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Comment on "Development of Murine Lupus Involves the Combined Genetic Contribution of the *SLAM* and *FcγR* Intervals within the *Nba2* Autoimmune Susceptibility Locus"

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Letters to the Editor

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Comment on "Cutting Edge: Depletion of Foxp3$^+$ Cells Leads to Induction of Autoimmunity by Specific Ablation of Regulatory T Cells in Genetically Targeted Mice"

The existence of FoxP3 expression in nonhematopietic tissues is hotly debated. A recent report published in *The Journal of Immunology* provided evidence from a genetic animal model excluding expression of FoxP3 in nonhematopietic tissues (1), which is in contrast to various other reports suggesting expression of FoxP3 in murine and human breast and prostate tissues (2–5).

Using our previously established breast cancer tissue microarray (6), a large screening for FoxP3 expression in benign and malignant human breast tissue was performed. As control, we used conventional full tissue slides from five patients with breast cancer containing both tumor and adjacent normal tissue. The tissue microarray contained 2200 evaluable cores from different breast cancer subtypes covering almost all histological subtypes, 108 cores from precancerous lesions, and 151 from fibro-cystic breast disease or normal healthy tissue. Two different mAbs (ab20034 and ab22510 from Abcam, Cambridge, U.K.) were used (7).

FoxP3$^+$ lymphocytes served as internal positive controls (Fig. 1*A*). FoxP3 protein expression was negligible in primary samples from healthy as well as cancerous breast tissue. Only 13 out of 2200 cases (6‰) displayed weak (Fig. 1*B*) to moderate (Fig. 1*C*) but inhomogenous expression (maximum of 15% of total tumor cells, mean of 6%). We were not able to detect any positive signal in normal breast epithelial cells, in precancerous tissue cores, or in the cases studied on conventional full tissue slides. Thus, we conclude that, at least in healthy and cancerous human breast tissue, FoxP3 protein expression is negligible.

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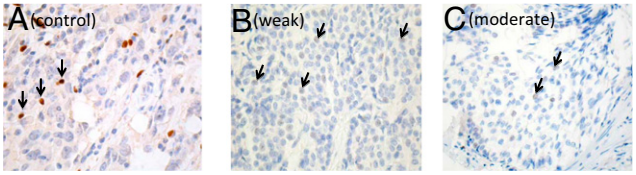

FIGURE 1. *A*, Internal control by strong nuclear staining for FoxP3 in tumor infiltrating regulatory T cells is marked by arrows. *B*, Inhomogenous and faint nuclear expression of FoxP3 in breast cancer epithelial cells is marked by arrows. *C*, FoxP3 expression with moderate intensity in a proportion of breast cancer cells is marked by arrows. Original magnification ×200.

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The conclusion by Jørgensen et al. (1) that the *Ifi200*-cluster genes within the *Nba2* locus are dispensable for ANA production is not supported by experimental data (see below). The cluster includes IFN-inducible candidate lupus susceptibility genes, including the *Ifi202*, *Ifi203*, and *Aim2* (2–4), which encode proteins (the p200-proteins) that regulate inflammation (4).

In the absence of appropriate controls for the p202 protein, the immunoblotting data in Fig. 3*B* does not establish the identity of the protein (1). This is because commercially available p202 Ab (S-19, from Santa Cruz Biotechology, Santa Cruz, CA) nonspecifically detects other proteins (the Ab data sheet).

Notably, in contrast to the 10–100-fold difference in the steady levels of *Ifi202* mRNA between C57BL/6 (B6) and B6.*Nba2* splenic B cells (2, 3), levels of the p202 protein differ by only 2- to 3-fold (Fig. 1), thus suggesting that the mouse strain-dependent posttranscriptional mechanisms regulate the levels of p202 protein (3). Moreover, in B6 cells, the protein is detected primarily in the cytoplasmic fraction (Fig. 1). However, in B6.*Nba2* cells, the p202 protein (including its phosphorylated form) is detected in cytoplasmic and nuclear fractions. Because the IFN signaling-dependent nuclear

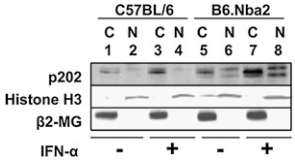

FIGURE 1. B6 and B6.*Nba2* splenic B cells differ with respect to steady-state levels of p202 protein and its subcellular localization. Splenic B cells were purified from age-matched B6 and B6.*Nba2* female mice (cells from four mice pooled; aged ~10 wk). Cells were either left untreated (control) or treated with IFN-α (1000 U/ml for 16 h). Cells were harvested and fractionated into cytoplasmic and nuclear fractions. The cytoplasmic (C) and nuclear (N) fractions containing approximately equal amounts of proteins were analyzed by immunoblotting using Abs specific to the indicated proteins [anti-p202 (2)]. Detection of β_2-microglobulin (β_2-MG) in the cytoplasmic fraction and histone H3 in the nuclear fraction served as the quality control for cell fractionation.

localization of p202 protein inhibits the transcriptional activity of p53 and E2F1 (3), it is necessary to compare the steady-state levels of p202 protein (and other p200-proteins), determine its subcellular localization, and compare its transcriptional modulatory activity in immune cells of the congenic mice (ABC, A, A′B, B, BC, and C) to determine the functional role of the p202 in ANA production.

