Interleukin-1 antagonism moderates the inflammatory state associated with Type 1 diabetes during clinical trials conducted at disease onset

Susanne M. Cabrera, Xujing Wang, Yi-Guang Chen, Shuang Jia, Mary L. Kaldunski, Carla J. Greenbaum, and the Type 1 Diabetes TrialNet Canakinumab Study Group, Thomas Mandrup-Poulsen and the AIDA Study Group, and Martin J. Hessner

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Handling Executive Committee members: Prof. Iain McInnes

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 – 8 September 2015

Dear Dr. Cabrera,

Manuscript ID eji.201546005 entitled "Interleukin-1 antagonism moderates the inflammatory state associated with Type 1 diabetes during clinical trials conducted at disease onset" 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. 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.**

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 Prof. Iain McInnes

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Reviewer: 1

Comments to the Author
The authors have examined the transcriptional effect of the serum from patients included in two RCT testing the efficacy of anakinra or canakinumab in patients with Type 1 diabetes. They observed that the sera of patients receiving the active treatment modulated the expression of different genes consistent with a global anti-inflammatory effect of both drugs.

The authors should be congratulated for the huge number of data collected and complex biostatistical analysis performed from these transcriptional studies. Sadly, the results of the clinical trials were negative and the observed transcriptional changes are only marginally associated with an effect on insulin production. We can only agree with the authors when they mention that additional research is needed to
identify other drugs targeting complementary pathways that may either alone or in combination with anti-IL-1 approaches lead to more effective preventive therapy.

My specific comments are the following:

Regarding the nomenclature: The authors should not use IL-1RN to design IL-1 receptor antagonist. IL1RN is usually used to describe the gene. IL-1Ra is generaly used for the protein.

The authors should provide more details regarding the cell line used in the studies. PBMC stands usually for peripheral blood mononuclear cells but as I understand they used a cell line. Is it an immortalized cell line? It corresponds to which cells: monocytes?

The authors should provide more details regarding the statistical analysis in the Methods or supplementary methods.

It is true that anakinra was shown to improve the beta-cell function in one trial. However, this finding was not confirmed in subsequent trials. The results with gevokizumab, an anti-IL-1b antibody, were negative despite a clear effect on inflammatory markers (CRP). The authors should also mention this result in their introduction to modulate the enthusiasm regarding anti-IL-1 therapies in this setting. It is of course still possible that IL-1a may be more important in Type 2 diabetes but this should also be discussed.

The authors mentioned that IL-1Ra has altered the inflammatory state in patients included in the RCT. What is exactly the level of inflammation in the patients at baseline? How does it compared to healthy controls?

Reviewer: 2

Comments to the Author
Cabrera and coworkers performed transcriptional analysis using plasma from diabetic individuals treated with Anakinra and anti-IL-1 beta versus placebo and reporter PBMCs from an unrelated subject. They concluded that inflammation was reduced and Treg activity was enhanced in both trials, as evidenced by the in vitro induction of IL-10 and TGF-β dependent genes. Major issues in this article are the data presentation and the experimental approach used (see below).
1) The data presented in the heat maps are difficult to follow. The authors should present those statistically significant findings (at least from pro-inflammatory and anti-inflammatory or other relevant genes) in a table together with raw numbers, fold increase or decrease and adjusted p values.
2) It is difficult to assess the significance of the data without any indication as to the plasma components that could be responsible for the changes detected in the reporter PBMCs.

3) The authors do not discuss potential effects induced by additives present in the agents used in the clinical trials.

4) The authors need to present validation data (from representative subjects) to demonstrate that their findings are a true reflection of the inflammatory status in treated versus untreated (placebo) subjects.

5) Were the data adjusted for variables such as age, disease duration, and hemoglobin A1C?

6) There are no data regarding the subject from whom the reporter PBMCs were taken.

First revision – authors’ response – 14 September 2015

Reviewer: 1

Comment: Regarding the nomenclature: The authors should not use IL-1RN to designate IL-1 receptor antagonist. IL1RN is usually used to describe the gene. IL-1Ra is generally used for the protein.
Response: The manuscript, figures and legends have been edited accordingly.

Comment: The authors should provide more details regarding the cell line used in the studies. PBMC stands usually for peripheral blood mononuclear cells but as I understand they used a cell line. Is it an immortalized cell line? It corresponds to which cells: monocytes?
Response: In the revised manuscript, we clarify that the reporter cell population are cryopreserved PBMC of a single well-characterized healthy blood donor.

