JCB

Signal strength controls the rate of polarisation within CTLs during killing

Gordon Frazer, Christian Gawden-Bone, Nele Dieckmann, Yukako Asano, and Gillian Griffiths

Corresponding Author(s): Gillian Griffiths, University of Cambridge

Review Timeline:

Submission Date:	2021-04-20
Editorial Decision:	2021-05-27
Revision Received:	2021-06-03
Editorial Decision:	2021-06-15
Revision Received:	2021-06-21

Monitoring Editor: Ira Mellman

Scientific Editor: Tim Spencer

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1083/jcb.202104093

May 27, 2021

Re: JCB manuscript #202104093-T

Prof. Gillian M Griffiths
University of Cambridge
Cambridge Institute for Medical Research
Cambridge Biomedical Campus
Cambridge CB2 0XY
United Kingdom

Dear Prof. Griffiths,

Thank you for submitting your manuscript entitled "Signal strength controls the rate of polarisation within CTLs during killing". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that Rev#1 and #3 are positive about the degree of advance, and so are we. Really, only Rev#2 suggested any major new experiments: enhancing the advance through functional perturbations of killing, e.g., by inhibiting or engaging co-stimulation or with immune checkpoint inhibition. These are interesting suggestions that we encourage you to consider seriously; however, we do not feel strongly about this additional line of investigation. Please do address the reviewers' other comments. Please do not hesitate to contact us to discuss the revisions or if you anticipate any issues addressing the reviewers' remarks.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for an Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, https://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data.

Articles may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors once your lab has reopened to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Ira Mellman, Ph.D.
Editor, Journal of Cell Biology

Melina Casadio, Ph.D.
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

This is an interesting study that utilizes 4D imaging to determine the impact on TCR signal strength (using OTI cells and altered peptide ligands [APL]) on various aspects of the cytolytic process - target engagement, centrosome polarization/docking at the synapse, granule polarization and calcium release. The overall conclusion from this study is that while the steps required for the development of cellular cytotoxicity are independent of TCR signal strength, the rate of their occurrence is significantly increased in T cells engaging high affinity MHCI-ligand complexes. Several recent publications from this group, aimed at examining the impact of APLs on TCR signaling, revealed that signaling and target gene transcription were similar between cells stimulated with APLs, but the rate and proportion of cells that got activated with high affinity ligand was significantly increased over those stimulated with low affinity ligand. Thus, this current work, is a natural extension of these studies.

This is a well-designed study and there are no specific concerns regarding the data or it's interpretation. However, a description of the statistical analysis used in Figure 2 to generate the ** shown in Figure 2c-f is not provided.

There are several typos throughout the document that should be fixed (e.g. first line top of page 10 - thee is a missing 'D' for CD8 T; Figure 1 legend - there is an extra OTI). In many instances micron is spelled 'um' and in other instances it has the correct 'mu' symbol.

Reviewer #2 (Comments to the Authors (Required)):

In this manuscript, Frazer et al analyze the intracellular steps of T cell killing after TCR engagement, comparing high intermediate and low avidity TCR ligands. They show that, as expected, high avidity ligands promote more efficient killing, increase interaction times with the targets and enhance depletion of actin from the synapse. Increasing TCR signal strength also increased the frequency of prolonged primary calcium flux. The results are consistent and straight forward. This is a nice cell biology study of synapse and post-synapse kinetics induced by TCR ligands of different avidities.

The conclusions of the study, however, are also a bit disappointing. The abstract concludes: "Hence TCR signal strength modulates the rate but not organisation of effector CTL responses." In fact, previous studies, cited by the authors, have shown differences in dwelling times and killing efficiencies according to the strength of TCR engagement. Showing that subsequent steps due to these interactions are also increased in rate is not trivial, but is still quite predictable.

Including the analysis of functional perturbations of killing, for example by inhibiting or engaging co-stimulation, or by immunecheckpoint inhibitors, would increase my enthusiasm for the manuscript.

Reviewer #3 (Comments to the Authors (Required)):

This is a well-done kinetic analysis of polarization of T cells in response to different strengths of T cell receptor stimulation based on live cell spinning disc confocal microscopy. While killing by the CD8 T cells decreased with decreasing potency of peptide-MHC ligands the key to this appeared to be the decreasing proportion of cells that carried out key steps including dwelling with the target for longer, clearing F-actin from the synapse, polarizing the centrioles and granules and sustaining a calcium flux. While the proportions changed, the many of steps took place with the same kinetics in cells that were engaging the target. Some specific points need to be addressed.
1. In relation to Figure 3c, I didn't understand how the description in the text that all the N4 activated cells docked their centrosomes at the synapse related to the data in Figure 3c that only about 20% of the cells scored positive for centrosome docking.
2. In supplementary figure 2 the analysis of the speed of centrosome movement is potentially very interesting, but I don't understand why the curves are so smoothed. Was a model applied to the data to draw these sinusoidal looking lines or is this just a plotting option. I would prefer to see the actual data points and perhaps a line could be drawn once the pattern of the data is clear.
3. Regarding discussion of prior literature, there has been quite a bit of work form Huse on both centrosome recruitment to the synapse (papers by Quann et al) and an interesting recent paper in PNAS that suggested that a centrosome is not needed for directed degranulation (Tamzalit et al, 2020). While the Quann et al data is collected using a photoactivatable DAG, the rate of movement of the centrosome looks to be similar to what is shown here.
4. Another recent paper that could be cited regarding regulation is from Hooikaas et al 2021 Elife identified KIF21B as an important proteins to enable rapid centrosome repositioning. This paper just provides additional insight into the process that is regulated by the pMHC potency.

