donors, Treg cell–depleted CD4+CD45RO+ cells induced high amounts of CCL3 (mean ± SEM 82.7 ± 14.7 pg/ml), CCL17 (81.9 ± 34.9 ng/ml), CCL19 (473.5 ± 79.4 pg/ml), and CCL22 (116 ± 27.4 ng/ml), Treg cells significantly inhibited the secretion of all of these chemokines (mean ± SEM 15.7 ± 4 pg/ml, 37.9 ± 13.4 ng/ml, and 70.7 ± 21.4 ng/ml for CCL3, CCL17, and CCL22, respectively, with CCL19 not induced at all). Thus, our results suggest that, in addition to suppressing T cells, Treg cells can diminish the inflammation and immune response by inhibiting the secretion of DC chemokines implicated in the migration of immune cells.

We then determined the effects of specific molecules on Treg cells in regulating DC chemokine secretion. We examined the effects of blocking/neutralizing antibodies to CTLA-4, transforming growth factor β (TGFβ), IL-10, and lymphocyte activation gene 3 (LAG-3), all of which have been previously reported to contribute to Treg cell activity in certain contexts (2,4,6). DCs were cocultured with Treg cells for 72 hours in the presence of individual blocking/neutralizing antibodies, after which secretion of chemokines was analyzed. Notably, CTLA-4, TGFβ, and LAG-3 blocking/neutralization partially (though nonsignificantly) reversed the Treg cell–mediated suppressive effect on the secretion of DC-derived CCL17 and CCL22 (Figure 1B); no single blocking/neutralizing antibody completely reversed the Treg cell effects. IL-10 neutralization had minimal effect on CCL17 and CCL22 secretion. None of the antibodies affected the secretion of CCL18 (Figure 1B), or of CCL3 and CCL19 (data not shown). Together, the data indicate that multiple mechanisms contribute to the modulation of DC chemokine secretion by Treg cells.

Several non–mutually exclusive mechanisms have been proposed to account for the maintenance of immune tolerance by Treg cells (2). Thus, Treg cells modulate the maturation, activation, and functions of both human and murine DCs (7–11). Recent reports suggest that CTLA-4 plays a major role in Treg cell–mediated immunosuppression in mice (10–12). We found that, in addition to CTLA-4, the modulation of DC chemokine secretion by human Treg cells may be partially regulated by TGFβ, LAG-3, and (to a lesser degree) IL-10. It is possible that each molecule associated with Treg cells has a distinct role in modulating various aspects of DC biology, such as maturation and expression of costimulatory molecules, secretion of cytokines and chemokines, expression of antiinflammatory molecules, and T cell stimulatory functions. Thus, CTLA-4 has a direct role in regulating DC stimulatory capacity (10,11), while the remaining Treg cell accessory molecules regulate other aspects of DC-mediated immune responses, such as secretion of cytokines and chemokines. The cumulative effect of all of these accessory molecules of Treg cells might impart potent tolerogenic properties to DCs. It remains to be determined, however, whether CTLA-4, TGFβ, IL-10, and LAG-3 represent components of the same pathway or of multiple pathways that converge at a common point.

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1. Kolar P, Knieke K, Hegel JK, Quandt D, Burmester GR, Hoff H, et al. CTLA-4 (CD152) controls homeostasis and suppressive capacity of regulatory T cells in mice. Arthritis Rheum 2009;60:123–32.
2. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133:775–87.
3. Valencia X, Lipsky PE. CD4+CD25+FoxP3+ regulatory T cells in autoimmune diseases. Nat Clin Pract Rheumatol 2007;3:619–26.
4. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol 2008;9:239–44.
5. Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. Annu Rev Immunol 2007;25:787–820.
6. Liang B, Workman C, Lee J, Chew C, Dale BM, Colonna L, et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. J Immunol 2008;180:5916–26.
7. Tang Q, Adams JY, Tooley AJ, Bi M, Fife BT, Serra P, et al. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. Nat Immunol 2006;7:83–92.
8. Houot R, Perrot I, Garcia E, Durand I, Lebecque S. Human CD4+CD25high regulatory T cells modulate myeloid but not plasmacytoid dendritic cells activation. J Immunol 2006;176:5293–8.
9. Bayry J, Triebel F, Kaveri SV, Tough DF. Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with CD4+CD25+ regulatory T cells. J Immunol 2007;178:4184–93.
10. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proc Natl Acad Sci U S A 2008;105:10113–8.
11. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322:271–5.
12. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, et al. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. J Exp Med 2009;206:421–34.

