo-HCT it resulted in a protective effect, evident by decreased GVHD development and decreased mortality (13). Similarly, in rodent heart transplant models, administration of IL-33 supported allograft survival (14). IL-33 is generally considered to be a locally acting cytokine, with multiple control mechanisms to limit its systemic exposure (1,3,15). Therefore, administration of large doses of recombinant, truncated IL-33 may not reproduce the activity, timing or location of the endogenous protein. Thus, more investigation of the complex biology underlying these observations is needed.

Since allograft rejection and risk of mortality in GVHD is associated with high serum sST2 (9), and the function of sST2 is to neutralise IL-33, Zhang *et al.* hypothesised that IL-33 may have a beneficial role in GVHD (11). They propose a mechanism whereby IL-33 stimulates and expands Th2 cells and ST2+FoxP3+Tregs, found to be protective in this model. In contrast, high levels of sST2 produced by intestinal stromal and alloreactive T-cells are suggested to contribute to disease progression by limiting the beneficial effects of IL-33 on ST2+FoxP3+Tregs. Anti-ST2 treatment is proposed to have its effect via specific blockade of sST2, releasing a pool of free IL-33 to act on Tregs and thereby reducing pro-inflammatory cytokines, GVHD severity and mortality (11).

In support of this hypothesis, anti-ST2-treated mice had an increased ratio of Th2 and Treg cells to Th1 and Th17 cells at day 10 post allo-HCT compared with the control antibody group. IL-33 is known to be a key activator of Th2 cells (2) and, consistent with other studies (16), Zhang *et al.* observed that wild type Tregs have a better suppressive

capacity than ST2^{-/-} Tregs, implying an important role for IL-33 in the function of these cells also. In addition, the authors showed that high levels of sST2 were produced by intestinal stromal cells and Th17 cells, which was reduced after anti-ST2 treatment. Likewise, the authors demonstrated that ST2-deficiency on either host cells or donor T cells had an overall protective effect, which they attributed to lack of sST2 in these cells. However, as ST2-null mice lack both sST2 and mST2, it is impossible to ascertain which of these most greatly contributed to the beneficial outcome.

Regulation of IL-33 has been widely studied and several approaches have been taken to disrupt the IL-33/ST2 axis (5,17-20). Of these approaches, highly selective blockade of ST2 through an anti-ST2 neutralising antibody is one of the most widely characterised methods. In Zhang *et al.* (11) the anti-ST2 neutralising antibody used is the mouse specific antibody CNT03914 developed by Centocor Biopharmaceuticals (now Janssen Biotech). This antibody specifically targets domain 1 of mST2L preventing the interaction of IL-33 and its binding site on ST2 [patent WO 2013165894, (21)]. Consistent with blockade of ST2, a significant increase in circulating IL-33 was observed following anti-ST2 mAb dosing. sST2 levels also increased after anti-ST2 treatment as expected due to the longer half-life of the sST2/antibody complex.

As the authors themselves highlight, their studies have a number of limitations, most importantly that they are using an antibody that does not differentiate between sST2 and mST2. The IL-33-binding region of ST2 is identical in both the soluble and membrane forms thus it seems unlikely that a specific mST2 blockade can be achieved. Significantly, the CNT03914 treatment regimen used by Zhang *et al.* (11) is comparable with doses of this antibody demonstrated to inhibit membrane ST2 signalling in other model systems [WO 2013165894, (22)]. Thus, while it is possible that selective inhibition of sST2 (but not mST2) suggested by Zhang *et al.* has occurred, more pharmacological evidence is needed to confirm this mechanism. The authors state in their introduction that they tested several doses and schedules to identify a treatment course that would inhibit sST2 without inhibiting mST2. Unfortunately, these data were not included in the manuscript to justify the proposed pharmacology. Demonstration of a bell shaped dose response would have been strong rationale to support the author's hypothesis. Therefore, given the findings of other studies (12) it seems plausible to suggest that the reduced severity of GVHD in this study may be due to blockade

of both mST2 and sST2. Perhaps the strongest evidence to support this hypothesis is that when similar models of GVHD were performed in ST2 knockout mice, lacking both mST2 and sST2, the disease is attenuated (11).

It is also noteworthy that inhibition of ST2 throughout the study via repeated antibody dosing produced similar efficacy to transient blockade during the peri-transplant period, suggesting that the benefit of ST2 blockade occurred very early, even before the elevation of sST2 levels was observed (by day 10 post allo-HCT). In this regard, it is interesting to note that IL-33 is an alarmin cytokine and has been shown to possess significant adjuvant activity (23). As IL-33 is released during chemotherapy (24) or UV radiation (25), it appears likely that cell damage by irradiation employed as part of the HCT conditioning protocol would release IL-33, which might play a role in overcoming tolerance to host tissues. Therefore, blockade of IL33/ST2 signalling, or perhaps even other alarmins, in the peri-transplant period might be beneficial. However, this remains to be further substantiated.

Anti-ST2 (RG6149/AMG282, Genentech; GSK3772847, GSK) and anti-IL-33 (ANB020, AnaptysBio) antibodies are currently in early clinical development as therapies for allergic and inflammatory diseases, with the aim of fully inhibiting IL-33 signalling. The conclusions of Zhang *et al*, if correct, highlight a potential risk that anti-ST2 molecules might in fact enhance IL-33 signalling through selective blockade of sST2. This could have significant implications for therapeutic targeting of the axis and it will be of interest to see how the efficacy and safety of anti-ST2 or anti-IL-33 neutralising antibodies compare as these molecules move forward in clinical trials.

