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Cutting Edge: The Idd3 Genetic Interval Determines Regulatory T Cell Function through CD11b+ CD11c− APC

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Abstract

The *Idd3* genetic interval confers protection against multiple autoimmune diseases, including type 1 diabetes and experimental autoimmune encephalomyelitis (EAE). The favored candidate gene in this interval is *Il2*, which is polymorphic between susceptible and resistant strains of mice. IL-2 regulates the growth/death of effector T cells as well as the generation/maintenance of regulatory T cells (Tregs) and recent studies have shown that NOD.*Idd3* Tregs are more suppressive than their NOD counterparts. We have further dissected the mechanisms underlying the differential suppression by NOD and NOD.*Idd3* Tregs and find that it is determined by $CD11b^{+}$ $CD11c^{-}$ antigen presenting cells (APC). Thus, contrary to what might be expected, our data suggest that the differential suppressive activity of NOD and NOD.*Idd3* Tregs is not due to an effect of the *Idd3* genetic interval on T cells but rather is due to differences in the antigen presenting cell compartment.

Keywords

T cells; Suppression; Autoimmunity

Introduction

While the etiology of most autoimmune diseases is unclear, it is clear that they all have a significant genetic component. Indeed, genome-wide scanning for loci that influence disease has led to the identification of multiple loci in both humans and in animal models of autoimmunity. Interestingly, many of the loci identified in these studies overlap (1-4), giving rise to the concept that there are ‘common autoimmune genes’ affecting susceptibility to multiple autoimmune diseases. One such locus that appears to influence susceptibility to multiple autoimmune diseases is the diabetes susceptibility locus, *Idd3* (1) (2) (5).

The *Idd3* locus is located on mouse chromosome 3 and has been identified as a susceptibility locus for several autoimmune diseases including type 1 diabetes (5), experimental autoimmune encephalomyelitis (EAE) (1) and autoimmune ovarian dysgenesis (AOD) (2). When the

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C57BL/6-derived *Idd3* interval is introgressed onto the NOD background, diabetes incidence is reduced by 75% (5). Similarly, we have found that NOD.*Idd3* mice are protected against EAE (1).

The *Idd3* genetic interval has been reduced to 0.15 cM (5) and contains five known genes: *Tenr, Il2, Il21, Cetn4 and Fgf2*, and two predicted genes of unknown function. Among these, *Il2* is an obvious candidate gene because of its established role in regulating T cell growth (6), death (7) (8) and the generation/maintenance of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) (9). The fact that there are *Il2* gene polymorphisms that are shared among autoimmune susceptible strains (10) and the recent identification of IL-2Ralpha (CD25) as a susceptibility gene for multiple human autoimmune diseases (11) (12) (13) support the hypothesis that *Il2* is an important determinant of autoimmune disease susceptibility.

While the functional role of polymorphic variants of *Il2* in suppressing autoimmunity has not been discerned (14), a recent study has suggested that an approximately two-fold difference in IL-2 production underlies the superior regulatory T cell function of NOD.*Idd3* Tregs relative to NOD Tregs (15). We have furthered addressed the mechanisms responsible for the differential suppressive activity of NOD and NOD.*Idd3* Tregs and find that it is determined by the antigen presenting cells (APCs) in NOD and NOD.Idd3 mice. We further identify $CD11b^+CD11c^-$ APC as the cell type mediating this effect.

Materials and Methods

Animals

Female NOD and NOD.B6*Idd3* mice (6−7 weeks) were purchased from Taconic (Germantown, NJ). All mice were screened for diabetes prior to use. Mice were housed in accordance with the guidelines established by the animal care and use committee at Harvard Medical School.

Flow cytometry

Single cell suspensions from the thymus, spleen, and lymph nodes were stained with antibodies against CD4, CD25 (BD Biosciences) and FoxP3 (Ebioscience). All data were collected on a BDFACSCalibur (BD Biosciences).

