Hematopoietic progenitors polarize in contact with bone marrow stromal cells in response to SDF1.

Thomas Bessy, Adrian Candelas, Benoit Souquet, Khansa Saadallah, Alexandre Schaeffer, Benoit Vianay, Damien Cuvelier, Samy Gobaa, Cecilia Nakid-Cordero, Julien Lion, Jean-christophe Bories, Nuala Mooney, Thierry Jaffredo, Jerome Larghero, Laurent Blanchoin, Lionel Faivre, Stephane Brunet, and Manuel Thery

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July 13, 2020

Re: JCB manuscript #202005085

Dr. Manuel Thery
CEA
IUH, Hopital Saint Louis, 1 Avenue Claude Vellefaux.
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France

Dear Dr. Thery,

Thank you for submitting your manuscript entitled "Hematopoietic progenitors polarize in contact with bone marrow stromal cells by engaging CXCR4 receptors". We sincerely apologize for the delay in sending our decision to you. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. The paper was assessed by JCB Senior Editor Dr. Ira Mellman. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that all the reviewers found the observations that HSPCs interact with various niche cells through a contact interface that involves polarization exciting. They made several suggestions to better understand the molecular basis of the interactions and their relevance. From their comments, we suggest you focus a revision on the following points:

1) We agree with Reviewer #1 that testing primary T cells, as opposed to Jurkat, would strengthen the work and be consistent with the other analyses presented. These studies would bolster your conclusion that "hematopoietic polarization is not a generic outcome but specific to defined interactions between hematopoietic progenitors and stromal cells". Similarly, we encourage you to add primary myeloid cells, such as a monocyte or granulocyte, too. Better testing the cell type specificity of the interactions would deepen the understanding of their role and provide a stronger test to your overall model.

2) On a related note, Reviewer #1 suggests looking at the distribution of SDF1 and CXCR4; this should be straightforward to do and would nail down just what cell types and lineages will be under this mechanism of development.

3) Please address Reviewer #3's points in full. In particular, the reviewer cites earlier work that questions whether the comparison to an IS is warranted. Rethinking the stalks in this context vs the immune synapse context is important, as Reviewer #1 also suggests in point #2.

4) Reviewer #2 raises important and interesting general questions about the basis of the interaction and its physiological role. JCB Reports must present definitive, highly novel observations of outstanding interest to a wide readership that have the potential to open up new avenues of research. Because of the emphasis on novelty and broad interest, Reports do not require the same mechanistic depth as full JCB Articles. Here, your study identifies a new relevant receptor-ligand interaction to trigger contact that may be an important HSPC activity. We feel that the work fulfills the editorial requirements of the format without substantial further mechanistic or functional
studies. However, determining whether polarization impacts or is needed for differentiation would certainly be very interesting. If you have such data, we encourage you to add them. If these studies are not already underway, we would not require the data for publication.

5) Lastly, Ira and I have one strong suggestion to increase the impact and relevance of the study: the drug AMD3100/plexafor is a well-known CXCR4 antagonist (CXCR7 agonist) that is used clinically to mobilize HSCs, but its mechanism of action is unclear. If you can obtain the drug and add it to your system to test if it blocks polarization or causes depolarization, it will yield significant insight that will increase the breadth and appeal of your work. It will also be an independent opportunity to confirm your hypothesis that the SDF1/CXCR4 interaction is predominant in generating HSC polarity. Since you do not investigate the functional consequences of polarity induction (e.g., with respect to triggering lineage differentiation), we believe this is an important addition.

We'd be happy to discuss the revision further if you anticipate any issues addressing the reviewers' comments or have any questions.

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 a Report is < 20,000, 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: Reports may have up to 5 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://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. Reports may have up to 3 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 the 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 the 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)):

The bone marrow, consisting of multiple types of stromal cells and soluble factors (diffusive signals), forms a niche to instruct the fate of HSPCs, which polarize both morphologically and biochemically during differentiation, but the exact cell types and signaling pathways causing HSPCs polarization is unknown. It is also difficult to dissect the contribution of cell migration and cell-cell interaction (anchorage) in a native or conventional assay system.

