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Response to Thum et al.

Thum et al. conclude that microRNA-21 (miR-21) is essential for cardiac hypertrophy and fibrosis in response to pressure overload (1). They also claim that our failure to observe a blockade to these processes in mice treated with an 8-mer locked nucleic acid–modified oligonucleotide against miR-21 (called Anti-21) (2) is due to the ineffectiveness of such inhibitors. We wish to point out several caveats to their study regarding the role of miR-21 in cardiac hypertrophy and their conclusions regarding the efficacy of the Anti-21 oligonucleotide.

First, we find that Anti-21 inhibits miR-21 with a half-maximal inhibitory concentration of 0.9 nM, indicating the efficacy of Anti-21. Second, Thum et al. do not state the method they used to measure miR-21 inhibition, though we assume it to be quantitative PCR (qPCR). In our hands, qPCR alone is unreliable for measuring miRNA inhibition, especially for 8-mer inhibitors, since they may be displaced during qPCR and thereby give an underrepresentation of miRNA inhibition. To demonstrate functional inhibition of a miRNA, it is important to show data from multiple assays, such as small RNA Northern blots, luciferase reporter assays, and target derepression, as shown in our study (2). Such data are lacking in the Thum et al. rebuttal, which makes comparison of the different chemistries impossible.

Thum et al. also state that we measured miR-21 inhibition on day 2 after dosing with Anti-21, when in fact we measured inhibition 3 weeks after dosing. At this time point, we observed inhibition of miR-21 in pressure-overloaded hearts at a level significantly below that of control mice. Thus, their approach for inhibition and/or measurement of miR-21 by their 8-mer inhibitors differs markedly from ours, since we observed robust miR-21 inhibition 3 weeks after injection, as demonstrated by multiple readouts (2). Using mismatched oligonucleotide controls is also important for interpreting miRNA inhibition studies in vivo, as described in our paper, rather than using PBS as a control, as reported by Thum et al. (1).

Finally, Thum et al. postulate that constitutive genetic deletion of miR-21 in mice may not reveal the functions of miR-21 in cardiac disease because of compensatory events that mask such functions. If such compensation occurs, it must be specific for the cardiac functions of miR-21, since miR-21 null mice are resistant to lung tumorigenesis (3), consistent with the documented pro-oncogenic functions of miR-21. To further address the possibility of genetic compensation, we have deleted a floxed miR-21 allele immediately prior to thoracic aortic constriction in mice using a ubiquitously expressed tamoxifen-regulated Cre transgene. These animals show cardiac hypertrophy and fibrosis comparable to that of their Cre-negative littermates. Genetic compensation therefore cannot account for the normal pathological cardiac remodeling response in miR-21 null mice. Moreover, functions of other miRNAs in heart disease can be revealed by genetic deletion in mice, as shown for miR-208 (4). Thus, while 22-mer oligonucleotide inhibitors against miR-21 are efficacious in inhibi-
Immunological tolerance is achieved through recessive and dominant mechanisms. In recessive tolerance the fate of self-reactive T cells is controlled in a cell intrinsic manner such that they undergo cell death or become anergic after exposure to self-antigen. In contrast, dominant tolerance is controlled in a cell intrinsic manner such that they undergo cell death or become anergic after exposure to self-antigen. In contrast, dominant tolerance is achieved through recessive and dominant mechanisms. Immunological tolerance is achieved through recessive and dominant mechanisms. In recessive tolerance the fate of self-reactive T cells is controlled in a cell intrinsic manner such that they undergo cell death or become anergic after exposure to self-antigen. In contrast, dominant tolerance is controlled in a cell intrinsic manner such that they undergo cell death or become anergic after exposure to self-antigen. In contrast, dominant tolerance is achieved through recessive and dominant mechanisms.

Expression of TNFRSF25 on conventional T cells and Tregs

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The authors investigated the expression of TNFRSF25 on conventional CD4+ T cells and Tregs, and our findings are remarkably different to those reported by Schreiber et al. We used two approaches to investigate expression of TNFRSF25 on T cell subsets (Figure 1). First, we showed that a different anti-TNFRSF25 antibody stains conventional CD4+ T cells and Tregs obtained from Foxp3-GFP knockin mice (S) with similar intensity (Figure 1, A–C). Second, we demonstrated that soluble recombinant TL1A (sTL1A), the ligand for TNFRSF25, binds equally well to conventional CD4+ T cells and Tregs (Figure 1D). We therefore conclude that Tregs and conventional CD4+ T cells express similar levels of functional TNFRSF25.

We agree with the findings of Schreiber et al. that TNFRSF25 triggering can expand Tregs. In fact, we recently showed that transgenic mice that constitutively express TL1A have increased numbers of Tregs (6). However, we also observed increased activation of conventional CD4+ T cells, elevated levels of IL-13 and IL-17, and small intestinal immune pathology that manifests as goblet cell and paneth cell hyperplasia (6). A similar phenotype was reported by Siegel and colleagues using mice that express the variant lacking the fourth cysteine-rich repeat (Figure 1E). We believe that caution should be applied in interpreting the findings obtained with anti-TNFRSF25 antibodies, as these may differ in their capacity to stimulate Tregs and effector T cells.

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