Immunity
Letters

Does Def6 Deficiency Cause Autoimmunity?

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Def6, also termed SLAT (Tanaka et al., 2003) or IBP (Gupta et al., 2003), is a Cdc42-Rac1-specific guanine nucleotide exchange factor (GEF) expressed predominantly in T cells. *Def6*$^{-/-}$ mice on a C57BL/6 background are resistant to T helper 1 (Th1) and Th2 cell-mediated inflammation (Becart et al., 2007). This resistance reflects the essential role of Def6 in promoting T cell receptor (TCR)-induced Ca^{2+} release from endoplasmic reticulum stores and, hence, subsequent steps in the Ca^{2+} signaling pathway, including activation of the transcription factor NFAT (Becart et al., 2007). More recently, we demonstrated that Def6 is activated by Lck-dependent phosphorylation of two defined tyrosine residues, an event that targets Def6 to the plasma membrane and, more specifically, to the immunological synapse, in antigen-stimulated T cells (Becart et al., 2008). Furthermore, the Ca^{2+} signaling function of Def6 leading to Th1 and Th2 cell differentiation depends on its GEF activity because a dominant-negative Cdc42 mutant blocked it and, conversely, a constitutively active Cdc42 mutant rescued the impaired NFAT activation and Th1-Th2 cell differentiation of *Def6*$^{-/-}$ T cells (Becart et al., 2008).

A recent paper (Chen et al., 2008) addressed the role of Def6 in regulating Th17 cell differentiation and function. The authors reported that *Def6*$^{-/-}$ (termed by the authors *Def6*$^{trap/trap}$) BALB/c mice crossed to DO11.10 transgenic (Tg) mice expressing an MHC class II-restricted ovalbumin-specific TCR spontaneously and rapidly develop rheumatoid-arthritis-like joint disease and large vessel vasculitis associated with enhanced IL-17 and IL-21 production. Of note, this was an early-onset disease because the mice started to show signs of joint disease at ~7 weeks of age and started to die at ~3 months, with > 90% of the mice dead by 12 months. The authors went on to show that Def6 sequesters, and negatively regulates, IRF-4 (Chen et al., 2008), a transcription factor recently found to control Th17 cell differentiation (Brustle et al., 2007). An earlier report by the same group showed that *Def6*$^{-/-}$ mice on a mixed 129 × C57BL/6 background spontaneously develop a systemic autoimmune disorder characterized by the accumulation of effector and memory T cells and IgG^+ B cells, profound hypergammaglobulinemia, and autoantibody production (Fanzo et al., 2006).

We question the biological and pathological relevance of the inflammatory and autoimmune manifestations that Chen et al. observed in *Def6*$^{-/-}$ mice (Chen et al., 2008; Fanzo et al., 2006). In the earlier study (Fanzo et al., 2006), the mixed genetic background of the mice could contribute to disease development, because epistatic interactions between the non-autoimmune-prone strains 129 and C57BL/6, used for generating gene-targeted animals, can induce a lupus-like disease (Bygrave et al., 2004). In that regard, the interchangeable use of *Def6*$^{-/-}$ mice on either a BALB/c or a C57BL/6 background in the latter study (Chen et al., 2008) is also a potential concern. In this regard, we have followed *Def6*$^{-/-}$ mice on a C57BL/6 background (Becart et al., 2007) up to an age of ~10 months and have not observed any signs of disease. We have also backcrossed *Def6*$^{-/-}$ mice to BALB/c mice for more than 10 generations and did not observe any signs of disease or sudden death up to an age of ~10 months (unpublished data).

We are also concerned with the recent study because the enhanced Th17 cell responses in young animals were only observed when the *Def6*$^{-/-}$ BALB/c mice were crossed to DO11.10 TCR-Tg mice (Chen et al., 2008). Indeed, the authors state that "in contrast to *Def6*$^{trap/trap}$ DO11.10 mice, young *Def6*$^{trap/trap}$ BALB/c mice do not develop obvious signs of arthritis." Thus, the reported inflammation (Chen et al., 2008) may be, in our opinion, an artifact of crossing the *Def6*$^{-/-}$ mice with TCR-Tg mice, raising two important, but unanswered, questions. First, what is the relationship between the antigen specificity of the TCR-Tg T cells (ovalbumin) and the putative self-antigen(s) that elicits the autoimmune-like inflammatory response? And, second, is the observed disease a unique reflection of the DO11.10 transgene expression or a phenotype that would be imposed by a TCR transgene of another antigen specificity? In this regard, we have followed *Def6*$^{-/-}$ C57BL/6 mice crossed to OT-II TCR-Tg mice (expressing the same antigen specificity as the DO11.10 mice used by Chen et al. (2008), i.e., ovalbumin, but on a different genetic background) up to an age of ~10 months, and have not observed any signs of disease or premature death. The mice appear healthy and do not display any signs of "impaired mobility," which would be expected if they were suffering from arthritis; similarly, *Def6*$^{-/-}$ C57BL/6 or BALB/c mice, which are not crossed to TCR-Tg mice, are also healthy (unpublished data).