In the current manuscript, Bessy et al. modeled the bone-marrow niche using an elegant microwell cell-culture system which promote long-term cell-cell interaction but prevents cell migration. This system allowed them to visualize in 3D, long-term interactions between human HSPCs and niche cells at a single cell level. They found that when an HSPC forms a small contact with an osteoblast, both centrosome and actin networks polarize to the contact zone while the nucleus polarizes to the distal end, resembling what was reported for an immunological synapse but not a uropod. The authors then tested the specificity of the HSPC polarization, by coculturing HSPCs with three types of stromal cells: umbilical vein endothelial cells, bone-derived osteoblast and skin fibroblast. The authors then provided evidence that polarization is cell-type specific. After assessing the cell morphology and centrosome positioning, the authors found that the HSPCs polarize upon contact with umbilical vein endothelial cells or with osteoblasts but not with fibroblasts. They further showed that HSPC polarization was correlated with stromal cell functions: AFT024, a murine stromal cell line known to support HSPC regeneration, induced HSPC polarization, whereas BFC012, a murine stromal cell line that does not support HSPC regeneration, failed to induce HSPC polarization. On the other hand, stromal cells induced polarization of HSPCs but not mature Jurkat T cell line.

Finally, the authors examined which molecular pathway(s) are responsible to HSPC polarization during HSPC-stromal cell contact. Using ligand-coated microwell, they provided evidence that VCAM-VLA4, ICAM-LFA1, or SDF1-CXCR4 pathway is sufficient for HSPCs to polarize morphologically, but only SDF1-CXCR4 pathway is sufficient for centrosome and microtubule polarization.
Overall this is a very nice paper. The study was well designed and executed. The findings are novel and interesting and presented in a logical manner. The methods are unique and powerful. The paper may be improved by providing some further molecular insights.

Major:

1. One intriguing aspect of this paper is the cell-type specificity. The authors also suggested that certain cell adhesion pathways, specifically the SDF1-CXCR4 axis, are central for HSPCs polarization. Are these pathways absent or downregulated in the fibroblast-HSPC, and stromal-lymphocyte context? Can the authors analyze the expression of SDF1, VCAM and ICAM in the skin fibroblast, and the expression of CXCR4 in HSPCs and mature lymphocytes? These experiments should enhance the molecular insights of the paper.

2. Formation of immune synapse involving a T cell is thought to require the stimulation of the antigen receptor, so I was left wondered why the HSPC polarization was induced in the absence of antigen. Or is the term "immune synapse" an overstatement as indicated by reviewer #3?

3. The authors showed that stromal cells did not induce polarization of Jurkat cells. Given Jurkat is a leukemia cell line that lack critical signaling components, such as PTEN, it will be worthwhile to determine whether the finding holds for primary T cells.

Minor:

1. Fig. 1C, centrosome shown in white in the image but black in the label.

2. Fig. 4A, no "murine liver-derived mesenchymal stromal cells" despite cited in the text (page 5).

3. I've found the terminology of stromal cells and endothelial cells a bit confusing. Are they referring to the same cell types? If so, why not combining these two terms?

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

This is a nice paper that reports a new phenomena: i.e. that hematopoietic progenitors polarize in response to contact with bone marrow stromal cells. The authors report that this seems to be specific to engagement to CXCR4 receptors. The paper is well written and the observation is interesting in that it extends the idea that cells of the hematopoietic lineage polarize the centrosome in response to signalling receptors. This would be the first report of centrosome polarization in response to a chemokine receptor. What the paper does not do is to explore the mechanism of triggering via this receptor beyond the experiment reported in Figure 5. Two ways in which they could do so would be

1. In NK cells, polarization has been shown to be triggered by integrins and one critical aspect of this has been that the tethering of ICAM seemed to be required for polarization (Gross et al J Imm 2010, 185: 2918). It would be interesting to know whether CXCR4 engagement requires tethering and whether it activates the integrins-ie could this mechanism be related?

2. Do the progenitor cells show signs of intracellular activation?

3. What would be the biological relevance of this polarization?

Minor comments:
The Arp2 labelling is not entirely clear. Is there a better antibody?
The authors refer to Figure 3E, when they must mean 3D.