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Response to Comment on "Development of Murine Lupus Involves the Combined Genetic Contribution of the *SLAM* and *FcγR* Intervals within the *Nba2* Autoimmune Susceptibility Locus"

The comment from Dr. Divaker Choubey and colleagues on our article (1) offers new compelling preliminary data suggesting a novel mechanism by which p202 protein may be involved in mouse lupus-like disease development. In contrast to previously known data, the observation suggests that the intracellular localization of p202 protein is strain dependent and that this, rather than the amount of total protein present in cells of different strains, may be responsible for driving autoantibody production in B6.*Nba2* congenic mice.

In our study, we conclude that genes expressed within the *Nba2*-C locus, comprising *Ifi202*, are dispensable for disease development. We would like to reiterate that this conclusion is based on the association between symptoms of disease (antinuclear Ab production and elevated proteinuria) and the genetic origin of the p200 cluster of genes (NZB versus B6). B6.*Nba2* subcongenic mice (strain A′B) contain the p200 cluster of genes from B6, yet develop symptoms of disease similar to parental B6.*Nba2* congenic mice that contain NZB-derived p200 genes. Therefore, it remains possible that p202 protein plays a role in the disease process even if the genetic origin is from B6. In this case, we suggest that epistatic regulation of B6- (and NZB-) derived p202 by NZB-derived genes located within the upstream *Nba2* congenic region (i.e., region A/A′, B, or both) could occur in B6.*Nba2* and B6.*Nba2*-A′B congenic mice. Further studies investigating the cellular localization of p202 protein in B6.*Nba2* subcongenic strains may help elucidate this possibility.

We greatly appreciate this comment and welcome further remarks from researchers that may help us define the factors contributing to mouse lupus-like disease development in B6.*Nba2* congenic mice.

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Comment on "CRTAM Confers Late-Stage Activation of CD8$^+$ T Cells to Regulate Retention within Lymph Node"

Takeuchi et al.'s elegant study in the October 1, 2009 issue of *The Journal of Immunology* (1) demonstrates that CD8 T cells, which express class I-restricted T cell-associated molecule (CRTAM) during activation, bind to CADM1 (Necl-2) expressing CD11c$^+$ dendritic cells in lymph nodes. Notably, this interaction is important for CD8$^+$ T cell retention within the lymph node and contributes to development of an autoimmune disorder. Given the critical in vivo role of CD8$^+$ T cell CRTAM-dependent dendritic cell binding, there may be therapeutic potential in blockade of CRTAM interactions. It is therefore of interest whether CRTAM might have ligands other than CADM1. We could demonstrate that a recombinant CRTAM-Fc

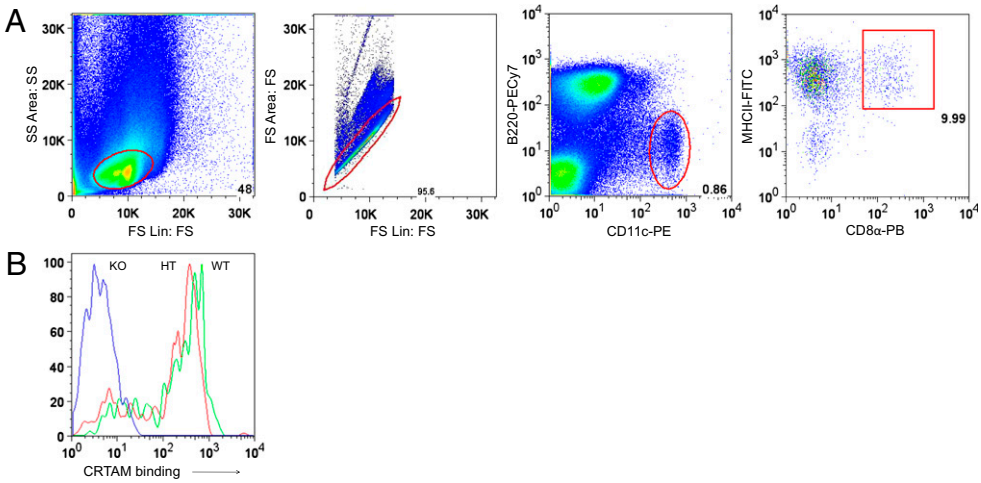

FIGURE 1. Cell surface expression of CRTAM-Fc binding activity. *A*, Gating strategy of splenic MHC II$^+$ CD11c$^+$ CD8α$^+$ dendritic cells. *B*, CRTAM-Fc binding activity in dendritic cells in wild-type (WT, green), heterozygous (HT, red), and knockout (KO, blue) mice.

protein bound to cells transfected with murine CADM1 isoforms (data not shown), confirming the reported CADM1–CRTAM interaction (2). Having generated *Cadm1*$^{-/-}$ animals (3), we were able to investigate *Cadm1* dependency of CRTAM-Fc binding. Consistent with the model of Takeuchi et al., CRTAM-Fc binding was observed on CD11c$^+$ dendritic cells (Fig. 1*A*) from wild-type animals, but not on those from *Cadm1*$^{-/-}$ animals (Fig. 1*B*). Specific CRTAM-Fc binding was not observed in any other splenic cell type (data not shown). Thus, the CRTAM–CADM1 interaction is nonredundant; and this, taken together with the data of Takeuchi et al., suggests its potential as a target for novel immunomodulatory agents.

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