In vitro proliferation assays

Suppression assay—We isolated $CD4^+CD25^-$ and $CD4^+CD25^+$ cells by sorting (BD FACSAria, BD Biosciences). $CD4^+25^+$ (2.5×10^4/well) and $CD4^+CD25^-$ (2.5×10^4/well) were cultured in triplicate in the presence of soluble anti-CD3 (1 μg/ml) and irradiated splenic APCs (1.25×10^5/well). After 48 hrs, plates were pulsed with 1 μCi/well of ^{3}H-thymidine and harvested 16 hours later. ^{3}H-thymidine incorporation was measured in a β scintillation counter (Wallac). Data are shown as mean of triplicate wells. For APC experiments, $CD3^+$ and $CD11c^+$, $CD11b^+CD11c^-$ or $CD19^+$ cells were depleted from spleen cells by cell sorting. Post-sort purity in every experiment was 100%. Percent suppression = 100- the mean CPM of wells with the indicated ratios of Effector:Tregs/ mean CPM of wells with $CD4^+CD25^-$ effectors alone.

Results and Discussion

Regulatory T cells in NOD and NOD.*Idd3* mice

IL-2 is known to be essential for the generation and maintenance/survival of naturally occurring regulatory T cells (Tregs) (9). A recent study reported a higher frequency of naturally occurring $CD4^+CD25^+FoxP3^+$ Tregs in NOD.*Idd3* mice (15) while another study reported no effect of

Idd3 on thymic generation of Tregs in fetal thymic organ cultures (16). To resolve whether *Idd3* affects thymic generation of Tregs and/or Treg numbers in the periphery, we examined the frequency of naturally occurring $CD4^+CD25^+FoxP3^+$ Tregs in the thymus and periphery of NOD and NOD.*Idd3* mice. We found no difference in the frequency or absolute number of Tregs generated in the thymus of NOD and NOD.*Idd3* mice (Figure 1 and data not shown). Similarly, the frequency and absolute number of $CD4^+CD25^+FoxP3^+$ Tregs in the spleen, lymph nodes, and pancreatic lymph nodes is not different between NOD and NOD.*Idd3* (Figure 1 and data not shown). We concluded from these data that the generation and maintenance of Tregs is not different between NOD and NOD.*Idd3* mice.

However, it was still possible that NOD and NOD.*Idd3* Tregs differed in their suppressive activity. Indeed, a recent study suggests that NOD.*Idd3* Tregs have superior regulatory T cell function relative to NOD (15). We have further examined to what extent the regulatory capacity of NOD and NOD.*Idd3* Tregs differs by performing an extensive titration of Tregs in *in vitro* suppression assays. It was clear from these assays that NOD.*Idd3* effector T cells ($CD4^+CD25^-$) proliferate more than NOD-derived effector T cells (Figure 2 and data not shown). In spite of these differences, we found that on a per cell basis NOD.*Idd3* Tregs were significantly more suppressive than NOD Tregs in that they exhibited more suppressive activity at all effector:Treg ratios tested and continued to exhibit suppressive activity even when present at a ratio of 1:0.0625 effector:Treg (16 effectors for one Treg) (Figure 2). In contrast, we observed an almost complete loss of suppressive activity in NOD Tregs when cultured at a ratio of 1:0.125 effector:Treg (8 effectors for one Treg). Thus, NOD.*Idd3* Tregs are more suppressive than NOD Tregs and this difference in suppressive activity is apparent in spite of the higher proliferative capacity of NOD.*Idd3* effector T cells.

NOD vs NOD. *Idd3* APCs determine Treg suppressor function

Recent data have shown that Tregs form lasting and stable contacts with dendritic cells (DCs), suggesting that DC-mediated activation of Tregs is critical for suppressive activity and/or Tregs mediate their suppressor function indirectly by inactivating DCs (17). In addition, DCs can produce IL-2 and both DCs and macrophages can express CD25 (18). While the functional role of CD25 on APCs has not been extensively studied, there are data that suggest that stimulation of macrophages with IL-2 induces anti-microbial activity (19) and that IL-2 synergizes with other activating signals to induce IFN in DCs (20). Given the connection of DCs with Treg function and that IL-2 can be produced by and may exert effects on APCs, we investigated whether the differential suppressive activity of NOD.*Idd3* Tregs was intrinsic to the Tregs themselves or whether APCs were playing an important role. We therefore cultured effectors and Tregs from NOD and NOD.*Idd3* mice with either NOD or NOD.*Idd3*-derived APCs. Surprisingly, we found that NOD Tregs cultured with NOD.*Idd3*-derived APC were able to suppress NOD effector cells even when cultured at a ratio of 1:0.03125 effector:Treg (32 effectors for one Treg), whereas NOD Tregs cultured with NOD APC lost most of their suppressive activity when cultured at a ratio of 1:0.125 effector:Treg (8 effectors for one Treg) (Figure 3A and B). Thus, NOD Tregs are not intrinsically defective as NOD.*Idd3* APCs can elicit potent suppressive activity from NOD Tregs. This is in spite of the increase in proliferation of NOD effectors cultured with NOD.*Idd3* APCs. In contrast, NOD.*Idd3* Tregs suppressed NOD.*Idd3* effectors equally well regardless of the source of APCs (Figure 3A and B), suggesting that the ability of NOD.*Idd3* Tregs to suppress NOD.*Idd3* effectors is fixed most likely as a result of their co-evolution.