To further clarify, we provide additional details here. To complete our seminal study (Wang et al., J Immunol. 2008; 180(3):1929-37), we used fresh PMBCs of >15 healthy donors. Although modest, responder cell-specific effects were evident. We have aimed to control the responder cell population in order to 1) reduce variability and improve our sensitivity to differentiate immune states; and 2) standardize the assay, making results generated over time more comparable than that of fresh cells collected from multiple donors or successive draws of the same individual.

We have published an unfavorable evaluation of lymphoid and myeloid cell lines as PBMC surrogates (Jia et al., Physiol Genomics. 2011; 43(11):697-709). We found that each cell line tested could differentiate recent onset (RO) T1D versus unrelated healthy control (uHC) plasma, but their responses were distinct from one another and that of fresh PBMC. Importantly the responses of the tested cell lines were less biologically informative compared to PBMC.

Commercial providers now offer highly viable, cryopreserved PBMC. In a single draw of a healthy well-characterized donor, billions of cells, sufficient for thousands of assays are collected by aphaeresis. We tested PBMC of 5 different donors. Donor UPN727 PBMC cells (Cellular Technology Ltd) were selected as they closely mimicked the mean response of the freshly isolated PBMC of multiple donors we used...
Previously (Levy et al. 2012; Genes Immun. 13(8):593-604). These cells were used in our most recent studies where we differentiated immune states among RO T1D probands, their autoantibody negative siblings, and uHC (Chen et al., Diabetes. 2014 63(11):3960-73). We have purchased the entire UPN727 lot and have been using them in our ongoing studies, including this one. We have also confirmed that the future identification of suitable PBMC donors will not be an obstacle going forward. In the assessment of the 5 donors, we found that the response of UPN524 PBMC correlated well with UPN727 when challenged with RO T1D and uHC plasma. Specifically the Pearson’s correlation coefficient between the union of the 1% tails of the RO T1D:uHC ratio distributions was 0.63. When the analysis was restricted to the intersection of commonly identified probe sets, the Pearson’s correlation coefficient was 0.85.

Comment: The authors should provide more details regarding the statistical analysis in the Methods or supplementary methods.
Response: The methods section of the manuscript has been edited accordingly.

Comment: It is true that anakinra was shown to improve the beta-cell function in one trial. However this finding was not confirmed in subsequent trials. The results with gevokizumab, an anti-IL-1b antibody, were negative despite a clear effect on inflammatory markers (CRP). The authors should also mention this result in their introduction to modulate the enthusiasm regarding anti-IL-1 therapies in this setting. It is of course still possible that IL-1a may be more important in Type 2 diabetes but this should also be discussed.
Response: Thank you for bringing up the study of gevokizumab in Type 2 diabetes. It offers interesting parallels to the findings of the canakinumab study in Type 1 diabetes. We have edited the introduction and discussion accordingly.

Comment: The authors mentioned that IL-1Ra has altered the inflammatory state in patients included in the RCT. What is exactly the level of inflammation in the patients at baseline? How does it compared to healthy controls?
Response: Respectfully, the analyses provided in the original manuscript have done this to the extent possible, as defined by the present limitations of our analytical approach. In our recent report (Chen et al., Diabetes. 2014 63(11):3960-73) we examined 47 RO T1D patients that were analyzed during the new onset period, corresponding to the baseline measurement in the AIDA and TN-14 trials. Currently, in contrast to an ELISA, our analyses using serum/plasma induced transcriptional analyses measure relative differences. These relative measurements are best done using samples collected under an identical protocol and analyzed by plasma induced transcription at the same time (versus years apart). Our local RO T1D and uHC were collected at a different time, under different conditions, and analyzed independently of those analyzed in the AIDA and TN-14 trials. These same limitations apply to direct comparisons of the samples collected in the AIDA and TN-14 trials. While this reality presently precludes
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direct comparisons of plasma induced transcription data collected under one study to that collected under another (e.g. directly comparing baseline samples in the trials to our local RO T1D samples), it is still possible, and appropriate, to compare relative changes between the studies. The analyses shown in Figures 2 and 5 are aimed at addressing the relative relationships between RO T1D and uHC analyzed in our published cross-sectional studies versus placebo and treated subjects analyzed in each of the two trials.