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

The paper describes the interaction of HSPCs with various potential niche cells in two microfabricated in vitro environments. One is a microfluidic system with 3D matrixes populated by endothelial cells, HSC and osteoblasts. The HSPC undergo motile or stable interactions with stromal cells and maintain normal self-renewal. Some HSPC undergo interactions through the stalk like attachments with apparently small contact areas and strong polarization. A second system was based on fabrication of glass bottom polyacrylamide wells with adhesive ligands grafted to the glass surface only. In this way the niche defining adherent cells remained in the well that could also contain a small number of HSC. In this setting, an interesting mode of interaction is described and investigated. This mode involves strong cell polarization with a stalk like appendage that forms a small contact about which the cell pivots. The authors find that ezrin, LFA-1, VLA-4, CXCR4 is also associated with this type of stalk like attachment. The author concludes that these interfaces have some similar to immunological synapses and may be important functional aspects of HSPC niche.

1. This stalk like attachment mode of attachment was described in 1992 (PMID: 1349320) for lymphoblastoid cells attaching to purified LFA-1 coated substrates through cellular ICAM1, one of the interaction systems studied here. The ICAM-1 localization leading to the localization was associated with α-actinin (PMID: 1355095). These earlier studies reporting a similar morphology, dynamic behavior and that started to unravel molecule mechanisms should be cited would seem to contribute to the discussion. At the same time, its exciting to find a biological context for this mode of interaction.

2. In Figure 1 would it be possible to quantify the different interaction modes? This would be important to anyone trying to reproduce this. This could be done in a tabular form. Similarly for Figure 3, what percentage of cells do this? The interaction mode should be quantified.

3. The authors conclude that the interaction is similar to an immunological synapse based on sharing some molecules and centrosome polarization. But this doesn't seem plausible. In fact, earlier work showed that this stalk like interaction could be replicated for cytotoxic T cells with surfaces presenting only pMHC (PMID: 15851656), but this was not a highly functional synaptic morphology as the cytotoxic granules weren't recruited towards the target. This study could also be cited to even contrast the stalk like interactions, that can also be generated by TCR signals in the absence of adhesion molecule engagement. So I think on balance, the literature would argue that the stalk like attachments are very distinct from the spreading posture of the T cell forming immunological synapses.
Dear Editors and Reviewers,
We thank you for your in-depth analysis of our work. Please find below our responses to your comments and suggestions.

Editors

You will see that all the reviewers found the observations that HSPCs interact with various niche cells through a contact interface that involves polarization exciting. They made several suggestions to better understand the molecular basis of the interactions and their relevance. From their comments, we suggest you focus a revision on the following points:

1) We agree with Reviewer #1 that testing primary T cells, as opposed to Jurkat, would strengthen the work and be consistent with the other analyses presented. These studies would bolster your conclusion that "hematopoietic polarization is not a generic outcome but specific to defined interactions between hematopoietic progenitors and stromal cells". Similarly, we encourage you to add primary myeloid cells, such as a monocyte or granulocyte, too. Better testing the cell type specificity of the interactions would deepen the understanding of their role and provide a stronger test to your overall model.

   We followed your recommendation and tested the polarity of human primary T cells (CD4+, activated and non-activated) and monocytes (CD14+) on osteoblasts (new Figure 3G). None of them were polarized, thus confirming that this property was specific to HSPC.

2) On a related note, Reviewer #1 suggests looking at the distribution of SDF1 and CXCR4; this should be straightforward to do and would nail down just what cell types and lineages will be under this mechanism of development.

   We performed these measurements with immuno-fluorescence and western-blots. With the western blots, we found that SDF1 is more expressed in stromal cells of the bone-marrow (osteoblast and endothelial cells), which can induce the polarization of HSPC, than on fibroblast, which can not induce their polarization (new Figure 5G, 5H). With immuno-fluorescence we found that SDF1 and CXCR4 are concentrated in but not limited to the point of interaction between HSPCs and osteoblasts. They were also present in vesicles located in close vicinity with the contact site (new Figure 4F).

3) Please address Reviewer #3’s points in full. In particular, the reviewer cites earlier work that questions whether the comparison to an IS is warranted. Rethinking the stalks in this context vs the immune synapse context is important, as Reviewer #1 also suggests in point #2.

   We apologize for the lack of clarity and the misunderstanding due to the fact that we did not named the structure properly. We now refer to the polarized structure as the “magnupodium”, a term that was proposed in earlier studies on HSPC (that were already cited in our initial manuscript). It refers to a structure that is closer to the one we investigated than the stalk described in T cells. We think the comparison of the magnupodium to the uropod and the immune synapse is relevant and useful. First because these are the two polarized structures of reference that have been described in the blood lineage. Both could be related to the magnupodium since they are all associated with the centrosome. But the uropod stands in the back of a moving cell, whereas the immune synapse is at the front of an anchored cell, so it is important to know to which one the magnupodium is closer. Second because the magnupodium has been lately considered to be closer to the uropod, as it was described in
moving HSPC, while we rather think it is closer to the immune synapse. We paid attention not to confuse the maguropodium with the immune synapse and clarified this in the discussion. However we kept open the possibility that it may be considered as an “hematopoietic synapse” in the future.