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Reply

To the Editor:

Naturally arising CD4+CD25+ Treg cells appear to be central control elements within immunoregulation. Treg cells can restrict most types of immune responses, ranging from

transplant rejection to tumor rejection to autoimmunity (1–3). The interesting findings of Navarrete and colleagues, showing the suppression of DC-secreted chemokines CCL3, CCL19, CCL17, and CCL22 by Treg cells, present a new aspect of Treg cell–mediated immunoregulation. Treg cell–induced inhibition of chemokine secretion is of special interest, since it would prevent recruitment of effector T cells to the site of inflammation, thus limiting unwanted local proinflammatory processes. Up to now, Treg cells have been thought to interfere directly with the activation of naive T cells, but recently, there has been evidence to suggest that Treg cell–mediated effects on DCs ultimately lead to suppression of T cell responses in vitro (4–6). It has been demonstrated that Treg cell–mediated effects on DCs rely on CTLA-4 engagement with CD80 and CD86 (7). However, convincing proof that CTLA-4 expression by Treg cells mediates repression of CD80 and CD86 on DCs as a suppressor-relevant action of Treg cells in vivo has not yet been presented.

Since Treg cells interfere with various activities of the immune system, it is likely that their mode of action involves down-regulation of different proinflammatory elements of an immune response. Consistent with the findings of Navarrete et al, this implies that Treg cells do not exert their inhibitory function on only one inflammatory target but instead might use more than one suppressor mechanism. Alternatively, a heterogeneous compartment of Treg cells could comprise subpopulations that fulfill different tasks. As we reported in our article, data supporting the existence of functionally distinct Treg cells show a dichotomy of Treg cells with regard to constitutive CTLA-4 expression at the cell surface. In addition, Treg cells that have been differentiated in the presence or absence of CTLA-4 seem to be similarly potent in vitro. CTLA-4–deficient Treg cells use a TGFβ-dependent mechanism for suppression (8). Interestingly, despite the presence of "potent" Treg cells in CTLA-4–deficient mice, their lifespan can be extended by adoptive transfer of CTLA-4–competent Treg cells, which illustrates their importance in vivo.

Navarrete and colleagues report that the application of blocking antibodies against TGFβ, IL-10, CTLA-4, or LAG-3 did not completely reverse the observed Treg cell–mediated suppression of secretion of chemokines by DCs. They conclude that Treg cells influence DCs via multiple mechanisms. The concept is appealing, but the experimental set-up has to be extended. It would be interesting to see whether administration of a combination of the blocking antibodies would reverse the effect of Treg cells on DCs. Furthermore, it is unusual to use the complete antibody to block CTLA-4; such an application often leads to equivocal results. Aggregation of the antibodies to each other or binding to the various Fc receptors on the DCs might actually crosslink CTLA-4, inducing CTLA-4 signaling. In our report, we found that Treg cells in particular are very sensitive to CTLA-4 crosslinking, since at low concentrations of anti–CTLA-4, Treg cells (but not CD4 effector T cells) do respond to the crosslinking. In addition, as reported by Navarrete et al, no T cell receptor (TCR) stimulus was present in the cocultures of DCs/Treg cells or DCs/Treg cells/CD4+CD45RO+ cells. This would mean that Treg cells "calm down" DCs in the absence of antigenic challenge. So far, CTLA-4 has not been shown to signal without a concomitant TCR signal. Thus, we are not surprised to see that blocking CTLA-4 was not able to result in a reversion of the Treg cell–induced suppression of DC-secreted chemokines in an antigen-free system.

