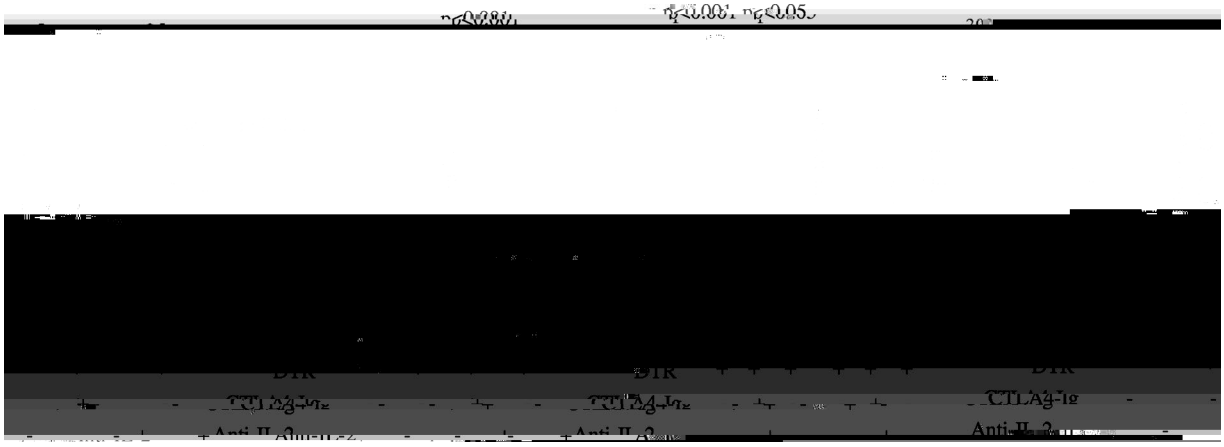
in the absence of Tregs through substitution with CTLA-4 or neutralization of IL-2.

$\bar{r}$ 3^{D} mice on the C57BL/6 background[6] were analyzed at 8-12 weeks of age. Doses of 0, 2.5, 5.0, 7.5, 10, or 20 g/kg diphtheria toxin (DT; Sigma-Aldrich) were administrated intraperitoneally to $\bar{r}$ 3^{D} mice on days 0, 1, 3, and 6. BrdU (1 mg; Becton Dickinson) was administered daily

are consistent with the role Tregs play in suppressing the magnitude of immune responses; however, they are not sufficient to explain the Th1 bias caused by Tregs, because both Th1 and Th2 were affected equally.

expression of activated caspase 3, which sensitively marks cells in the early stages of apoptosis.[30] There was no significant change in the proportion of Th1 cells expressing activated caspase 3 in wild-type versus DT-treated *F 3D* mice (Figure 4C). However, in wild-type mice the proportion of Th2 cells staining positive for activated caspase 3 was 2-fold higher than that of Th1 cells, and in mice depleted of Tregs the proportion of apoptotic Th2 cells dropped to below that of Th1



DT-treated *Foxp3*^{DTR} mice were treated with CTLA4-Ig on day 5 or anti–IL-2 Ab on days 0-8. (A) Frequency of IL-4 and IFN producers among CD4 CD44^{hi}Foxp3 T cells and (B) Th2/Th1 ratio in wild-type mice, DT-treated *Foxp3*^{DTR} mice, DT-, and CTLA4-Ig–treated *Foxp3*^{DTR} mice and DT- and anti–IL-2-treated *Foxp3*^{DTR} mice, after 9 days of treatment with DT. All data are from the spleen (n 3-4/group). (C) The effect of CTLA4-Ig or anti–IL-2 Ab on apoptosis in Th1 and Th2 subsets in the absence of Tregs, measured by calculating the percentage change in activated caspase 3 expression in DT-treated mice (diamonds), DT-treated CTLA4-Ig–treated mice (squares) or DT-treated anti–IL-2-treated mice (circles), compared with wild-type mice (dashed line at 100%). Each symbol represents an individual mouse, *P* values represent significant differences in CTLA4-Ig– or anti–IL-2 Ab-treated mice versus Treg depletion alone. Error bars indicate SD.

(supplemental Figure 7B). Because day 0 treatment may interfere with initial priming[34] and day 7 treatment had reduced effectiveness, treatment on day 5 was analyzed in greater detail. CTLA4-Ig treatment on day 5 of DT treatment had no effect on the magnitude of the Th1 response at day 9 but sharply limited Th2 expansion (Figure 5A). The net effect of CTLA4-Ig was to return the Th2/Th1 ratio from 1:1 in Treg-deficient mice to the "wild-type" ratio of 5:1 (Figure 5B). Notably, as observed with the Treg-reconstitution experiment, injection of CTLA4-Ig on day 5 kinetically separated the "magnitude control" and "Th bias" regulatory effects. Further supporting the parsimonious conclusion that Treg suppression of Th2 responses operates through CTLA-4 was the effect of CTLA4-Ig on apoptosis. Compared with the Treg-depleted state, CTLA4-Ig treatment in Treg-depleted mice had no effect on Th1 apoptosis but significantly increased Th2 apoptosis to levels approaching that of Treg-sufficient mice (Figure 5C).