4) Reviewer #2 raises important and interesting general questions about the basis of the interaction and its physiological role. JCB Reports must present definitive, highly novel observations of outstanding interest to a wide readership that have the potential to open up new avenues of research. Because of the emphasis on novelty and broad interest, Reports do not require the same mechanistic depth as full JCB Articles. Here, your study identifies a new relevant receptor-ligand interaction to trigger contact that may be an important HSPC activity. We feel that the work fulfills the editorial requirements of the format without substantial further mechanistic or functional studies. However, determining whether polarization impacts or is needed for differentiation would certainly be very interesting. If you have such data, we encourage you to add them. If these studies are not already underway, we would not require the data for publication.

We appreciate your understanding. Adrian Candelas started its PhD in 2019 to investigate the potential effect of HSPC polarization on their differentiation. He first duplicated all the key experiments we described in this manuscript and performed all the experiments suggested by the reviewers (primary cells, SDF1 quantification and immunostainings, SDF1 agonist in cell-cell and cell-substrate interactions). He is thus signing the manuscript as a co-first author. He will now focus on its initial project.

5) Lastly, Ira and I have one strong suggestion to increase the impact and relevance of the study: the drug AMD3100/plexafor is a well-known CXCR4 antagonist (CXCR7 agonist) that is used clinically to mobilize HSCs, but its mechanism of action is unclear. If you can obtain the drug and add it to your system to test if it blocks polarization or causes depolarization, it will yield significant insight that will increase the breadth and appeal of your work. It will also be an independent opportunity to confirm your hypothesis that the SDF1/CXCR4 interaction is predominant in generating HSC polarity. Since you do not investigate the functional consequences of polarity induction (e.g., with respect to triggering lineage differentiation), we believe this is an important addition.

We followed your recommendation and used AMD3100 to block CXCR4 interaction with SDF1 in both HSPC-osteoblast and HSPC-ligand assay. In both cases the polarization of HSPC was impaired (new Figure 5D, 5E).

We’d be happy to discuss the revision further if you anticipate any issues addressing the reviewers’ comments or have any questions.
The bone marrow, consisting of multiple types of stromal cells and soluble factors (diffusive signals), forms a niche to instruct the fate of HSPCs, which polarize both morphologically and biochemically during differentiation, but the exact cell types and signaling pathways causing HSPCs polarization is unknown. It is also difficult to dissect the contribution of cell migration and cell-cell interaction (anchorage) in a native or conventional assay system.

In the current manuscript, Bessy et al. modeled the bone-marrow niche using an elegant microwell cell-culture system which promote long-term cell-cell interaction but prevents cell migration. This system allowed them to visualize in 3D, long-term interactions between human HSPCs and niche cells at a single cell level. They found that when an HSPC forms a small contact with an osteoblast, both centrosome and actin networks polarize to the contact zone while the nucleus polarizes to the distal end, resembling what was reported for an immunological synapse but not a uropod. The authors then tested the specificity of the HSPC polarization, by coculturing HSPCs with three types of stromal cells: umbilical vein endothelial cells, bone-derived osteoblast and skin fibroblast.

The authors then provided evidence that polarization is cell-type specific. After assessing the cell morphology and centrosome positioning, the authors found that the HSPCs polarize upon contact with umbilical vein endothelial cells or with osteoblasts but not with fibroblasts. They further showed that HSPC polarization was correlated with stromal cell functions: AFT024, a murine stromal cell line known to support HSPC regeneration, induced HSPC polarization, whereas BFC012, a murine stromal cell line that does not support HSPC regeneration, failed to induce HSPC polarization. On the other hand, stromal cells induced polarization of HSPCs but not mature Jurkat T cell line.

Finally, the authors examined which molecular pathway(s) are responsible to HSPC polarization during HSPC-stromal cell contact. Using ligand-coated microwell, they provided evidence that VCAM-VLA4, ICAM-LFA1, or SDF1-CXCR4 pathway is sufficient for HSPCs to polarize morphologically, but only SDF1-CXCR4 pathway is sufficient for centrosome and microtubule polarization.