To further examine to what extent NOD versus NOD.*Idd3* APCs determine Treg function and to address the possibility of intrinsic differences in the Tregs themselves, we performed additional comparisons. First, we compared the ability of NOD.*Idd3* Tregs to suppress NOD effectors in the presence of NOD versus NOD.*Idd3* APC and of NOD Treg to suppress

NOD.*Idd3* effectors in the presence of NOD versus NOD.*Idd3* APC. We found that NOD.*Idd3* Tregs suppress NOD effectors even when cultured at a ratio of 1:0.03125 effector:Treg (32 effectors for one Treg) when NOD.*Idd3* APCs are present while NOD.*Idd3* Tregs lose the ability to suppress NOD effectors completely when cultured at a ratio of 1:0.0625 effector:Treg (16 effectors for one Treg) when NOD APCs are present (Figure 3C). We observed a similar trend with NOD Tregs in that NOD Tregs show a small but consistent increase in the ability to suppress NOD.*Idd3* effectors when NOD.*Idd3* APCs are present. To address the possibility of intrinsic differences in the Tregs themselves, we compared the ability of NOD Tregs versus NOD.*Idd3* Tregs to suppress NOD effectors in the presence of NOD APC and NOD.*Idd3* effectors in the presence of NOD.*Idd3* APC (Figure 3D). We found that NOD Tregs and NOD.*Idd3* Tregs were equivalent at suppressing NOD effectors in the presence of NOD APC. Similarly, NOD and NOD.*Idd3* Tregs were equivalent at suppressing NOD.*Idd3* effectors in the presence of NOD.*Idd3* APC. Thus, if the source of APCs and effector cells is kept constant, NOD and NOD.*Idd3* Tregs suppress equally well. Collectively, all of our data suggest that the source of APCs is the most important determinant of Treg suppressor function..

In order to identify the APC population responsible for determining Treg suppressor function, we depleted $CD11c^+$, $CD11b^+CD11c^-$, or $CD19^+$ cells from NOD and NOD.*Idd3* splenocytes and used the depleted fraction as APC in a suppression assay with NOD effectors and NOD Tregs. We found that NOD Tregs still exhibited poor suppression in cultures with either $CD11c^+$ or $CD19^+$ depleted NOD APC relative to cultures with $CD11c^+$ or $CD19^+$ depleted NOD.*Idd3* APC (Figure 4 A and B). However, when $CD11b^+CD11c^-$ cells were depleted, the suppressive capacity of NOD Tregs in cultures with NOD APC was almost identical to that in cultures with NOD.*Idd3* APC (Figure 4C). Interestingly, the suppression by NOD Tregs in cultures with $CD11b^+CD11c^-$ depleted NOD.*Idd3* APC was similar to that observed with whole splenic NOD.*Idd3* APC (Figure 3B). Taken together, these data suggest that it is the $CD11b^+CD11c^-$ APC in NOD mice that impair the ability of NOD Tregs to suppress either directly by inhibiting the suppressor function of Treg and/or rendering effector T cells resistant to suppression or indirectly by affecting the ability of other APC to activate Treg function. Direct comparison of the ability of NOD versus NOD.*Idd3* $CD11b^+CD11c^-$ APC to activate Treg function shows that $CD11b^+CD11c^-$ APC from NOD are poor at stimulating both effector T cell proliferation and Treg suppression relative to $CD11b^+CD11c^-$ APC from NOD.*Idd3* (Supplementary Figure 1). These data support the possibility that the $CD11b^+CD11c^-$ APC in NOD determine Treg function by acting directly on T cells; however, we cannot distinguish between the possibilities that NOD-derived $CD11b^+CD11c^-$ APC inhibit the function of Treg or protect effector cells from Treg-mediated inhibition.