The involvement of CTLA-4 in enhanced Th2 regulation may be explained by the effect of Treg CTLA-4 expression on the tonic dampening of CD80/CD86 expression on APCs,[13] because anti-CD28 blockade sharply reduces the Th2 response through a disproportionate and irreplaceable role for IL-2 on Th2 differentiation.[35] This model would imply that IL-2 neutralization during Treg depletion would limit the Th2 response, similar to CTLA4-Ig provision. This was tested through the treatment of DT-treated Foxp3DTR mice with neutralizing anti–IL-2 Ab. IL-2 neutralization from day 0 of Treg depletion was unable to limit the size of the day 9 Th1 response but was, by contrast, highly effective in limiting the Th2 response (Figure 5A). As with CTLA4-Ig, this restored the Th2/Th1 ratio back to the Th1 bias observed in wild-type mice (Figure 5B) and was associated with a restoration of wild-type levels of Th2 apoptosis (Figure 5C).

These results show an asymmetric regulation of Th cells by Tregs. Tregs not only have a greater in vivo suppressive effect on the Th2 response than the Th1 response but also have a more complex relationship. These data indicate that suppression of Th1 responses by Tregs operates along simple principles, with frequency-proportional suppression. By contrast, Th2 suppression by Tregs is disproportionate to frequency, with even moderate Treg numbers capable of efficiently quenching a Th2 response. The model generated here suggests that the simple proportional component of Treg suppression is mediated by a reduction in effector T-cell proliferation, whereas the heightened suppression of Th2 responses is associated with the additional effect of enhanced apoptosis in the presence of Tregs (Figure 6). Because the Th1 bias and Th2 apoptotic effect generated by Tregs can be replicated through CTLA4-Ig (Figure 5) and loss of CTLA-4 on Tregs results in Th2-associated autoimmune disease,[13] CTLA-4 expression on Tregs is a probable mediator of this asymmetric behavior. The involvement of CTLA-4 in enhanced Th2 regulation may be explained by the effect of Treg CTLA-4 expression on the tonic dampening of CD80/CD86 expression on APCs,[13] because anti-CD28 blockade sharply reduces the Th2 response through a disproportionate and irreplaceable role for IL-2 on Th2 differentiation,[35] a trait which is shared with Tregs.[36] This model is also consistent with the reversal of hyper IL-4 production in Treg-deficient *F* 3 mice crossed onto the CD28-deficient or IL-2–deficient backgrounds.[34,37] In addition to the indirect effect of Tregs on IL-2 levels, by CTLA-4–mediated reductions in T-cell priming, Tregs have been shown to directly reduce IL-2 availability by enhanced cytokine consumption[38] and trans-reduction in IL-2 signaling capacity.[39] Pandiyan et al[38] demonstrated that the IL-2 consumption mechanism increases T-cell apoptosis, the results shown here suggest that this effect is primarily directed toward the Th2 lineage.

These data build on recently emerging evidence that Tregs use distinct molecular mechanisms to control effector T-cell subsets, with T-bet-, IRF4-, Stat3-, and Bcl6-deficient Tregs exhibiting poor control over Th1, Th2, Th17, and Tfh subsets, respectively, while leaving the remainder subsets unchanged.[9-11,14] Various cellular mechanisms have been invoked to explain these subset-specific activities. For example, T-bet is up-regulated on a subset of Tregs during Th1 responses and is probably driven by Th1-mediated IFN production because up-regulation occurs by IFN R and Stat6 signaling.[9] Unlike

T-bet, Stat3 is expressed by most Tregs. However, activation of Stat3 depends on the appropriate cytokine signal, such as the Th17 cytokine IL-6, making it probable that enhanced Th17-regulation by Tregs is induced by a Th17 microenvironment.[10]

CTLA-4 expression on Tregs releases autoreactive Th2 cells. Because *C A4* is a candidate susceptibility gene for rheumatoid arthritis, type 1 diabetes, systemic lupus erythematosus, celiac disease, Graves disease, and Hashimoto thyroiditis,[45-48] partial CTLA-4 deficiency on Tregs may be a common contributor to multiple diseases. These studies suggest that such a defect may translate to disproportionate autoreactive Th2 activation and raise the potential that CTLA4-Ig treatment may prove a rapid and efficacious treatment.

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Foxp3$^+$ regulatory T cells exert asymmetric control over murine helper responses by inducing Th2 cell apoptosis

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