Overall this is a very nice paper. The study was well designed and executed. The findings are novel and interesting and presented in a logical manner. The methods are unique and powerful. The paper may be improved by providing some further molecular insights.

We thank the reviewer for these positive comments and for the great suggestions of additional experiments which did reinforce our conclusions.

Major:

1. One intriguing aspect of this paper is the cell-type specificity. The authors also suggested that certain cell adhesion pathways, specifically the SDF1-CXCR4 axis, are central for HSPCs polarization. Are these pathways absent or downregulated in the fibroblast-HSPC, and stromal-lymphocyte context? Can the authors analyze the expression of SDF1, VCAM and ICAM in the skin fibroblast, and the expression of CXCR4 in HSPCs and mature lymphocytes? These experiments should enhance the molecular insights of the paper.
We followed reviewer’s advice and compared the expression SDF1 in bone-marrow stromal cells and in skin fibroblast. We found that it was much less expressed in skin fibroblast (new Figure 5G, 5H), which is consistent with their lower ability to induce the polarization of HSPCs.

2. Formation of immune synapse involving a T cell is thought to require the stimulation of the antigen receptor, so I was left wondered why the HSPC polarization was induced in the absence of antigen. Or is the term "immune synapse" an overstatement as indicated by reviewer #3?

This is correct. The polarized structure of HSPC we described is not an immune synapse. We apologize for the lack of clarity. We argued that, based on the localization of specific proteins, it resembles more to an immune synapse than a uropod. Indeed, the polarized structures of HSPC have often been compared to uropod-like structures since they were observed in migrating cells. Here we provide evidences that the polarized structures that HSPCs form in contact with osteoblast or endothelial cells do not contain some of the characteristic markers of uropod, and instead share more features with immune synapse. Our new immunostainings of SDF1 and CXCR4 revealed some vesicles in the vicinity of the contact site, further supporting the possibility that the two cell types are actually exchanging signals and materials. However we agree that the structure we described is not an immune synapse and made this point clear in the revised main text and discussion.

3. The authors showed that stromal cells did not induce polarization of Jurkat cells. Given Jurkat is a leukemia cell line that lack critical signaling components, such as PTEN, it will be worthwhile to determine whether the finding holds for primary T cells.

We followed reviewer’s advice and tested the polarity of human primary T cells (CD4+, activated and non-activated) and monocytes (CD14+) on osteoblasts (new Figure 3G). None of them were polarized, thus confirming that this property was specific to HSPC.

Minor:

1. Fig. 1C, centrosome shown in white in the image but black in the label.

   We corrected this.

2. Fig. 4A, no "murine liver-derived mesenchymal stromal cells" despite cited in the text (page 5).

   We apologize for the lack of clarity due to the use of abbreviations. AFT and BFC are murine liver-derived stromal cells (see Charbord et al, Cell Stem Cell, 2014).

3. I’ve found the terminology of stromal cells and endothelial cells a bit confusing. Are they referring to the same cell types? If so, why not combining these two terms?

   We thank the reviewer for pointing at this confusion. The term “stromal cells” was intended to refer to both endothelial cells and osteoblasts at once and remain concise. We made this clearer in the revised text.
Reviewer #2

This is a nice paper that reports a new phenomena: i.e. that hematopoietic progenitors polarize in response to contact with bone marrow stromal cells. The authors report that this seems to be specific to engagement to CXCR4 receptors. The paper is well written and the observation is interesting in that it extends the idea that cells of the hematopoietic lineage polarize the centrosome in response to signalling receptors. This would be the first report of centrosome polarization in response to a chemokine receptor. What the paper does not do is to explore the mechanism of triggering via this receptor beyond the experiment reported in Figure 5. Two ways in which they could do so would be:

We thank the reviewer for these positive comments and constructive criticisms which have helped us to improve our study.

1. In NK cells, polarization has been shown to be triggered by integrins and one critical aspect of this has been that the tethering of ICAM seemed to be required for polarization (Gross et al J Imm 2010, 185: 2918). It would be interesting to know whether CXCR4 engagement requires tethering and whether it activates the integrins- ie could this mechanism be related?