Previous work has shown that NOD APCs are defective in eliciting suppression from NOD effectors compared to B6 APCs (21). Our studies further narrow this effect to the B6-derived *Idd3* interval and isolate the effect to $CD11b^+CD11c^-$ APC. While our data do not prove or disprove that *Il2* is responsible for the protective effect of *Idd3*, they do ascribe the protective effect of *Idd3* primarily to APCs and only indirectly to T cells. It is possible that differences in IL-2 direct the functional responses of effector T cells, Tregs, and possibly APCs. Further investigation of the mechanisms and genes responsible for alterations in APC function will provide insight into the potent protective effect of this genetic interval.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abreviations used in this paper

Treg, regulatory T cells; APC, antigen presenting cell.

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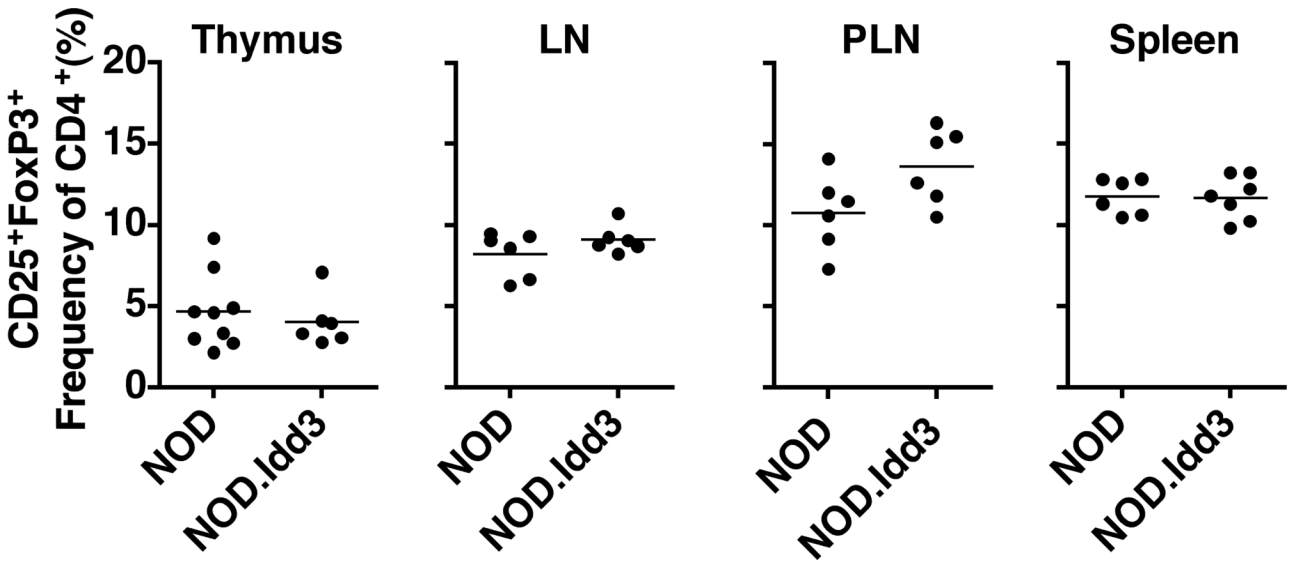

Figure 1. Regulatory T cell frequency in NOD vs NOD.*Idd3* mice

Frequency of $CD25^+FoxP3^+$ thymocytes in the CD4 single positive gate and frequency of $CD25^+FoxP3^+$ cells in the $CD4^+$ gate from axillary lymph nodes (LN), pancreatic lymph nodes (PLN), and spleen.