Indeed, the placebo treated subjects were more correlative to RO T1D patients while the treated subjects were more similar to uHC; specifically the RO T1D and placebo arms exhibited greater inflammatory bias whereas the uHC and treated arms exhibited greater regulatory bias. As described in this study and our recent report (Chen et al., Diabetes. 2014 63(11):3960-73), we are developing algorithms for scoring plasma induced signatures. However, at this time, newer approaches that are based solely on scoring of fluorescence intensities of a single array to allow for independent analysis, are not refined enough to allow us to confidently compare scores between studies. This is an ongoing line of development.

Reviewer: 2
Comment: The data presented in the heat maps are difficult to follow. The authors should present those statistically significant findings (at least from pro-inflammatory and anti-inflammatory or other relevant genes) in a table together with raw numbers, fold increase or decrease and adjusted p values.
Response: In the revised manuscript, additional tabulated data has been provided to accompany the annotated heat maps in Figures 1, 2, and 5. Specifically, in Figure 1G, the fold of change, p-value and false discovery rate (used to assess impact of multiple testing) are now provided for the annotated transcripts at the 1 month time point. In the original manuscript, the annotated heat maps shown in Figures 2 and 5 reflected the average change observed in the treatment and placebo arms at all the time points. In the revised Figures 2 and 5, the average fold of change between the two treatment arms at all time points is now shown both in the heat map and is tabulated. As in the original manuscript, all data is downloadable from GEO, and Supplement 2 has been revised to include all fold of change, p-values and FDRs. This file is provided as a PDF, however it states that a sortable spreadsheet will be available upon request for readers, making it possible to rapidly call up any of the data subsets presented in any of the heat maps.

Comment: It is difficult to assess the significance of the data without any indication as to the plasma components that could be responsible for the changes detected in the reporter PBMCs.
Response: In T1D, cytokines, chemokines, and other mediators may be at locally high concentrations within the islets and associated lymph nodes, but are often too dilute in the periphery for direct measurement. Further, our published studies (namely, Chen et al., Diabetes 2014 63(11):3960-73) support that measurement of a single or few cytokines may be uninformative or even misleading due to important combinatorial effects. In fact, we did not detect any differences in a multiplex cytokine analysis
between RO T1D probands and their healthy autoantibody negative siblings (Chen et al., Diabetes 2014). In that study, we used receptor blockade studies to confirm the IL-1 dependence of the RO T1D signature and the IL-10 and TGF-β dependencies we observed in the continuum of immune states seen in their healthy siblings and unrelated healthy controls.

In this currently submitted report, the amount of material available from the AIDA and TN-14 trials precluded the extensive multiplex ELISA and cytokine add-back and receptor blockade studies we had previously performed in Chen et al. Respectfully, we submit that the findings of this report are reasonably supported and significant when viewed from the foundation established by our previous array-based, cytokine-based, and flow-cytometry studies, which are described in more detail with in the introduction, and because the varying levels of the predicted immunoregulation were observed among the treated subjects.

Comment: The authors do not discuss potential effects induced by additives present in the agents used in the clinical trials.

Response: Reviewer 2 raises an interesting point in mentioning possible effects of additives used in the AIDA and TrialNet Canakinumab trials. While we feel any effects on the signatures would be negligible as additives are largely assumed to be biologically and immunologically inert, it is a variable to be considered in this and future studies.

Specific to TN-14, canakinumab was administered in a solution containing sucrose, L-histidine, and polysorbate 80. The placebo was an identical isotonic histidine buffered sucrose solution containing polysorbate 80. In the unlikely event these additives exerted any effect on the signature, it would have been an identical effect between placebo and canakinumab arms, making any differences in the signature due solely to the therapeutic agent and not the additives.

The excipient for anakinra used in the AIDA trial is proprietary but contains disodium EDTA, sodium chloride, anhydrous citric acid, and polysorbate 80 in water. It was developed originally for the infusion bags needed for intravenous administration in sepsis clinical trials, as the acid buffer is thought to stabilize the anakinra protein at room temperature. This excipient was also used for the subcutaneously delivered anakinra preparations used in AIDA trial as the manufacturer (Amgen) did not want to go through an additional approval for a new formulation.

