European Journal of Immunology

Changes in T-cell subsets identify responders to FcR non-binding anti-CD3 mAb (teplizumab) in patients with Type 1 diabetes

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Handling Executive Committee member: Dr. Lucienne Chatenoud

Please note that the correspondence below does not include the standard editorial instructions regarding preparation and submission of revised manuscripts, only the scientific revisions requested and addressed.

First Editorial Decision - 28-Apr-2015

Dear Prof. Herold,

Manuscript ID eji.201545708 entitled "Changes in T cell subsets identify responders to FcR non-binding anti-CD3 mAb (teplizumab) in patients with Type 1 diabetes" which you submitted to the European Journal of Immunology has been reviewed.

The comments of the referees are included at the bottom of this letter. Even though the three referees think that your study is interesting, they have some concerns about it. A revised version of your manuscript that takes into account the comments of the referees will be reconsidered for publication. Should you disagree with any of the referees' concerns, you should address this in your point-by-point response and provide solid scientific reasons for why you will not make the requested changes.

You should also pay close attention to the editorial comments included below. In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Failure to do this will result in delays in the re-review process.

European Journal of Immunology

Please note that submitting a revision of your manuscript does not guarantee eventual acceptance, and that your revision will be re-reviewed by the referees before a decision is rendered.

If the revision of the paper is expected to take more than three months, please inform the editorial office. Revisions taking longer than six months may be assessed by new referee(s) to ensure the relevance and timeliness of the data.

Once again, thank you for submitting your manuscript to European Journal of Immunology and we look forward to receiving your revision.

Yours sincerely,
Laura Soto Vazquez

On behalf of
Dr. Lucienne Chatenoud

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Reviewer: 1

Comments to the Author

In this manuscript, the authors studied the in vitro and vivo effects of teplizumab, an anti-CD3 antibody, using cells and data from type 1 diabetes patients enrolled in 2 clinical trials. They first stratified patients according to clinical response (using a different criteria than the one originally used in the two trials) and showed that clinical response was associated with lower proportions of CD8 EM and CD4 EM two weeks after completion of treatment, and with higher proportions of CD8 CM at month-2 (Delay trial) or month-3 (AbATE trial). They further showed that teplizumab in vitro induces an expansion of CD8CM T cells from PBMCs from healthy donors. Finally, a gene expression study performed in CD8 CM T cells sorted from samples collected in clinical responders of the Delay trial at month-2, revealed downregulated expression of genes involved in T cell activation, as compared to placebo-treated controls. From these results, the authors

conclude that CD8 CM T cells constitute a biomarker of therapeutic response to teplizumab as well as a preferential target of the antibody acting through inactivation of T cells, presumably including pathogenic autoimmune T cells.

Major comments:

1. The expansion and preferential in vitro proliferation of cells showing a CD8 CM phenotype at the end of a 3-day culture of PBMCs in the presence of teplizumab and anti-CD28 is interpreted as a preferential effect of teplizumab on this cell subset, explaining the association between prolonged increase of CD8 CM T cells and clinical response. However,

- CD3-mediated T cell activation in vitro impact the phenotype of T cell subsets present in the culture, especially the expression of CD45RO. It is thus likely that naïve CD8 T cells that proliferate vigorously in culture, acquired a CM phenotype in culture. Only the analysis of the effect of teplizumab on sorted CD8 subsets could support this conclusion.
- Second, transient viral reactivation has been observed a few weeks after the end of the treatment in the AbATE trial and other CD3 antibody trials, associated in some cases with an expansion of virus-specific T cells with an effector/memory phenotype. Such cells could readily account for the expansion of CD8 CM T cells observed at month-1 in responders and non responders. This should be discussed.

2. The gene expression profile of CD8 CM T cells 2-months post therapy was performed in responders versus placebo-treated controls. Additional comparison of the CD8 CM expression data in responders versus non responders would be useful to support the hypothesis that tepluzimab acts in responders through impairment of CD8 CM responsiveness. In fact, a sentence page 12, line 30 of the discussion alludes to the existence of this data in non responders.

Minor comment:

The timing of the first sampling for T cell analysis after the end of treatment in the Delay trial is unclear: 2 months as stated page 7, or 2 weeks as apparently shown in figure 2C?