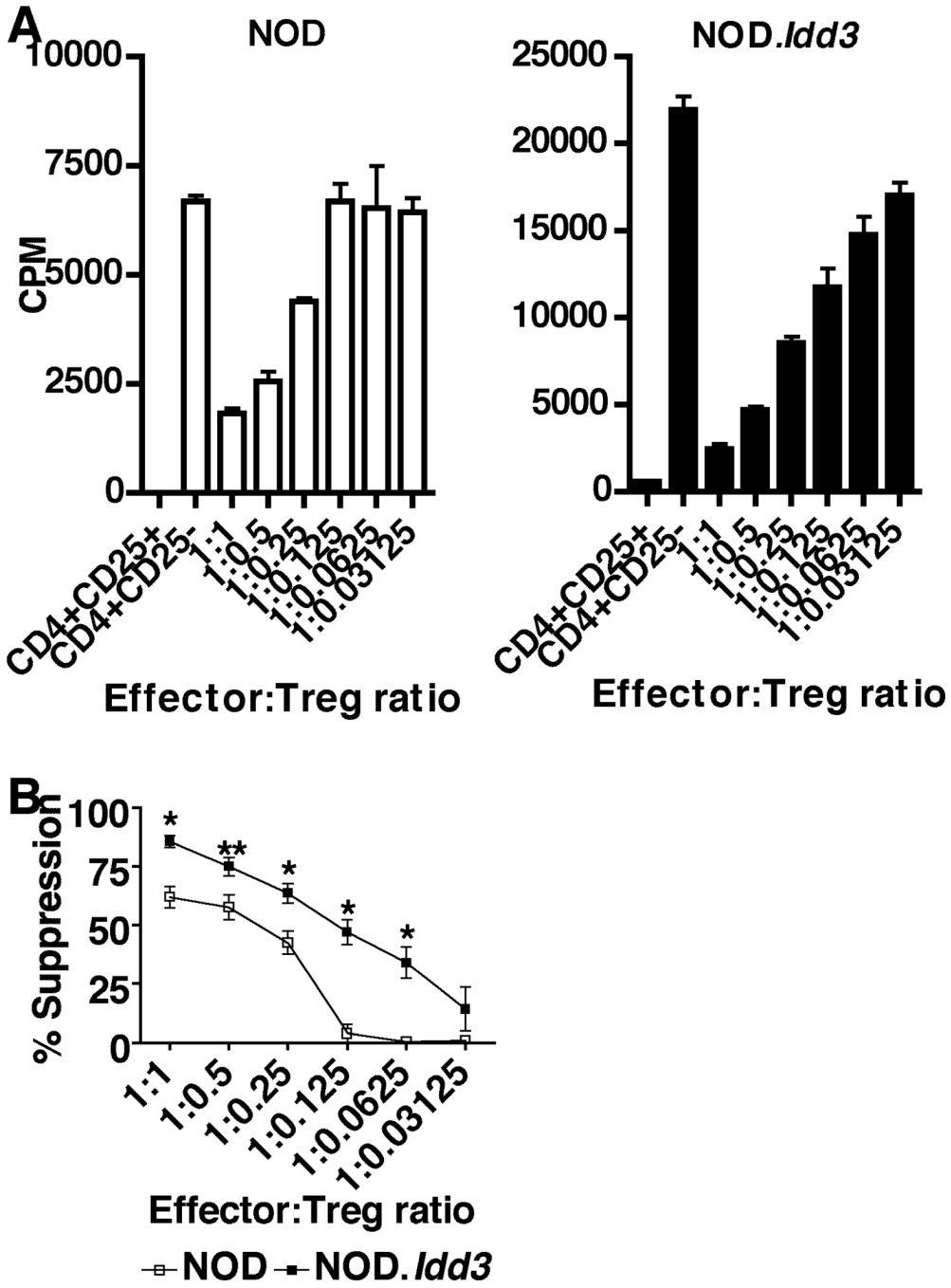

Figure 2. NOD.*Idd3* Tregs are more suppressive

A) $CD4^+CD25^+$ Tregs and $CD4^+CD25^-$ effectors from NOD (left panel) or NOD.*Idd3* (right panel) were cultured with irradiated syngeneic APC and soluble anti-CD3. Similar results were obtained in 5 independent experiments. B) Percent suppression of NOD and NOD.*Idd3* Tregs. The mean of five independent experiments is shown. *$p<0.0159$; **$p<0.03$.