The roles of CTLA-4 in Treg cells are varied, but all of them are conducive to the fulfillment of the Treg cells' suppressive tasks. CTLA-4 polymorphisms provide a good idea of this cytokine's profound action on autoimmune diseases (9). It is likely that altered CTLA-4 molecules exert their effects on CD4+CD25+ Treg cells in particular. Importantly, in addition to their suppressive capacity, Treg cells require abilities such as low or no proliferation and enhanced survival in an environment of aggressive inflammation. We clearly showed that CTLA-4 confers these basic properties to Treg cells. Thus, although indirectly (not via the suppressor function of Treg cells), CTLA-4 controls Treg cells prior to suppression. In addition, we (in our report) and others have provided in vivo data showing that Treg cells that are competent to express CTLA-4 are superior to CTLA-4–deficient Treg cells, thus implementing the suppressor mechanism (7,10).

The challenging data presented by Navarrete and colleagues certainly open the question of the relevance of Treg cells in vivo. The importance of CTLA-4 expression on Treg cells is clearly shown in the severe lymphoproliferative disorder of CTLA-4–knockout mice, which closely resembles autoimmune diseases such as disseminated encephalomyelitis, Crohn's disease, and ulcerative colitis (1,11,12). The ameliorative effect of CTLA-4–competent Treg cells on the symptoms and lifespan of CTLA-4–deficient mice nicely demonstrates the powerful immunoregulatory effect of CTLA-4+ Treg cells, and this model has an advantage over other models that use monospecific, nonphysiologic T cell repertoires (13). It will be interesting to see whether CTLA-4+ Treg cell treatment causes reduced chemokine expression of DCs in vivo. Basic studies on the role of CTLA-4 in Treg cells in a model with a complete T cell repertoire are important to explain phenomena that are observed with current CTLA-4–based therapies. For example, the therapeutic agent CTLA-4Ig not only abrogates the activation of effector T cells but could also affect the function of Treg cells (13).

Many studies were needed to provide convincing data demonstrating the in vivo relevance of the suppressive capacity of CTLA-4 on Treg cells. We are now looking forward to learning more about the regulation of chemokines by Treg cells in vivo, regardless of whether this turns out to be CTLA-4 dependent.

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1. Gartner D, Hoff H, Gimsa U, Burmester GR, Brunner-Weinzierl MC. CD25 regulatory T cells determine secondary but not primary remission in EAE: impact on long-term disease progression. J Neuroimmunol 2006;172:73–84.
2. Sutmuller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of

CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med 2001;194:823–32.
3. Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nat Rev Immunol 2003;3:199–210.
4. Bayry J, Triebel F, Kaveri SV, Tough DF. Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with CD4+CD25+ regulatory T cells. J Immunol 2007;178:4184–93.
5. Houot R, Perrot I, Garcia E, Durand I, Lebecque S. Human CD4+CD25high regulatory T cells modulate myeloid but not plasmacytoid dendritic cells activation. J Immunol 2006;176:5293–8.
6. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proc Natl Acad Sci U S A 2008;105:10113–8.
7. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322:271–5.
8. Tang Q, Boden EK, Henriksen KJ, Bour-Jordan H, Bi M, Bluestone JA. Distinct roles of CTLA-4 and TGF-β in CD4+CD25+ regulatory T cell function. Eur J Immunol 2004;34:2996–3005.
9. Brunner-Weinzierl MC, Hoff H, Burmester GR. Multiple functions for CD28 and cytotoxic T lymphocyte antigen-4 during different phases of T cell responses: implications for arthritis and autoimmune diseases. Arthritis Res Ther 2004;6:45–54.
10. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, et al. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. J Exp Med 2009;206:421–34.
11. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 1995;3:541–7.
12. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 1995;270:985–8.
13. Verhagen J, Gabrysova L, Minaee S, Sabatos CA, Anderson G, Sharpe AH, et al. Enhanced selection of FoxP3+ T-regulatory cells protects CTLA-4-deficient mice from CNS autoimmune disease. Proc Natl Acad Sci U S A 2009;106:3306–11.