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Response to reviewer's comments:

We were delighted with the comments from the reviewers and thank them for their time.

Reviewer 1:

We have added a description of the statistical methods used for Figure 2 in the legend and also corrected as many typos as we could find between us.

Reviewer 2:

We appreciate that it is possible to view the results as not perhaps as exciting as if, for example, the centrosome moved faster with stronger signals. However, the biological answer is much more elegant. Rather than changing the machinery and mechanics to vary intracellular polarisation, CTLs simply vary the rate at which individual cells proceed to initiate the mechanics that are already in place to trigger polarisation. Our data points to this at each stage of polarisation and supports a rate-based mechanism for TCR signalling controlling polarisation. We have tried to emphasise this very novel (and unexpected) finding in revisions in the discussion, and added a heading to the final part of the discussion where this concept is described.

Reviewer 3:

1. *In relation to Figure 3c, I didn't understand how the description in the text that all the N4 activated cells docked their centrosomes at the synapse related to the data in Figure 3c that only about 20% of the cells scored positive for centrosome docking.*

We agree that this was not clear in the text and have now clarified the difference between the data shown in Figure 3b and 3c in the text on pages 4-5. Figure 3b shows the analysis from "tracking the centrosome within individual CTLs that formed a stable conjugate with a target cell," while Figure 3c shows "a population of CTLs interacting with targets over a 40-minute period, including transient interactions (Figure 3c)"

2. *In supplementary figure 2 the analysis of the speed of centrosome movement is potentially very interesting, but I don't understand why the curves are so smoothed. Was a model applied to the data to draw these sinusoidal looking lines or is this just*

a plotting option. I would prefer to see the actual data points and perhaps a line could be drawn once the pattern of the data is clear.

This was indeed a plotting option, selected as having the actual time points measured made the figure rather crowded and so more difficult to see. We are including both options to allow an editorial decision on which to include.

3. Regarding discussion of prior literature, there has been quite a bit of work form Huse on both centrosome recruitment to the synapse (papers by Quann et al) and an interesting recent paper in PNAS that suggested that a centrosome is not needed for directed degranulation (Tamzalit et al, 2020). While the Quann et al data is collected using a photoactivatable DAG, the rate of movement of the centrosome looks to be similar to what is shown here.

Thanks for pointing this out! Quann et al was originally there, but was somehow deleted during revisions. We have now included reference to these papers in the revised discussion on centrosome polarisation on p9-10.

4. Another recent paper that could be cited regarding regulation is from Hooikaas et al 2021 Elife identified KIF21B as an important proteins to enable rapid centrosome repositioning.

We have included reference to this paper in the revised discussion on centrosome polarisation on p9-10.

June 15, 2021

RE: JCB Manuscript #202104093R

Prof. Gillian M Griffiths
University of Cambridge
Cambridge Institute for Medical Research
Cambridge Biomedical Campus
Biomedical Campus
Cambridge CB2 0XY
United Kingdom

Dear Gillian:

Thank you for submitting your revised manuscript entitled "Signal strength controls the rate of polarisation within CTLs during killing". We've now had an opportunity to assess your revisions and we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).
With regard to the specific query concerning Supplementary Figure 2, we feel that the second version (2B) would be the preferred presentation.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

1) Text limits: Character count for Articles is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, and acknowledgments. Count does not include materials and methods, figure legends, references, tables, or supplemental legends. You are below this limit at the moment but please bear it in mind when revising.

2) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

3) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (both in the figure legend itself and in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, since you used parametric tests in your study (e.g. t-tests, ANOVA, etc.), you should have first determined

whether the data was normally distributed before selecting that test. In the stats section of the methods, please indicate how you tested for normality. If you did not test for normality, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

4) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions (at least in brief) in the text for readers who may not have access to referenced manuscripts. The text should not refer to methods "...as previously described."

5) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies.

6) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

7) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication (do not use numbered references). Abbreviate the names of journals according to PubMed.

8) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. At the moment, you are below this limit but please bear it in mind when revising.
Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material (that is, other than the supplementary figure legends) should appear at the end of the Materials and methods section.

9) eTOC summary: A ~40-50 word summary that describes the context and significance of the findings for a general readership should be included on the title page. We realize that you have provided one already but we ask that the statement refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.

10) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

11) A separate author contribution section is required following the Acknowledgments in all

research manuscripts. All authors should be mentioned and designated by their first and middle initials and full surnames. We encourage use of the CRediT nomenclature (https://casrai.org/credit/).

12) ORCID IDs: ORCID IDs are unique identifiers allowing researchers to create a record of their various scholarly contributions in a single place. At resubmission of your final files, please consider providing an ORCID ID for as many contributing authors as possible.

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and MP4 video files: See our detailed guidelines for preparing your production-ready images, https://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g. if you cannot retrieve necessary files from your laboratory, etc.), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Ira Mellman, Ph.D.
Senior Editor
The Journal of Cell Biology

Tim Spencer, PhD

Executive Editor
Journal of Cell Biology