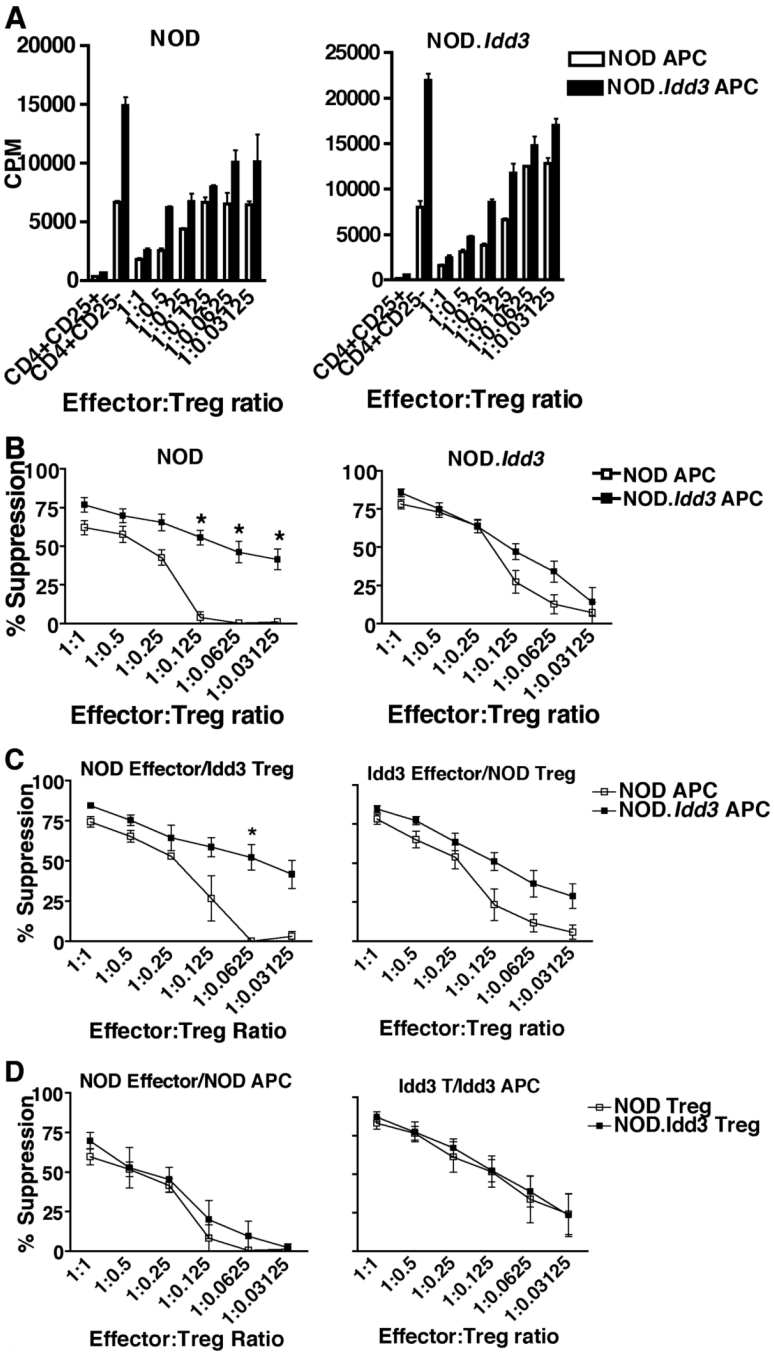

Figure 3. NOD.*Idd3*-derived antigen presenting cells drive enhanced suppression

A) $CD4^+CD25^+$ Tregs and $CD4^+CD25^-$ effectors from NOD (left panel) and NOD.*Idd3* (right panel) were cultured with soluble anti-CD3 and irradiated APC from either NOD (open bars) or NOD.*Idd3* (closed bars). Similar results were obtained in 5 independent experiments. B) Percent suppression of NOD (left panel) and NOD.*Idd3* (right panel) Tregs cultured with NOD (open squares) and NOD.*Idd3* (closed squares) APC. The mean of five independent experiments is shown. *p=0.0079. C) Percent suppression of NOD effectors by NOD.*Idd3* Tregs (left panel) and of NOD.*Idd3* effectors by NOD Tregs (right panel) cultured with NOD (open squares) or NOD.*Idd3* (closed squares) APC. The mean of three (left panel) and 5 (right panel) independent experiments is shown. *p=0.02. D) Percent suppression by NOD (open

squares) versus NOD.*Idd3* (closed squares) Tregs of either NOD effectors cultured with NOD APC (left panel) or NOD.*Idd3* effectors cultured with NOD.Idd3 APC (right panel). The mean of 4 (left panel) and 3 (right panel) experiments is shown.