More recently, we analyzed the development of myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) in *Def6*$^{-/-}$ C57BL/6 mice. In contrast to the claim by Chen et al. (2008) that *Def6*$^{-/-}$ mice express enhanced amounts of IL-17 (and IL-21), we consistently observe the opposite results. Thus, MOG-primed *Def6*$^{-/-}$ mice are resistant to EAE development and display highly reduced amounts of IL-17 in the CNS and draining lymph nodes, consistent with defective MOG-peptide-induced proliferation and cytokine (IL-2, IFN-γ, IL-17) production by $CD4^+$ T cells from these mice (unpublished data). Irrespective of the difference in experimental systems used in different studies, the resistance to EAE development puts into question the validity and physiological relevance of the observations in *Def6*$^{-/-}$ DO11.10 mice. Thus,

although the biology of Def6 may be complex, it is hard for us to see how it could be a negative regulator of IL-17 in *Def6*$^{trap/trap}$ DO11.10 mice and, at the same time, a positive regulator of IL-17 in *Def6*$^{-/-}$ C57BL/6 mice (unless, of course, the DO11.10 transgene expression resulted in an artifact).

We are also concerned about other data presented in the study by Chen et al. (2008). As the authors show (Figure 4 of Chen et al. [2008]), and consistent with other published reports (Ivanov et al., 2006; Yang et al., 2008) and our own experience, in vitro stimulation of naive CD4$^+$ T cells with CD3 and CD28 antibodies in the absence of antigen-presenting cells or the prerequisite Th17 cell-polarizing cytokines (defined as Th0 conditions by Chen et al. [2008]) fails to induce substantial IL-17 expression. However, Figure 7A of the same paper shows substantial IL-17 expression by wild-type C57BL/6 mice cultured under Th0 conditions, which is intrinsically inconsistent with the data in Figure 4A, as well as with other published studies.

In summary, we have doubts about whether studies documenting enhanced susceptibility of *Def6*$^{-/-}$ mice to systemic autoimmunity and inflammation (Chen et al., 2008; Fanzo et al., 2006) reflect the true biology of Def6, given obvious issues related to the genetic background of the mice used in these studies.

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Response to Letter from Altman and Bécart

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We are glad to have the opportunity to clarify the points that Drs. Altman and Bécart have raised regarding our manuscript. Because *Def6* is the official name of the gene, we will henceforth use this nomenclature rather than its alternate names, IBP and SLAT.

Gene deletion that leads to autoimmunity such as lupus-like syndrome occur only in selected strains of mice because the development of autoimmunity usually requires interaction of the effects of the single gene deletion with those mediated by various strain-specific background loci (Jorgensen et al., 2004). We were thus not surprised to observe, as Altman and Bécart have, that *Def6*$^{trap/trap}$ mice on a C57BL/6 background do not develop a lupus-like syndrome. We have, however, observed that CD4$^+$ T cells from *Def6*$^{trap/trap}$ mice backcrossed onto either a C57BL/6 or a BALB/c background (for > 10 generations) always exhibit deregulated expression of IL-17 and IL-21 under neutral conditions in vitro (although the absolute amounts can vary depending on the strain). Furthermore, deregulated expression of IL-17 and IL-21 can be observed in both transgenic (DO11.10) and nontransgenic *Def6*$^{trap/trap}$ T cells, indicating that this effect is not due to an artifact imposed by the DO11.10 transgene. For these reasons, we have felt comfortable using CD4$^+$ T cells from *Def6*$^{trap/trap}$ mice on either a C57BL/6 or a BALB/c background in our manuscript. We believe that this is a strength of the system rather than a concern because the presence of similar abnormalities in CD4$^+$ T cells from *Def6*$^{trap/trap}$ mice from multiple strains strongly supports the idea that this defect is truly due to the absence of Def6 and not to the presence of strain-specific modifiers (or to the presence of the DO11.10 transgene). Furthermore, our data on the role of Def6 on the production of IL-17 and IL-21 were backed by substantial mechanistic studies, which could not have been influenced by the strain background of our mice. In particular, these studies linked Def6 to the regulation of interferon-regulatory factor-4 (IRF-4), a transcription factor whose role in the regulation of IL-17 and IL-21 has been