In summary, recent findings strongly support a functional role for the IL-33/ST2 axis in animal models of GVHD and present an exciting opportunity for intervention in human disease. However, due to the complexity of the pharmacology, the required mechanism of action and dosing regimen for an effective and safe IL-33/ST2 therapeutic in GVHD remains to be resolved. Nevertheless, with various molecules for IL-33/ST2 axis modulation emerging in clinical development, there might be a future possibility to translate these findings from animal models of GVHD into therapeutics for human disease.

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Footnote

Conflicts of Interest: I.C.S, K.F.H and E.S.C contributed equally to plan and write this manuscript. The authors are employees of the AstraZeneca Group and have stock/stock options in AstraZeneca.

References

1. Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol* 2016;17:122-31.
2. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol* 2016;16:676-89.
3. Cohen ES, Scott IC, Majithiya JB, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun* 2015;6:8327.
4. Lefrançois E, Cayrol C. Mechanisms of IL-33 processing and secretion: differences and similarities between IL-1 family members. *Eur Cytokine Netw* 2012;23:120-7.
5. Kearley J, Silver JS, Sanden C, et al. Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. *Immunity* 2015;42:566-79.
6. Kaur D, Gomez E, Doe C, et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. *Allergy* 2015;70:556-67.
7. Donovan C, Bourke JE, Vlahos R. Targeting the IL-33/IL-13 Axis for Respiratory Viral Infections. *Trends Pharmacol Sci* 2016;37:252-61.
8. Salvagno GL, Pavan C. Prognostic biomarkers in acute coronary syndrome. *Ann Transl Med* 2016;4:258.
9. Vander Lugt MT, Braun TM, Hanash S, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. *N Engl J Med* 2013;369:529-39.
10. Qian L, Wu Z, Shen J. Advances in the treatment of acute graft-versus-host disease. *J Cell Mol Med* 2013;17:966-75.
11. Zhang J, Ramadan AM, Griesenauer B, et al. ST2 blockade reduces sST2-producing T cells while maintaining protective mST2-expressing T cells during graft-versus-host disease. *Sci Transl Med* 2015;7:308ra160.
12. Reichenbach DK, Schwarze V, Matta BM, et al. The IL-33/ST2 axis augments effector T-cell responses during acute GVHD. *Blood* 2015;125:3183-92.
13. Matta BM, Reichenbach DK, Zhang X, et al. Peri-alloHCT IL-33 administration expands recipient T-regulatory cells that protect mice against acute GVHD. *Blood* 2016;128:427-39.
14. Turnquist HR, Zhao Z, Rosborough BR, et al. IL-33

- expands suppressive CD11b⁺ Gr-1(int) and regulatory T cells, including ST2L⁺ Foxp3⁺ cells, and mediates regulatory T cell-dependent promotion of cardiac allograft survival. *J Immunol* 2011;187:4598-610.
15. Palmer G, Lipsky BP, Smithgall MD, et al. The IL-1 receptor accessory protein (AcP) is required for IL-33 signaling and soluble AcP enhances the ability of soluble ST2 to inhibit IL-33. *Cytokine* 2008;42:358-64.
 16. Schiering C, Krausgruber T, Chomka A, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* 2014;513:564-8.
 17. Fursov N, Johnston E, Duffy K, et al. Generation and characterization of rat anti-mouse ST2L monoclonal antibodies. *Hybridoma (Larchmt)* 2011;30:153-62.
 18. Percival-Alwyn JL, England E, Kemp B, et al. Generation of potent mouse monoclonal antibodies to self-proteins using T-cell epitope "tags". *MAbs* 2015;7:129-37.
 19. Liu X, Li M, Wu Y, et al. Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma. *Biochem Biophys Res Commun* 2009;386:181-5.
 20. Lipsky BP, Toy DY, Swart DA, et al. Deletion of the ST2 proximal promoter disrupts fibroblast-specific expression but does not reduce the amount of soluble ST2 in circulation. *Eur J Immunol* 2012;42:1863-9.
 21. Liu X, Hammel M, He Y, et al. Structural insights into the interaction of IL-33 with its receptors. *Proc Natl Acad Sci U S A* 2013;110:14918-23.
 22. Ramaprakash H, Shibata T, Duffy KE, et al. Targeting ST2L potentiates CpG-mediated therapeutic effects in a chronic fungal asthma model. *Am J Pathol* 2011;179:104-15.
 23. Mehraj V, Ponte R, Routy JP. The Dynamic Role of the IL-33/ST2 Axis in Chronic Viral-infections: Alarming and Adjuvanting the Immune Response. *EBioMedicine* 2016;9:37-44.
 24. Guabiraba R, Besnard AG, Menezes GB, et al. IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice. *Mucosal Immunol* 2014;7:1079-93.
 25. Byrne SN, Beaugie C, O'Sullivan C, et al. The immune-modulating cytokine and endogenous Alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation. *Am J Pathol* 2011;179:211-22.

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