The placebo arm of the AIDA trial received only normal saline (0.9% sodium chloride) because the AIDA ethical committee did not approve the use of the excipient as placebo. This decision was based upon the thought that the acidic excipient solution, versus the anakinra protein itself, was responsible for causing the transient local injection reactions (skin lesions) observed in about 15% of cases in the intravenously administered anakinra and placebo in the clinical sepsis trial (Opal SM et al., Crit Care Med. 1997 Jul;25(7):1115-24). Larsen et al (N Engl J Med. 2007; 356(15):1517-26) also found 40% of adults with type 2 diabetes developed transient injection-site reactions with anakinra versus 0% in the saline placebo group, but that these disappeared after 19-38 days of daily subcutaneous injections. Importantly, the therapeutic response (measured by glycated hemoglobin) did not correlate with the local injection
reaction, suggesting the acid excipient did not meaningfully mitigate the systemic benefits provided by the anakinra protein. Given this finding, the degree of dilution of excipient within the volume of the body, as well as the buffering capacity of plasma, we feel it is unlikely that the excipient the anakinra-treated subjects received resulted in any significant changes to our signatures. Given the length and density of the manuscript, and the other additions made in response to the review, we have not brought this important, but likely not data influencing, issue into the discussion section. If the reviewer feels strongly about this point, we are more than willing to edit this into the discussion.

Comment: The authors need to present validation data (from representative subjects) to demonstrate that their findings are a true reflection of the inflammatory status in treated versus untreated (placebo) subjects.
Response: As mentioned above, the amount of material available from the AIDA and TN-14 studies precluded extensive follow-up studies. The analyses shown in Figures 2 and 5 are aimed at addressing the relative relationship between RO T1D and uHC (where extensive validation was conducted and is now more fully described in the introduction, Chen et al., Diabetes. 2014 63(11):3960-73) versus placebo and treated subjects in each of the two trials. These analyses show that the placebo treated subjects more closely correlate with RO T1D patients and the treated subjects more closely correlate with uHC, supporting that varying levels of the predicted immunoregulation was observed.

Comment: Were the data adjusted for variables such as age, disease duration, and hemoglobin A1C?
Response: As reflected by the edited methods section in the revised manuscript, while thoroughly investigated, the data were not adjusted for variables such as age, disease duration, and HbA1c levels.

Comment: There are no data regarding the subject from whom the reporter PBMCs were taken.
Response: The manuscript has been revised accordingly. Please see above as this inquiry was extensively addressed for reviewer 1.

Second Editorial Decision – 8 October 2015

Dear Dr. Cabrera,

Thank you for submitting your revised manuscript ID eji.201546005.R1 entitled "Interleukin-1 antagonism moderates the inflammatory state associated with Type 1 diabetes during clinical trials conducted at disease onset" to the European Journal of Immunology. Your manuscript has been re-reviewed and the comments of the referees are included at the bottom of this letter.
Unfortunately, Referee 1 was not satisfied with the revisions made and further major revision is requested. In particular, this referee feels that the way the data are presented are not optimal to come to any confident/credible conclusion from the data. The Executive Editor and I tend to agree: the submission shows many heatmaps with significances reported only for some of the data. However, I do understand that you may have wanted to include all data in the spirit of transparency. We ask that you provide adjusted p values for all heatmap data and, to draw attention to the main findings and message of your manuscript, a "roadmap" type table to show the genes they feel were upregulated/downregulated.

The journal does not encourage multiple rounds of revision and you should fully address the concerns of the referee in this final round of revision. 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.*

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 referees 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,
Karen Chu

On behalf of Prof. Iain McInnes

Dr. Karen Chu
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Reviewer: 1

Comments to the Author
The main issue with this manuscript is the way the data are presented, as it is currently buried under an immense amount of heat maps and numbers (Supplementary Table 2). For example, it is currently unclear what genes were significantly up-regulated or down-regulated. The heat maps can be moved to the supplementary section whereas the raw data on which the authors base their conclusions should be clearly presented in tables with the fold increase, p values and adjusted p values.

The authors need to base their conclusions on statistically significant data (adjusted p values). For example, they state that “An intersection of 75 probe sets, regulated between the two arms at all time points was identified that consisted of transcripts down-regulated by plasma of IL-1Ra-treated participants (Figure 1G). These were primarily transcripts encoding inflammatory mediators regulated by IL-1…” The data shown in Figure 1G are not convincing, since the adjusted p values indicate that only the level of CXCL1 was significantly altered.

Figures 2 and 5: Why aren’t the adjusted p values shown?
The authors should discuss the limitations of their experimental approach.
Supplementary Table 2 should present only those genes that were significantly altered. All the other numbers confuse and hinder the potentially important observations.

