Comment on "Type 1 Diabetes in BioBreeding Rats Is Critically Linked to an Imbalance between Th17 and Regulatory T Cells and an Altered TCR Repertoire"

Jan-Luuk Hillebrands, Jan Rozing and Jeroen T. J. Visser

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Comment on “Type 1 Diabetes in BioBreeding Rats Is Critically Linked to an Imbalance between Th17 and Regulatory T Cells and an Altered TCR Repertoire”

In their recent paper, van den Brandt et al. (1) conclude that diabetes development in diabetes-prone BioBreeding (DP-BB) rats is due to an imbalance between Th17 effector (Th17_{eff}) and regulatory T cells (T_{reg}) in favor of the Th17_{eff} cells. DP-BB T_{reg} function by itself was not hampered, which stands in contrast to previous reports by us (2, 3) and others (4). However, to expand the T_{reg} population they treated DP-BB rats with the superagonistic αCD28 Ab JJ316 (5). The authors note that a JJ316-induced improvement of T_{reg} function cannot be excluded but consider this unlikely (1).

On the basis of data presented in Fig. 4A of their paper (1), however, we calculated that T_{reg} isolated from JJ316-treated DP-BB rats are significantly less suppressive compared with DR-BB T_{reg} at 1:1 and 1:5 T_{reg}/indicator ratios. These data are in line with our observations that JJ316 treatment in BB-DP rats indeed increases the number of CD4^{+}CD25^{+}Foxp3^{+} T_{reg} (Fig. 1A), but also increases their suppressor function (Fig. 1B). This functional improvement cannot be fully explained by the increase in CD4^{+}CD25^{+}Foxp3^{+} T_{reg}. 

**FIGURE 1.** Treatment with the superagonistic αCD28 Ab JJ316 improves CD4^{+}CD25^{+} T_{reg} function in DP-BB rats. DP-BB rats were daily injected i.p. with JJ316 or the IC Ab 3H5-1 (both 1.25 μg/g body weight) for 4 d. Rats were then sacrificed, and single-cell suspensions were prepared from mesenteric and cervical lymph nodes. Flow cytometry for CD4^{+}CD25^{+}Foxp3^{+} was performed, and CD4^{+}CD25^{+} T cells were purified using a high-speed MoFlo cell sorter for subsequent functional analysis. For comparison, DR-BB CD4^{+}CD25^{+} T cells were sorted and analyzed. A, Compared with DR-BB rats, IC-treated DP-BB rats have reduced numbers of CD4^{+}CD25^{+} T cells containing substantially fewer Foxp3-expressing T_{reg}. Following JJ316 treatment in DP-BB rats, levels of CD4^{+}CD25^{+} T cells greatly increased, with only a relatively small increase in Foxp3-expressing T_{reg}. Graphs display representative results obtained in three separate experiments. B. For functional analyses, in each experiment lymph node cells of two DP-BB rats were pooled to get sufficient cells for cell sorting. Data shown were obtained in three independent experiments. In vitro suppression assays were performed as described previously (2, 3). Briefly, 5×10⁴ CD4^{+}CD25^{neg} (indicator) T cells isolated from DR-BB rats were either cultured alone (in the presence of 5×10⁵ irradiated APCs and 2.5 μg/ml ConA) or cocultured with purified CD4^{+}CD25^{+} T cells in a 1:1 ratio. CD4^{+}CD25^{+} T cells were purified from DR-BB, naive DP-BB, IC-treated DP-BB, and JJ316-treated DP-BB rats. All cultures were conducted for 72 h at 37°C, 5% CO₂, and 95% air. Proliferation was determined using [³²P]thymidine incorporation, and results are expressed as the percentage proliferation (mean ± SEM) relative to CD4^{+}CD25^{neg} (indicator) T cells only. *p < 0.05 versus JJ316 DP-BB; **p < 0.05 versus naive and IC DP-BB; ***p < 0.001 versus naive and IC DP-BB. IC, isotype control.
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Response to Comment on “Type 1 Diabetes in BioBreeding Rats Is Critically Linked to an Imbalance between Th17 and Regulatory T Cells and an Altered TCR Repertoire”

In our recent paper, we investigated the suppressive capacity of Treg cells in DP-BB rats and concluded that they are functional (1). Due to severe lymphopenia, CD4+CD25+ T cells in DP-BB rats contain a high proportion of Foxp3+ effector T cells. To enrich the Treg cells, we had treated DP-BB rats with the superagonistic anti-CD28 Ab JJ316. As clearly stated, this also enhances the function of Treg cells (1). Nevertheless, we doubt that JJ316 is able to convert completely defective Treg cells into fully functional ones. Apart from this, we consider it reasonable to analyze Treg cells from prestimulated animals as this also mimics Ag-driven immune responses.