We performed additional immuno-stainings and found that ICAM-1 is enriched in the part of the stromal cells facing the HSPC and that LFA-1 is concentrated in the HSPC protrusion (new Figure 4F) so their interaction may be involved in the polarization process. However this interaction is not sufficient since HSPC did not polarize in contact with a surface coated with ICAM-1 only (Figure 5C). We added the reference to the work of Gross et al to discuss the difference with NK cells.

2. Do the progenitor cells show signs of intracellular activation?

3. What would be the biological relevance of this polarization?

We fully agree with the reviewer that it would be required to investigate these two questions to further understand the potential impact of the polarization event we described. We considered, in agreement with the editors, that this would represent too much work. However, we highlighted your suggestion in the revised discussion.

Minor comments:
The Arp2 labelling is not entirely clear. Is there a better antibody?

We performed additional immuno-stainings with antibodies against the p34 subunit of Arp2/3 and obtained a clearer labelling.

The authors refer to Figure 3E, when they must mean 3D.

This is now corrected.
Reviewer #3

The paper describes the interaction of HSPCs with various potential niche cells in two microfabricated in vitro environments. One is a microfluidic system with 3D matrixes populated by endothelial cells, HSC and osteoblasts. The HSPC undergo motile or stable interactions with stromal cells and maintain normal self-renewal. Some HSPC undergo interactions through the stalk like attachments with apparently small contact areas and strong polarization. A second system was based on fabrication of glass bottom polyacrylamide wells with adhesive ligands grafted to the glass surface only. In this way the niche defining adherent cells remained in the well that could also contain a small number of HSC. In this setting, an interesting mode of interaction is described and investigated. This mode involves strong cell polarization with a stalk like appendage that forms a small contact about which the cell pivots. The authors find that ezrin, LFA-1, VLA-4, CXCR4 is also associated with this type of stalk like attachment. The author concludes that these interfaces have some similar to immunological synapses and may be important functional aspects of HSPC niche.

1. This stalk like attachment mode of attachment was described in 1992 (PMID: 1349320) for lymphoblastoid cells attaching to purified LFA-1 coated substrates through cellular ICAM1, one of the interaction systems studied here. The ICAM-1 localization leading to the localization was associated with α-actinin (PMID: 1355095). These earlier studies reporting a similar morphology, dynamic behavior and that started to unravel molecule mechanisms should be cited would seem to contribute to the discussion. At the same time, its exciting to find a biological context for this mode of interaction.

We thank the reviewer for pointing at this earlier description of a similar structure in lymphoblastoid cells. The polarized structure we described may be different since the centrosome did not locate in it when cells were plated on ICAM-1 coated glass (as it was done in the paper mentioned by the reviewer). In the text we referred to several earlier studies who described very similar structures in human HSPC (see below the images extracted from Freund et al, Stem Cell Dev, 2006). We think these are the closest descriptions of the structure we investigated here.
In the paper mentioned by the reviewer the elongated part of the cell is named a “stalk”. In previous works in HSC it was referred to as a “longpodium”, which could evolved either as a “tenupodium” or a “magnupodium” (Francis et al., Blood, 1998, Holloway et al., Cytometry, 1999). The laboratory of Denis Corbeil confirmed the frequent occurrence of the “magnupodium” (Freund et al., Stem Cell Dev 2006, see image above). The analysis of the molecular composition of the “magnupodium” led them to conclude it was closer to the uropod of immune cells (Fonseca et al., J Biol Chem, 2010 and Fonseca et al, Commun Integr Biol, 2011) since it was associated to the motion of HSC. Hence our discussion whether it is closer to a uropod, in the back of the moving cell, or an immune synapse, in the front of an anchored cell. Our data suggest it is closer to the latter, however, we did not have enough time to investigate whether HSC and stromal cells do actually exchange materials (as it was described in Gillette et al., Nat Cell Biol, 2009). Since we could not confirm the “synaptic” aspect of it we refrained from calling it an “hematopoietic synapse” but mentionned this possibility in the revised discussion. However we agree with the reviewer that we should adopt a better qualification than the term “protrusion” we used and should choose a word that echoes the earlier descriptions we mentioned in the text. We decided to adopt the term “magnupodium” initially proposed by Francis et al and further used by the laboratory of Denis Corbeil (BIOTEC, Dresden, Germany).

2. In Figure 1 would it be possible to quantify the different interaction modes? This would be important to anyone trying to reproduce this. This could be done in a tabular form. Similarly for Figure 3, what percentage of cells do this? The interaction mode should be quantified.