Reviewer: 2

Comments to the Author

The authors seek to explain the immunomodulatory effect of teplizumab in two trials in type 1 diabetes (ABATE and DELAY). They show that responders to therapy demonstrate an increase in CD8CM T-cells in peripheral blood, following an initial reduction in CD4EM. There is an absolute increase in CD8CM numbers in responders, and teplizumab appears to expand (or generate from naive cells) this subset in vitro (healthy control data). Subsequently they use nanostring to examine gene expression in CD8CM cells

from 6 responders and 5 controls from DELAY. They also illustrate upregulation of CCL20 protein, predominantly in CD8CD45RA- cells. Their overall conclusion is that teplizumab expands CD8CM T-cells and, at the same time, reduces the effector function of these cells. This could be consistent with their previous findings that there is no absolute reduction in antigen-specific T-cells following teplizumab therapy.

CRITIQUE:
This is an interesting story, although not entirely consistent with effects seen with other immunomodulatory drugs in diabetes. There are two main deficiencies in the work. Firstly, the authors do not provide any information about gene expression in non-responders to teplizumab. Unless this is distinct to the effects seen in responders then the therapeutic effects cannot specifically be attributed to these changes. Secondly, there are no functional data to corroborate the authors' suggestion that these changes at transcriptional level are ultimately down-regulatory. Thus intuitively one might have expected changes in EM T-cells to be more important, but conceivably CM cells might regulate EM for example. Without these two pieces of information the conclusions are speculative.

Specific comments:
P3, first sentence of second paragraphs - not all of the trials referenced used teplizumab.

P8. The absolute number of CD8CM cells in DELAY is not mentioned - did they increase also?

P9, Figure 4 and Figure 5. Is the P value corrected for multiple testing? I cannot find this information in the manuscript. As above, without seeing equivalent expression profiles for non-responders, it is not possible to attribute the consequences of therapy to these changes. For example a 2-fold rise in IL10 when responders are compared to placebo is not large (P = 0.15, so also not significant). Why do the authors think that the response to teplizumab differs between responders and non-responders? In this regard it would be of interest also to see data on gene expression in control CD8CM cells pre- and post-teplizumab exposure in vitro.

Discussion - one explanation for the increase in CD8CM cells in peripheral blood is presumably redistribution out of the pancreas. This possibility is not discussed by the authors.

Reviewer: 3

Comments to the Author

This study addresses the mechanism of action of Teplizumab in reducing progression of beta cell loss in

about half of the subjects in 4 clinical trials. The paper ignores the Phase III data and does not attempt to discuss why that trial did not meet its primary endpoint. In so doing it could be seen as a little misleading and some discussion of the negative trial and the current status of the drug would make the manuscript clearer especially to inexperienced readers. I note various mechanisms of action of anti-CD3 are proposed - should the recent studies of You and Chatenoud be cited?

In looking for an immunological biomarker of response the study examines the effect on CD8 T cell subsets in peripheral blood ""especially central-memory T cells. It confirms previous indications about changes in CD8 CM T cells but the changes are quite subtle and therefore it is important not to make too strong a case for this being "the"• mechanism. For example in Delay there is only a difference at one time point and in Abate also only significant at one time point.
In Fig 2 was blood not drawn prior to commencement of treatment why is there no "before"• sample shown are these data already published somewhere?

In Fig 3 in vitro studies are shown with anti-CD3 and anti-CD28 treatment of peripheral blood cells taken not from study subjects or people known to respond or not or even people with type 1 diabetes but from healthy controls. Unclear how these would relate to study subjects in age etc. Three controls were studied so one would not be confident that both responder types and non-responder types would be represented. The cells were incubated with both anti-CD3 and anti-CD28. As far as I can see no explanation is given for the addition of anti-CD28 which of course was not part of the treatment in Abate or Delay. The possibility that the cells change phenotype occurred to me and I see it is mentioned briefly in the Discussion.
I think a stronger rationale needs to be made for the experiments shown in Figure 3.