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A meta-analysis of the effect size of rheumatoid arthritis on left ventricular mass: comment on the article by Rudominer et al

To the Editor:

We appreciate the work of Rudominer et al, who recently published a report describing the association of rheumatoid arthritis (RA) with increased left ventricular mass (1). The findings of Rudominer and colleagues are consistent with the findings of our own previous study of RA patients, which is cited by the authors (2). Patients with RA have both structural and functional left ventricular involvement. In these patients, we have previously demonstrated that diastolic dysfunction (detected as impaired relaxation abnormalities during left ventricular filling) is directly correlated with structural changes in the left ventricle, specifically, changes involving left ventricular mass, interventricular septal thickness, and left ventricular posterior wall thickness (2). In RA patients, these all seem to be features of the systemic disease process, and diastolic dysfunction seems to be a consequence of left ventricular structural changes. However, it is probable that these findings only partially explain the cardiovascular morbidity in RA.

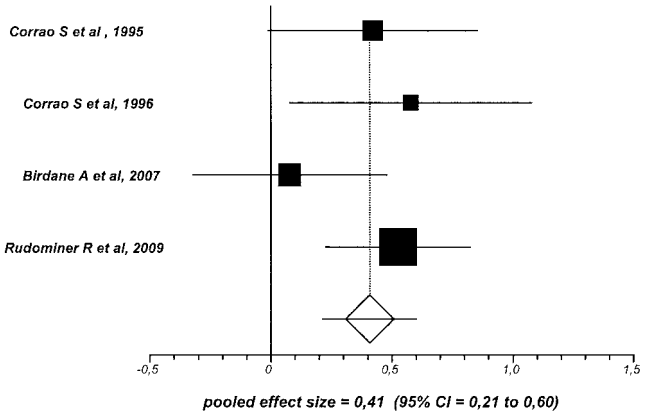

Figure 1. Forest plot of the effect size in the meta-analysis, using a fixed-effects model. Squares represent the effect sizes in the previous studies. The diamond and dotted line represent the pooled effect size in the meta-analysis. Horizontal lines represent the 95% confidence interval (95% CI). The Glass statistic (mean difference standardized by pooled standard deviation) and 95% CI are shown as the pooled mean effect size estimate. For the overall fixed-effects model, $P < 0.0001$.

Indeed, in a study of RA patients (with no exclusion criteria), we observed a peculiar cardiac picture among patients who had no symptoms of cardiac disease (3). The population of RA patients in that study seemed to share 3 characteristics: minimal posterior pericardial effusion, alteration of the aortic root (detected as an increased prevalence of Valsalva sinus aneurysms), and both valvular and valvular cord thickening. However, even though left ventricular mass was greater overall in the RA group, the difference was not statistically significant in patients versus controls. We believe that heart involvement in RA results from the disease's systemic inflammatory process, which explains the high rates of cardiac mortality in RA patients. Thus, we completely agree with the conclusions discussed by Rudominer et al and think that the collective scientific community has achieved an important goal in demonstrating that different populations with RA (both Mediterranean [2,3] and North American [1]) have the same clinical cardiac features (i.e., a higher left ventricular mass index and abnormalities consistent with impaired left ventricle relaxation).

We undertook a meta-analysis of previously published reports (1–4) to summarize the effect size of RA on left ventricular mass index in patients compared with healthy controls. We searched for data pertaining to left ventricular mass index, which had been determined using echocardiography. Unfortunately, in the studies by Arslan et al (5) and Gonzalez-Juanatey et al (6), left ventricular mass index was not calculated; therefore, we excluded these studies from our analysis. We computed the modified Glass statistic using pooled sample standard deviation and Cochran's Q statistic. The pooled mean effect size estimate was calculated according to methods described by Hedges and Olkin (7). Figure 1 shows the pooled estimation of effect size using a fixed-effects model