Second revision – authors’ response – 10 November 2015
Reviewer 1

Comment: The main issue with this manuscript is the way the data are presented, as it is currently buried under an immense amount of heat maps and numbers (Supplementary Table 2). For example, it is currently unclear what genes were significantly up-regulated or down-regulated. The heat maps can be moved to the supplementary section whereas the raw data on which the authors base their conclusions should be clearly presented in tables with the fold increase, p values and adjusted p values.

Response: We agree with Reviewer 1 that, by itself, an initial threshold of $|\log_2 \text{ratio}| > 0.263$, 1.2-fold; ANOVA $p<0.05$ is modest. This threshold was not randomly applied, but rather used under several relevant contexts. In blinded analysis, 70.2% ($p=0.006$) of AIDA and 68.9% ($p=0.02$) of TN-14 participants were correctly called to their treatment arm. Further, significant proportions of the probe sets regulated between the treatment and placebo arms of the AIDA ($n=358/827$, 43.3%, $X^2 p<10E-06$) and TN-14 ($n=238/602$, 34.8%, $X^2 p<10E-6$) trials at this modest threshold were previously identified in our cross-
sectional analyses of new onset cases and healthy controls (Chen et al., 2014 Diabetes 63: 3960-3973) using thresholds of $|\log_2 \text{ratio}| >0.263$, 1.2-fold; ANOVA $p<0.05$; FDR $<20\%$. As part of all global gene expression analyses, we do calculate a false discovery rate (FDR) as a means of multiple test correction. Among the regulated transcripts respectively identified the AIDA and TN-14 trials, 495/827 (59.9\%) and 323/602 (53.7\%) exhibited an FDR $\leq30\%$ at $\geq1$ time point, respectively. In the AIDA study 156 probe sets (18.9\%) met only the $|\log_2 \text{ratio}| >0.263$, ANOVA $p<0.05$ threshold. In the TN-14 study 149 probe sets (24.8\%) met only the $|\log_2 \text{ratio}| >0.263$, ANOVA $p<0.05$ threshold.

It is the opinion of the authors that there is no hard consensus within the field of functional genomics as to what FDR level must be applied, rather it is most appropriate to make an informed decision based upon what might be acceptable for a given experiment. In this case, where we examined 2 clinical trials that failed to meet their primary endpoints with the objective of determining whether there was any evidence of immunomodulation among the treated subjects, a significant proportion of the identified transcripts (AIDA: $>81\%$, TN-14: $>75\%$) either passed a $\leq30\%$ FDR cut off and/or were previously identified in our studies comparing new onset T1D patients to related and unrelated control cohorts at an FDR $<20\%$.

Another important consideration relevant to our analysis is the fact that the expression level of a given gene is dependent on its ontology/function. Our colleague and coauthor Dr. Wang (formerly with our group in Wisconsin and now director of the Systems Biology Core at NIH/NHLBI), was among the first to identify this important, now recognized relationship. Please see:

Y. Xie et al., 2004. The dynamic range of gene expressions depend on their ontology. Proceedings of the 2004 IEEE Computational Systems Bioinformatics Conference (CSB 2004). (Attached).

J. Li et al., 2010. Exploiting the determinants of stochastic gene expression in Saccharomyces cerevisiae for genome-wide prediction of expression noise. PNAS: 107(23): 10472–10477.

J.C. Mar et al., 2011. Variance of Gene Expression Identifies Altered Network Constraints in Neurological Disease. PLoS Genet 7(8): e1002207. doi:10.1371/journal.pgen.1002207.

