nature research

Peer Review File

Manuscript Title: Structural basis of malaria RIFIN recognition by LILRB1-containing antibodies

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

Previous work has shown that insertions of the LAIR1 immune regulator receptor into antibodies occurs naturally and is linked to recognition of plasmodium infected erythrocytes by these antibodies. The LAIR1 recognition of the parasite is mediated through RIFIN. LILRB1 is recognized by different RIFIN as well and the authors in the paper by CHEN and all have now shown that antibodies that have a LISLRB1 receptor insertion can be found in malaria endemic regions. Chen et al. then show that LILRB1 recognition of RIFIN is mediated through a D3 domain and they identify key conserved signature amino acids in RIFIN that are important for binding. This motif was then used to identify other LILRB1 interacting RIFIN. The structural basis for binding indicates similarities in the binding to LAIR1 by RIFIN.
This study is interesting and provides important new insights on firstly the extend of insertions of immune regulators into antibodies as a unique recognition mechanism and secondly a highly similar structural basis for the recognition of these receptors by RIFIN.
There are though a number of issues that need to be addressed.
1. Broadly, I found the paper difficult to follow and confusing in some parts. Some effort should be made to ensure that the text and figure legends are clear and easy to follow.
2. line 22. I am not sure whether adhesion to endothelial cells by RIFIN has been clearly demonstrated.
3. line 75-78. The identification of shared hotspots is confusing. A sequence analysis showing how these 14 spots have been identified would be very helpful.
4. Figure legends are cryptic and require a significant amount of extra effort to figure out exactly what is shown

Referee #3 (Remarks to the Author):

This is a beautiful paper that studies LILRB1-containing antibodies that bind to RIFIN malarial antigens. The authors first identify B cell clones from malaria-positive donors to find LILRB1-containing IgGs. Some of the interesting findings of their sequence analyses of these clones include that the VH regions were somatically mutated, but the LILRB1 inserts were usually not. They then go on to identify the targets of the LILRB1-containing antibodies by LC-MS/MS, screening a panel of RIFINS, and identifying which domain of LILRB1 is involved in binding. In addition, they present a cryo-EM structure of an insert-containing Fab bound to a RIFIN domain. The cryo-EM structure well-done, revealing the interactions at the LILRB1-RIFIN interface and also showing a very interesting and previously-unseen architecture for the elbow angle where the LILRB1 domain is inserted. Also, the Fab makes a homodimer through its light chains. Taken together, these results show why solving a RIFIN structure that includes the Fab as well as the inserted domain. Because of flexibility that resulted in lower resolution for the RIFIN portion of the structure, the authors solved a crystal structure of a LILBR1-RIFIN complex to 2.6 Å, which they used in separate analyses and to interpret their cryo-EM structure. The studies allow the authors to propose how antibodies with LILRB1 or LAIR-1 insertions could hijack RIFIN binding to host

inhibitory receptors.

Taken together, this is an excellent paper that is very appropriate for publication in Nature, either on its own, or in combination with the structures reported in the accompanying paper. My only suggestion is to move Extended Data Figure 8 to the main text, since it is a very nice summary of the results and an interesting hypothesis.

Author Rebuttals to Initial Comments:

Referee #1 (Remarks to the Author):

Previous work has shown that insertions of the LAIR1 immune regulator receptor into antibodies occurs naturally and is linked to recognition of plasmodium infected erythrocytes by these antibodies. The LAIR1 recognition of the parasite is mediated through RIFIN. LILRB1 is recognized by different RIFIN as well and the authors in the paper by CHEN and all have now shown that antibodies that have a LISLRB1 receptor insertion can be found in malaria endemic regions. Chen et al. then show that LILRB1 recognition of RIFIN is mediated through a D3 domain and they identify key conserved signature amino acids in RIFIN that are important for binding. This motif was then used to identify other LILRB1 interacting RIFIN. The structural basis for binding indicates similarities in the binding to LAIR1 by RIFIN.
This study is interesting and provides important new insights on firstly the extend of insertions of immune regulators into antibodies as a unique recognition mechanism and secondly a highly similar structural basis for the recognition of these receptors by RIFIN.
There are though a number of issues that need to be addressed.

1. Broadly, I found the paper difficult to follow and confusing in some parts. Some effort should be made to ensure that the text and figure legends are clear and easy to follow.

We thank the Referee for bringing up this point and agree that certain parts, in particular that dealing with RIFIN identification, were difficult to follow. We have extensively reviewed figure legends and text to ensure a smooth flow of information. Figure 2 has been improved so that the workflow could be better appreciated.

2. line 22. I am not sure whether adhesion to endothelial cells by RIFIN has been clearly demonstrated.

The Referee correctly points out that there is no solid evidence of RIFIN binding to endothelial cells, which we found just mentioned in a review. We therefore corrected the text accordingly.

3. line 75-78. The identification of shared hotspots is confusing. A sequence analysis showing how these 14 spots have been identified would be very helpful.

We thank the Referee for suggesting how to improve the presentation of the data. We have added the alignment and the annotation in Fig. 2d and improved the table layout in Fig. 2e.

4. Figure legends are cryptic and require a significant amount of extra effort to figure out exactly what is shown

The figure legends have been extensively revised. To summarize the findings on LAIR1 and LILRB1 antibodies, we also improved the summary scheme in Extended Data Fig. 10 (that could be added as Fig. 5 in the main text).

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structure that includes the Fab as well as the inserted domain. Because of flexibility that resulted in lower resolution for the RIFIN portion of the structure, the authors solved a crystal structure of a LILBR1-RIFIN complex to 2.6 Å, which they used in separate analyses and to interpret their cryo-EM structure. The studies allow the authors to propose how antibodies with LILRB1 or LAIR-1 insertions could hijack RIFIN binding to host inhibitory receptors.

Taken together, this is an excellent paper that is very appropriate for publication in Nature, either on its own, or in combination with the structures reported in the accompanying paper. My only suggestion is to move Extended Data Figure 8 to the main text, since it is a very nice summary of the results and an interesting hypothesis.

omments:

We thank the Referee for the positive evaluation of our study. As suggested, we now provide an updated version of Extended Data Fig. 8 (now Extended Data Fig. 10) that could be added to the main text as Fig. 5.