In Figure 4 gene expression in CD8 CM T cells from responders are compared with that population from untreated controls. What was the reason not to test cells from responders versus cells from non-responders i.e. both treated?
Can you show the comparison of gene expression between responders and no-responders? Is there any difference in gene expression between responders and non-responders prior to treatment?

First Revision – authors' response - 30-Aug-2015

European Journal of Immunology

Reviewer 1

Major comments:

1. The expansion and preferential in vitro proliferation of cells showing a CD8 CM phenotype at the end of a 3-day culture of PBMCs in the presence of teplizumab and anti- CD28 is interpreted as a preferential effect of teplizumab on this cell subset, explaining the association between prolonged increase of CD8 CM T cells and clinical response. However, CD3-mediated T cell activation in vitro impact the phenotype of T cell subsets present in the culture, especially the expression of CD45RO. It is thus likely that naïve CD8 T cells that proliferate vigorously in culture, acquired a CM phenotype in culture.
Only the analysis of the effect of teplizumab on sorted CD8 subsets could support this conclusion.

Response: We agree with this Reviewer's point that we did not know the source of the CD8 Central Memory (CD8CM) T cells that were expanded at the end of the cultures (Figure 3). To address this question, we tracked CD8CM T cells in the cultures that are shown in this Figure. (Had we just studied the CD8CM T cells we would not have been able to determine whether other populations differentiate into CM cells with anti-CD3 mAb.) We performed new experiments with PBMC from healthy control subjects, in which we sorted and labeled CD8CM T cells with Cell Tracer violet, labeled the remaining cells with CFSE, and then placed the two populations in culture for 3 days with teplizumab and anti-CD28 mAb or control Ig. After 1, 2, and 3 days, we determined the proportion of the CD8CM T cells that were labeled with CellTracer Violet or CFSE. At the end of the cultures, all of the cells still retained dye enabling us to identify their origin.

In the cultures with control Ig, about ½ of the CD8CM T cells were CellTracer Violet and about ½ were CFSE+ at each of the culture days. However, in the cultures with teplizumab and anti-CD28, there was a reduced proportion of CellTracer Violet CD8CM T cells (44% vs 34%). This suggests that the CD8CM T cells at the end of the culture, and presumably in vivo after treatment, are derived from the original CD8CM T cell pool as well as cells that become CD8CM cells when activated with anti-CD3 mAb. When we isolated and labeled naïve CD8+ T cells (i.e. CC45RO-CCR7+) with Cell Tracer, and added them to the cultures, we found that 3-9% of the cells became CD8CM cells after 3 days compared to < 1% of originally naïve cells in the control wells.

These new experiments are discussed in the revised "Results" section under "Source of CD8CM T cells" and in the new Figure 3D. We also have revised the Discussion to address this point.

2. Second, transient viral reactivation has been observed a few weeks after the end of the treatment in the AbATE trial and other CD3 antibody trials, associated in some cases with an expansion of virus-specific T cells with an effector/memory phenotype. Such cells could readily account for the expansion of CD8 CM T cells observed at month-1 in responders and non responders. This should be discussed.
Response: We agree. We did not measure viral antigen reactive T cells in the CD8 population and therefore cannot exclude that some of the CD8CM T cells were reactive with EBV or CMV.

However, data from the AbATE trial suggests that while there is an increase in the CD8CM population in patients who are EBV sero+, previous infection cannot account for the increase in the CM cells. The following are data from the AbATE trial showing the frequency of CD8CM T cells in responders who are EBV sero+ (n=9), EBV sero- (n=10), and 16 EBV sero- non-responders. (There was only 1 EBVsero+ non-responder.)

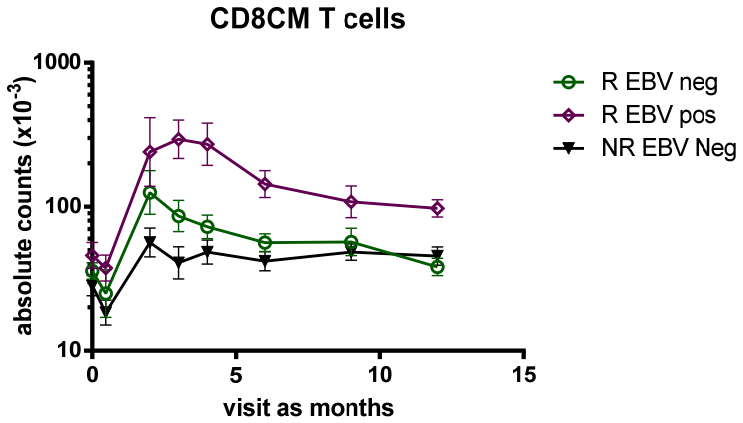

Figure 1: CD8 CM T cells in AbATE participants. Data are only available on 35 subjects who were enrolled after the institution of the appropriate flow panel. There was one EBVsero+ non-responder (not shown).