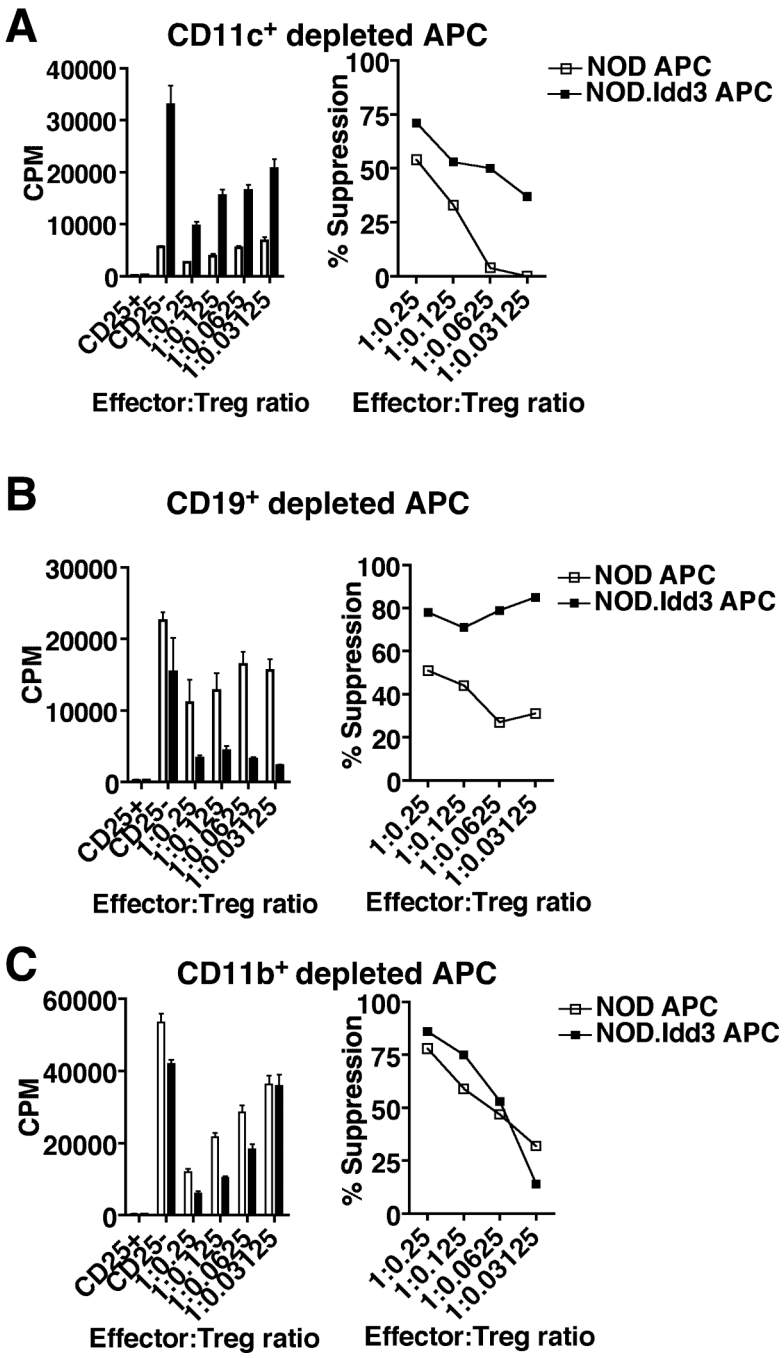

Figure 4. CD11b$^+$CD11c$^-$ cells determine regulatory T cell function

NOD CD4$^+$CD25$^-$ effectors (7.5×10^4/well) were stimulated in the presence of soluble anti-CD3 with NOD or NOD.*Idd3* splenic APC depleted of CD3$^+$ cells and either A) CD11c$^+$ cells (2.25×10^5/well), B) CD19$^+$ (7.5×10^4/well), or C) CD11b$^+$CD11c$^-$ cells (2.25×10^5/well). NOD APC (open bars/squares) and NOD.Idd3 APC (closed bars/squares). Representative data of 2−3 independent assays is shown.