This is a great suggestion. We repeated the experiments of Figure 1 and quantified the occurrence of polarized HSC in the bone marrow (new Figure 1C). The quantification of the polarized phenotypes of Figure 3 were quantified in Figure 4.

3. The authors conclude that the interaction is similar to an immunological synapse based on sharing some molecules and centrosome polarization. But this doesn't seem plausible. In fact, earlier work showed that this stalk like interaction could be replicated for cytotoxic T cells with surfaces presenting only pMHC (PMID: 15851656), but this was not a highly functional synaptic morphology as the cytotoxic granules weren’t recruited towards the target. This study could also be cited to even contrast the stalk like interactions, that can also be generated by TCR signals in the absence of adhesion molecule engagement. So I think on balance, the literature would argue that the stalk like attachments are very distinct from the spreading posture of the T cell forming immunological synapses.

We fully agree with the reviewer and apologize for the misunderstanding. We acknowledged in the text that the exchange of signals, which is required to use the term “synapse”, deserves further characterization. We also specifically mentioned the absence of spreading that distinguish the magnupodium from an immunological synapse:

“The contact site was restricted to a small area estimated to be around 1–2 µm². However, HSPCs were not observed to spread on osteoblasts, in contrast to a lymphocyte forming immune synapse on a target cell (Ritter et al., 2013).”

In our revised discussion we made clearer that the magnupodium is distinct from lymphocyte’s uropod and immune synapse, but that in term of anchorage, internal architecture and composition it resembles more the latter.
August 18, 2021

RE: JCB Manuscript #202005085R

Dr. Manuel Thery
CEA Grenoble
IUH, Hopital Saint Louis, 1 Avenue Claude Vellefaux.
Paris 75010
France

Dear Dr. Thery:

Thank you for submitting your revised manuscript entitled "Hematopoietic progenitors polarize in contact with bone marrow stromal cells by engaging CXCR4/SDF1 axis". The paper has now been seen again by two of the original reviewers, both of whom now support acceptance so we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

**As you will see, reviewer #3 continues to feel that it is important for you to cite the 1992 study that s/he indicated in the previous round of review "both for reporting the mode of adhesion and demonstrating a priori that its not HSC specific". We feel that while adding this citation may not conclusively establish identity between these previous observations and your own, we do think it would be interesting for the community and our readership for you to refer to this paper for purposes of completeness and scholarship. Please be sure to indicate in your final cover letter or rebuttal document how you addressed this point.**

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.**

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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. Please be sure to add molecular weight markers to the gels in figure 5I.

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."

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e. Fluorochromes
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g. Acquisition software
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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. The statement should be written in the present tense and refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.

11) 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."

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

The authors have fully addressed my concerns. I support the publication of this novel and interesting work.

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

The authors have improved the paper and its very nice that the CXCR4 antagonist blocks development of this morphology in relation to the osteoblasts. The use of a term proposed in 1998 and 1999 to describe this behaviour in HSC is fine. I think its interesting that the authors working model is that this is a HSC version of synapse. Nonetheless, this mode of adhesion was described in 1992 in PMID: 1349320. The paper describes the attachment through a uropod-like stalk on supported lipid bilayers containing purified LFA-1, the integrin family adhesion receptor. So the cell surface receptor is ICAM-1, which was subsequently shown to interact with ezrin, like CD44. So the model for this novel adhesion mode described in 1992 fits the model in Fig 3g of this paper, but specific surface receptors involved are not the same. The implication of this is that there is a receptor(s) like LFA1 (receptor for ERM linked cell surface ligand) on the osteoblasts and a ligand like ICAM1 (ERM binding transmembrane protein) present on the polarised HSC. While the 1992 paper didn't have movies, the description of the behaviour is very clear.

PMID: 1349320 is important in the context of this study both for reporting the mode of adhesion and demonstrating a priori that its not HSC specific, but other hematopoietic cells could do this given the correct signals. Its appropriate to cite this paper as its a specific precedent for this adhesion mode and a molecular pathway to trigger it. If the authors think this is an important adhesion mode then is really useful that you can re-create it with one adhesion molecule and a readily available hematopoietic cell line. It suggests that the behaviour in HSC could be reconstituted on defined substrates once the relevant signals are identified, including CXCL12 interaction with CXCR4.