These data shows that in both the sero+ and sero- responders there is an increase in the frequency of CD8CM T cells compared to the sero- non-responders. The persistence of the CD8CM T cells was longer in the EBV sero+ individuals but the differences in the sero+ and sero- individuals compared to non-responders was seen at 2-3 months, when our studies were done, and both are clearly elevated to the baseline level which is similar to the frequency in the control subjects. We cannot exclude that responders are more likely to be EBV sero+ and that some of the CM T cells are EBV reactive. However, experience from the trial of otelixizumab, in which all participants were EBVsero+ suggests that this feature alone does not account for responsiveness to non-FcR binding anti-CD3 mAb (Keymeulen et al NEJM 2005). We have added text to the Discussion to address this limitation.

The gene expression profile of CD8 CM T cells 2-months post therapy was performed in responders versus placebo-treated controls. Additional comparison of the CD8 CM expression data in responders versus non responders would be useful to support the hypothesis that tepluzimab acts in responders through impairment of CD8 CM responsiveness. In fact, a sentence page 12, line 30 of the discussion alludes to the existence of this data in non responders.

Response: We agree that the in order to fulfill our objective to identify a signature of responders, a comparison to non-responders is needed. However, these data did not exist previously. Accordingly, we sorted CD8CM T cells from the samples of drug treated non-responders, responders and placebo treated patients, that we had, and analyzed gene expression by Nanostring. We normalized the data as described in the Methods, to eliminate any technical artifacts that may be introduced because of plate effects. Of the samples from 4 non-responders that we had available, one could not be adequately normalized and therefore this sample was not included in the analysis. We analyzed the data with three strategies. First, we were interested in identifying genes that were associated with clinical responses and therefore, pooled the data from the non-responders and placebo treated subjects. We compared the values of gene expression in these two groups using Wilcoxon tests. The results of this analysis, which identified 53 genes with significantly different levels of expression (i.e. at least a 1.5 fold change and $p<0.05$) are

shown in the revised Figure 4 and Table 2. Although we did not correct for multiple comparisons, 21 of the 53 comparisons had p values < 0.01 as shown in Figure 4. These genes fell into 3 categories suggesting reduced activation of CD8CM T cells from the responders, differentiation towards cells with regulatory function, and others. To identify differences between drug treated responders and non-responders, we then compared the data from the responders, non-responders, and placebo treated subjects by Kruskal-Wallis ANOVA. There were similar differences between the responders and the placebo treated non-responders and placebo treated subjects involving genes associated with cell activation and differentiation. These data are shown in Figures 5A and B. Finally, we used these data to perform a principle component analysis. This analysis identified two dimensions that separate the groups (Figure 6 and Table 3). This new analysis, which we feel is much superior to the analysis used previously for comparison of the data and includes non-responders, is presented in the Results section and the Figures (4-6) and Tables (2, 3) and Supplemental Figure 1 display the data. The Discussion has been extensively rewritten to reflect this new analysis.

Minor comment:

The timing of the first sampling for T cell analysis after the end of treatment in the Delay trial is unclear: 2 months as stated page 7, or 2 weeks as apparently shown in figure 2C?

Response: We have clarified this wording. The first timing of the first sample from Delay was at 2 mos. In the AbATE trial there was a sampling at 2 weeks.