Hillebrands et al. argue that the suppressive capacity of Treg cells from DP-BB rats treated with JJ316 is lower compared with similarly treated DR-BB rats. This is true with regard to proliferation and IFN-γ secretion, but not with regard to IL-17 secretion (1). Furthermore, it is reasonable that Treg cells from JJ316-treated DP-BB rats are slightly less suppressive because IL-2 levels are generally lower in lymphopenic animals. Nevertheless, at least with regard to the critical parameter of suppressing Th17 cells, the capacity of Treg cells from DP-BB and DR-BB rats is comparable.

Of note, Hillebrands et al. did not use optimal conditions to expand the Treg cells. The amount of JJ316 was relatively low (2), and injection was done i.p. instead of i.v. (1). Furthermore, JJ316 was administered daily, although Treg cell expansion is known to be most pronounced 3 d after application (2, 3). This explains the minor increase in CD4+CD25+ T cells from 2.2 to 3.4% after JJ316 application, whereas we observed an increase from 2.7 to 10.3% (Fig. 1). Moreover, Hillebrands et al. found that 59% of the CD4+CD25+ T cells are Foxp3+, whereas we achieved a purity of 90% after JJ316 treatment (1).

We believe our data demonstrate that Treg cells from DP-BB rats have potent suppressive capacity after JJ316 treatment and are thus not generally defective. We do not know whether this also applies to Treg cells from naive rats, but we consider it likely. Nevertheless, we agree that the final answer to this debate has to await the generation of Foxp3 knockin rats, allowing for the purification of naive Treg cells from DP-BB rats.

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Comment on “Flagellin as an Adjuvant: Cellular Mechanisms and Potential”

Mizel and Bates (1), in their review on flagellin as an adjuvant, state that “a number of flagellin-based vaccines for infectious diseases have already entered clinical trials.” I would like to add a cautionary note concerning the evaluation of the efficacy of these trials. Some people are naturally high responders to flagellin, whereas others are low responders to this Ag. This feature is likely to influence immune response to the Ag linked to this adjuvant. The results of ongoing clinical trials cannot be properly evaluated without taking this possible confounding variable into account. Almost four decades ago, in The Journal of Immunology, Wells et al. (2) reported significant associations between highly polymorphic IgG markers (GM) and Ab responses to flagellin. Subsequent studies have confirmed these findings (3). Mechanisms underlying the association of GM allotypes and Ab responses could include GM determinants as part of the recognition structure for flagellin epitopes on B cells. Perhaps B cell membrane-bound IgG molecules expressing certain GM determinants are more compatible receptors for flagellin epitopes and thus provoke a strong humoral immunity, whereas those expressing other GM alleles form a less compatible receptor for the critical epitopes of this Ag. In addition—and contrary to the prevalent belief in immunology—these constant-region determinants could directly influence anti-flagellin Ab specificity by causing conformational changes in the Ag binding site in the IgV region (4). These alleles could also influence the expression of idiotypes involved in flagellin immunity, as shown in the T15 system in mice (5).

Janardan P. Pandey

Response to Comment on “Flagellin as an Adjuvant: Cellular Mechanisms and Potential”

We sincerely appreciate Dr. Pandey’s interest in our review on flagellin and its adjuvant property. With regard to his comments, it is important to note that we were referring to clinical trials that assess the adjuvant property of flagellin and not necessarily its immunogenic potential. We agree with Dr. Pandey that there is likely to be substantial variability in flagellin responsiveness in humans. Although early phase I studies will provide important information on safety, we must clearly wait for the results from larger studies to assess the extent to which low responsiveness is present in the human population.

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