These studies show that genes occupying important positions in interaction networks (e.g. those that interact with many, or influence or regulate the expression of other genes) exhibit smaller dynamic ranges of expression. This relationship was evident in our previous report (Chen et al., 2014 Diabetes 63: 3960-3973) where the induction of transcripts encoding proinflammatory mediators by T1D plasma was more robust than the induction of regulatory transcripts by plasma of the related and unrelated healthy control cohorts in cross-sectional analyses. In this study, we observe the induction of many of the same IL-10/TGFB dependent “regulatory” transcripts in subjects treated with either IL-1Ra or Cankinumab. Here,
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this group of transcripts also exhibited less robust induction among treated patients as compared to the reductions observed in induction of transcripts encoding inflammatory mediators (as reflected by those annotated Figures 2 and 5). In contrast to our previous study of new onset cases versus healthy controls, here when applying the (| log2 ratio | >0.263, 1.2-fold; ANOVA p<0.05) threshold, proportionally fewer transcripts were annotated as “regulatory” versus “inflammatory”. Given these were immunomodulatory clinical trials that did not meet their primary endpoint, this is not surprising. However it, does present problems in the pathway analyses and in the application of our gene ontology-based scoring algorithm which is needed to correlate residual beta cell function with the plasma induced signatures. Specifically, in past analyses, we have typically found pathway analyses most informative when we are able to use inputs of >500 transcripts; this is because reasonable coverage of the pathways is necessary to statistically assess their significance. These factors further influenced our use of the data sets defined by | log2 ratio | >0.263, 1.2-fold; ANOVA p<0.05. As reflected in by the GO Terms tabulated Figures 2 and 5, co-expression of inflammatory and regulatory transcripts regulated at these thresholds led to significant identification of many of the same or terms previously defined by the data set described in Chen et al., 2014 (Diabetes 63: 3960-3973).

These are the rationale we used to approach the data sets generated from these completed T1D clinical trials. In the revised manuscript, the statistical relationships are now clearly indicated in Figures 1 and 4, as well as in the narrative. We have retained the heat maps in Figures 1 and 4, as we feel it is important to show all the data on which the pathway analyses and relationships with stimulated C-peptide are based; further it is not feasible to tabulate this quantity of information within the manuscript itself. To further clarify transcripts differentially regulated between the treatment and placebo arms of the AIDA and TN-14 trials, transcripts meeting (| log2 ratio | >0.263, 1.2-fold; ANOVA p<0.05; FDR≤30%) are now tabulated in Supplements 3 and 4, respectively. Respectfully, we find the significantly identified GO Terms and the relationships between the Inflammatory Indices and preservation of stimulated C-peptide nonrandom and evidence of modest immune modulation within subset of treated patients.

Comment: The authors need to base their conclusions on statistically significant data (adjusted p values). For example, they state that “An intersection of 75 probe sets, regulated between the two arms at all time points was identified that consisted of transcripts down-regulated by plasma of IL-1Ra-treated participants (Figure 1G). These were primarily transcripts encoding inflammatory mediators regulated by IL-1...”The data shown in Figure 1G are not convincing, since the adjusted p values indicate that only the level of CXCL1 was significantly altered.
Response: Respectfully, as discussed above, it is the author’s opinion that an FDR of 1% is too stringent for these data sets, as we are examining two clinical trials that failed to meet their primary endpoint for evidence of immune modulation.

Comment: Figures 2 and 5: Why aren’t the adjusted p values shown?
Response: In Figures 2 and 5, p-values and adjusted p-values were not shown because the annotated heat maps illustrated were of the mean fold of change response observed for all time points measured in each of the two trials. The tabulated data accompanying the heat maps were also the mean fold of change between the two arms at all time points. In the revised Figures 2 and 5, letters are used to denote which transcripts reach FDR≤30% and at what time points.

Comment: The authors should discuss the limitations of their experimental approach.
Response: In the discussion we now include text that speaks to one possible limitation of the plasma induced transcription assay, which is the necessity to identify a representative responder cell population, as heterogeneity may exist between fresh cells collected from different healthy individuals or even between successive draws of the same person. Vendors now offer highly viable, cryopreserved peripheral blood mononuclear cells (PBMC) that have been collected by aphaeresis. Quantities of cells, sufficient for thousands of assays, can be prepared from a single draw of a healthy well-characterized donor. This greatly simplifies the process of testing a panel of candidates and identifying a representative responder cell donor.

Comment: Supplementary Table 2 should present only those genes that were significantly altered. All the other numbers confuse and hinder the potentially important observations.
As described above, transcripts meeting ( |log2 ratio| >0.263, 1.2-fold; ANOVA p<0.05; FDR≤30%) are now tabulated in Supplements 3 and 4, respectively.

Third Editorial Decision – 26 November 2015

Dear Dr. Cabrera,

It is a pleasure to provisionally accept your manuscript entitled "Interleukin-1 antagonism moderates the inflammatory state associated with Type 1 diabetes during clinical trials conducted at disease onset" 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.
Peer review correspondence

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

Yours sincerely,
Karen Chu

on behalf of Prof. Iain McInnes

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