Reviewer 2

This is an interesting story, although not entirely consistent with effects seen with other immunomodulatory drugs in diabetes. There are two main deficiencies in the work. Firstly, the authors do not provide any information about gene expression in non- responders to teplizumab. Unless this is distinct to the effects seen in responders then the therapeutic effects cannot specifically be attributed to these changes. Secondly, there are no functional data to corroborate the authors' suggestion that these changes at transcriptional level are ultimately down-regulatory. Thus intuitively one might have expected changes in EM T-cells to be more important, but conceivably CM cells might regulate EM for example. Without these two pieces of information the conclusions are speculative.

Response: We were glad to learn that the Reviewer finds the work of interest. We do not mean to imply that this drug and others that have been tested in T1D have similar mechanisms of action. We refer to the studies with CTLA4Ig and LFA3Ig (alefacept) in the introduction and point out the differences with anti-CD3 mAb. It should be noted, however, that to the best of our knowledge, studies similar to those reported here, such as the genetic profiling, have not been done with samples from these other clinical trials.

We agree with the need for comparison to non-responders. Please see the discussion above and the new Figures 4-6 and Tables 2,3 and Supplementary Figure 1.

We agree that functional studies of the CD8CM cells would be useful. However, in previous studies of T cells from patients treated with teplizumab and in humanized mice, we have shown that the CD8+ T cells have regulatory properties (Ablamunits EJI 41:1832, 2011, Waldron-Lynch Sci Trans Med 4: 118, 2012, and Bisikirska J Clin Invest 115:2904, 2005). Indeed, these new data support those previously reported functional studies using CD8+ T cells from patients in showing increased expression of IL10 and family member transcripts.

The study protocol did not permit recovery of a sufficient number of PBMC to perform additional functional studies and we do not have sufficient samples to carry out more extensive studies in vitro with purified cells. Most of the subjects were children (mean age =12 for both studies), and the volume of blood that is available after the flow analyses that were performed is insufficient to isolate sufficient numbers of cells for conventional suppression or similar assays. The CD8CM T cell subpopulation represents a relatively small fraction of the total CD8+ T cells.

Nonetheless, we confirmed findings from the nanostring analysis by showing that there was decreased expression of IL7r on the surfaces of CD8CM T cells from the responders (Supplementary Figure 1). In addition, we also found that the expression of CCL20 followed a pattern similar to the nanostring analysis. In the previous version of the manuscript we discussed and showed a difference in the expression of CCL20 in the responder and placebo groups, the addition of the non-responders showed that increased CCL20 expression was due to the anti-CD3 mAb treatment and did not discriminate responders and non-responders. We were unable to show a difference in the expression of CD3 on the surfaces of the CD8CM T cells. A number of the components of the CD3 complex may control the expression of CD3. These findings are highlighted in the Discussion.

Specific comments:

1. P3, first sentence of second paragraphs –not all of the trials referenced used teplizumab.

Response: Thank you for pointing this out. We have changed the wording.

2. P8. The absolute number of CD8CM cells in DELAY is not mentioned – did they increase also?

Response: Yes they did – this has previously been published (Diabetologia 56:391, 2013).

3. P9, Figure 4 and Figure 5. Is the P value corrected for multiple testing? I cannot find this information in the manuscript.

Response: The data in revised Figure 4 and Table 2 were not corrected for multiple testing. The data in Figures 5A and B, showing the Kruskal-Wallis ANOVAs were corrected for multiple comparisons. Table 2 shows the actual p values. If we were to use a threshold of 0.01, which would have been a reasonable threshold for the multiple testing (rather than a Bonferroni correction) we would have still identified 21 genes, in the same categories, that differed significantly. These include the following:

	pvalue	fold (2^)
CD3D	0.000	0.432495
IKZF1	0.000	0.292752
ITGA6	0.001	0.37246
KLRC2	0.001	0.182511
LCK	0.002	0.485966
IRF5	0.003	3.140155
TGFBR1	0.004	1.068
HLA-B	0.005	0.665225
CCND3	0.005	0.463681
IKBKB	0.005	0.401938
EBI3	0.005	0.563579
BAX	0.007	0.614124
SLAMF6	0.007	1.006649
ATG10	0.008	0.104377
ETS1	0.008	0.557285
IL22	0.009	0.580058
IL20	0.009	2.506867
RAG1	0.010	0.609769
CD34	0.012	0.56079
IDO1	0.012	0.641236
IL7R	0.014	0.475256

Table 1: Genes that show differences in expression with a p value of < 0.01 in a comparison of responders vs non-responders+placebo

These include the genes associated with signaling (red) and differentiation (blue) as we describe in the Results.

4. As above, without seeing equivalent expression profiles for non-responders, it is not possible to attribute the consequences of therapy to these changes. For example a 2-fold rise in IL10 when responders are compared to placebo is not large (P = 0.15, so also not significant).Why do the authors think that the response to teplizumab differs between responders and non-responders? In this regard it would be of interest also to see data on gene expression in control CD8CM cells pre- and post-teplizumab exposure in vitro.

Response: We agree with the need to compare the gene expression in responders and non-

responders. Please see above response to Reviewer 1 and the new data presented in Figures 4, 5, 6 and Tables 2,3 and Supplementary Figure 1. We agree that studies pre and post treatment in the same individual would be informative, but we do not have sufficient samples from the trial participants pre-treatment evaluate the responses in vitro to the drug prior to treatment. Moreover, our objective was to identify differences in gene expression that are associated with clinical responses and therefore, the time point after treatment, when the differences are seen seem most relevant.

Discussion – one explanation for the increase in CD8CM cells in peripheral blood is presumably redistribution out of the pancreas. This possibility is not discussed by the authors.

Response: This mechanism does not appear to be a likely explanation. First, the number of T cells in the islets of patients with T1D is relatively small and we believe it is unlikely that even if the drug could cause egress of the cells from the pancreas that it would significantly change the number of cells in the peripheral blood. Second, the drug affects all T cells. We agree with the Reviewer that the drug causes redistribution of T cells. We have included references to our previous work showing migration of T cells to the lamina propria in response to teplizumab.

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Reviewer 3

This study addresses the mechanism of action of Teplizumab in reducing progression of beta cell loss in about half of the subjects in 4 clinical trials.

1. The paper ignores the Phase III data and does not attempt to discuss why that trial did not meet its primary endpoint. In so doing it could be seen as a little misleading and some discussion of the negative trial and the current status of the drug would make the manuscript clearer especially to inexperienced readers. I note various mechanisms of action of anti-CD3 are proposed – should the recent studies of You and Chatenoud be cited?

Response: The Phase III trial (Protégé) did not meet its primary endpoint as indicated by the Reviewer. However the trial did show statistically significant improvement in C-peptide for 2 years and other markers of clinical efficacy such as the frequency of non-insulin requiring remissions and the frequency of subjects taking small doses of insulin with HgbA1c < 7%. There are a number of reasons why the Protégé trial did not meet its endpoint including: 1) the particular endpoint chosen for that trial had been unproven and was not based on experience from trials in T1D. (Had a different criteria been selected (insulin use that had been given to non-diabetic subjects in the Diabetes Prevention trial and a HgbA1c level corresponding to the ADA recommendations for treatment) the primary endpoint would have been met.) 2) the trial was done in Western and Eastern countries. The characteristics of patients with diabetes in the eastern countries (India, Eastern Europe) was very different from those in Western countries. These included lower C-peptide levels at study entry, extremely high levels of insulin usage, and poor glycemic control in the participants from India and Eastern Europe. The most significant effects on C-peptide responses were in participants from Western countries. These analyses have been published (Hagopian Diabetes 2013) and we refer to this paper in the revised Introduction and Discussion. Indeed, the findings from Protégé are completely consistent with those shown by Keymeulen et al (NEJM 2005) showing that enrollees with higher C-peptide levels at entry were more likely to respond to anti-CD3 mAb or, in earlier studies, to other immunologics (Bougneres NEJM 1988).

We agree that the work of Chatenoud and more recently of You and Chatenoud regarding the timing of the anti-CD3 mAb may be very relevant to our observations. We have, as suggested, added references to this work in reference to understanding which cells are affected by the drug in the Discussion. We also refer to the work of You et al concerning adaptive CD4+ Tregs induced with anti-CD3 mAbs.

2. In looking for an immunological biomarker of response the study examines the effect on CD8 T cell subsets in peripheral blood – especially central-memory T cells. It confirms previous indications about changes in CD8 CM T cells but the changes are quite subtle– and therefore it is important not to make too strong a case for this being "the" mechanism. For example in Delay there is only a difference at one time point and in Abate also only significant at one time point.

Response: We agree that this may not be "the" mechanism of anti-CD3 mAb and that others, such as deletion and induction of other regulatory cell populations have been proposed. Our new analysis of the CD8CM T cells from drug-treated responders and non-responders, and placebo treated subjects supports at least two mechanisms including effects of the anti-CD3 mAb on regulatory cells and mediators. We have modified the Discussion (4th para) to indicate this.

3. In Fig 2 – was blood not drawn prior to commencement of treatment – why is there no "before" sample shown – are these data already published somewhere?
Response: Samples were analyzed prior to the first dose of study drug. The data shown in Figure 2 are corrected for the baseline values. The baseline levels of cell subsets were not significantly different in the responders and non-responders in AbATE or Delay (p=0.55 and p=0.57 for CD8CM T cells in AbATE and Delay by Student's t-test).

4. In Fig 3 in vitro studies are shown with anti-CD3 and anti-CD28 treatment of peripheral blood cells taken not from study subjects or people known to respond or not or even people with type 1 diabetes but from healthy controls. Unclear how these would relate to study subjects in age etc. Three controls were studied so one would not be confident that both responder types and non-responder types would be represented. The cells were incubated with both anti-CD3 and anti-CD28. As far as I can see no explanation is given for the addition of anti-CD28 which of course was not part of the treatment in Abate or Delay. The possibility that the cells change phenotype occurred to me and I see it is mentioned briefly in the Discussion. I think a stronger rationale needs to be made for the experiments shown in Figure 3.
Response: The goals of the studies performed in vitro, shown in Figure 3, were to determine the effects of teplizumab and the source of the CD8CM T cells. Please see our response to the point raised by Reviewer 1 regarding the changes of the T cells in culture. To address this question we have performed additional studies to identify the source of the CD8CM cells that expand in culture. We are unable to perform similar lineage tracing studies in vivo and therefore, needed to rely on the studies in vitro which appear to reflect the changes in vivo. We used cells from the blood bank because a large number of PBMC were needed to sort subpopulations. We believe it is unlikely that the cells from patients would differ and it is not feasible to obtain large volumes of blood from children (age 12) to do these mechanistic studies. The revised Figure 3 includes the new experiments to determine the source of the CD8CM T cells

We, and others, routinely add anti-CD28 mAb to cultures to provide co-stimulatory signals. CD28 co-stimulatory signals are expected to be more available to cells in vivo than in vitro because of the widespread expression of CD80.

5. In Figure 4 gene expression in CD8 CM T cells from responders are compared with that population from untreated controls. What was the reason not to test cells from responders versus cells from non-responders i.e. both treated? Can you show the comparison of gene expression between responders and no-responders? Is there any difference in gene expression between responders and non-responders prior to treatment?
Response: We agree this comparison is important to do. Please see our response to Reviewer 1 and the new studies, shown in Figures 4, 5, and 6, Tables 2 and 3 and Supplementary Figure 1, which include a comparison of gene expression of CD8CM T cells to non-responders. We are unable to determine whether there are differences in gene expression at baseline but agree that this would be a very worthwhile experiment to do in a future study.

European Journal of Immunology

Second Editorial Decision - 02-Oct-2015

Dear Prof. Herold,

It is a pleasure to provisionally accept your manuscript entitled "Changes in T cell subsets identify responders to FcR non-binding anti-CD3 mAb (teplizumab) in patients with Type 1 diabetes" for publication in the European Journal of Immunology.

For final acceptance, please follow the instructions below and return the requested items as soon as possible as we cannot process your manuscript further until all items listed below are dealt with.

Please note that EJI articles are now published online a few days after final acceptance (see Accepted Articles: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1521-4141/accepted). The files used for the Accepted Articles are the final files and information supplied by you in Manuscript Central. You should therefore check that all the information (including author names) is correct as changes will NOT be permitted until the proofs stage.

We look forward to hearing from you and thank you for submitting your manuscript to the European Journal of Immunology.

Yours sincerely,
Laura Soto Vazquez

on behalf of
Dr. Lucienne Chatenoud

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