RIFINing Plasmodium–NK Cell Interaction

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Spotlight

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Abstract

Among many *Plasmodium* proteins involved at the erythrocytic cycle, some exposed on the erythrocyte surface drive the antigenic variability. Recently, Harrison et *al.* elucidated the structural basis by which RIFINs activate LILRB1 and suppress the immune cell function. This breakthrough points out an additional strategy to survive into the human host.

Malaria still remains a major public health issue in the tropics and a potentially fatal disease caused by protozoan parasites from the genus *Plasmodium,* although major progresses have been achieved in the last two decades, with the scaling up of priority interventions (prevention and management of malaria cases). Severe malaria, mainly due to *Plasmodium falciparum* infections, is responsible for nearly half million deaths annually, affecting mostly children under five years old and pregnant women living in sub-Saharan Africa, as well as non-immune travelers [1]. Although *P. falciparum* is the most prevalent and dangerous species, other *Plasmodium* species including *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* account for a non-negligible impact on human health. Malaria symptoms, ranging from cyclic febrile episodes to life-threatening complications (severe anemia, respiratory distress and coma) are mainly linked with the parasite development into human red blood cells (RBCs) [2]. Studying the complex host-pathogen interactions are of major importance to decipher cellular and molecular mechanisms involved in malaria infections and to develop more effective therapies and vaccine [3,4].

To date, many parasite proteins have been described as essential for *Plasmodium* parasites to achieve its complex life cycle in the human host during various steps. One of the main challenges of the malaria parasites for an effective proliferation and differentiation into the RBCs is to escape the human immune system. The selection of highly polymorphic sequences at the end of some chromosomes (*i.e.* subtelomeres) encoding variant polypeptides of multicopy gene families seems to explain how malaria parasites have adapted to this barrier [5]. Some of these variant polypeptides have been shown to be involved in host cell adhesion, notably in promoting parasite invasion into RBCs. However, the major role of these variants, exposed on the surface of infected RBCs, is to ensure the antigenic variation and therefore to circumvent the host immunity response. Among hundreds of adhesive proteins, three main families are well-documented [6]. These include the *var* family encoding variant antigens *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), the subtelomeric variant open reading frame family (*stevor*) encoding STEVOR proteins, and variant polypeptides from the repetitive interspersed family (*rif*) encoding RIFINs [6]. PfEMP1, STEVOR, and RIFINS are responsible for characteristic protuberances on the surface of the RBCs in association with sub-membranous network of knob proteins (Figure 1A). This unique RBC membrane shape is known to promote the binding of infected erythrocytes to the endothelium (cytoadherence), a major mechanism to circumvent removal of infected RBCs by the spleen and the main pathophysiological phenomenon responsible for the severe forms of malaria [6]. Besides this function, variant surface antigens also play a crucial role in regulating interactions of infected RBCs with immune cells for dampening the host response.

In this respect, spectacular progresses had been done in the past years in our understanding of the function of RIFINs, used by *P. falciparum* to escape the host immune system. More precisely, some RIFIN variants were shown to be able to bind to either leucocyte immunoglobulin-like receptor B1 (LILRB1) or

leucocyte-associated immunoglobulin-like receptor 1 (LAIR1) [7-9] and inhibit the activation of LILRB1-expressing immune cells. However, one of the missing pieces of the puzzle was to comprehensively define the inhibitory function of RIFINs. This has been made recently in a striking study published by Harrison and colleagues. They elucidated the structural basis by which RIFINs activate LILRB1 by mimicking the binding mode of the natural ligand of LILRB1 for suppression of immune cell function [10].

The *Plasmodium* RIFIN family is composed of approximately 150-200 members, structurally categorized in two subtypes (A and B). Most of the RIFINs belongs to the A subtype. The canonical domain architecture of *P. falciparum* type-A RIFINs consists of a C-terminal signal peptide (SP), a first short variable domain (V1), a *Plasmodium* export element (PEXEL), a constant semi-conserved domain, a 25 amino acid-long insertion–deletion sequence (indel), a second large variable domain (VD), a transmembrane region (TM), and finally a basic terminal sequence (BTS) (Figure 1B). In their study [10], the authors demonstrated first that only the variable region of RIFINs binds to LILRB1. Intriguingly, they found that RIFINs binding affinities for LILRB1 are comparable to those previously observed with MHC class I molecules. Structure resolution approaches revealed that the LILRB1-binding region to RIFINs is composed of a loop linking a couple of helices albeit the contact surface of LILRB1 with RIFINs is located within the two N-terminal immunoglobulin-like domains (ILD1 and ILD2, Figure 1C). These structural predictions were then tested in a cellular context by using a reporter system in which fluorescence is emitted upon LILRB1 binding, and the authors confirmed that the predicted contact surface is involved in the induction of LILRB1-mediated signaling. Second, through mutagenesis studies, they provided final evidences demonstrating that RIFINs preserve their intrinsic function of adhesins despite high polymorphisms of the *rif* genes selected for promoting antigenic variation, and thus preventing the host immune detection, similar to what was observed for PfEMP1 proteins. They also indicated a common binding site for LILRB1 of the RIFIN and the MHC class I molecules (here HLA-A2) despite their very different structures (Figure 1D). Third, they proposed, by using a supported lipid bilayer system for triggering NK cell activation by antibody-dependent cell-mediated cytotoxicity (ADCC), an hypothetical mechanism by which this 3D similarity with MHC class I molecules is used by RIFINs at immunological synapse, formed between immune cells (mainly $CD4^+$ T cells and NK) and infected RBCs to prevent ADCC (Figure 1E).

In summary, Harrison and colleagues' findings nicely highlight the structural basis underlying RIFINs interaction with the prominent inhibitory immune receptor LILRB1. Importantly, this dampening function of RIFINs is ensured by a dedicated LILRB1-binding surface that commonly displays strong antigenic variation to avoid immune detection. This enlightening article provides additional evidences that *P. falciparum* multigene families of adhesins has evolved to disrupt the activation of immune cells, hereby

mimicking the structure of the natural ligand of LILRB1, *i.e.* MHC class I molecules. This study points out once again how *P. falciparum* parasites have deployed a plethora of strategies to survive in the human host and therefore to sustain *P. falciparum* life cycle in endemic areas.

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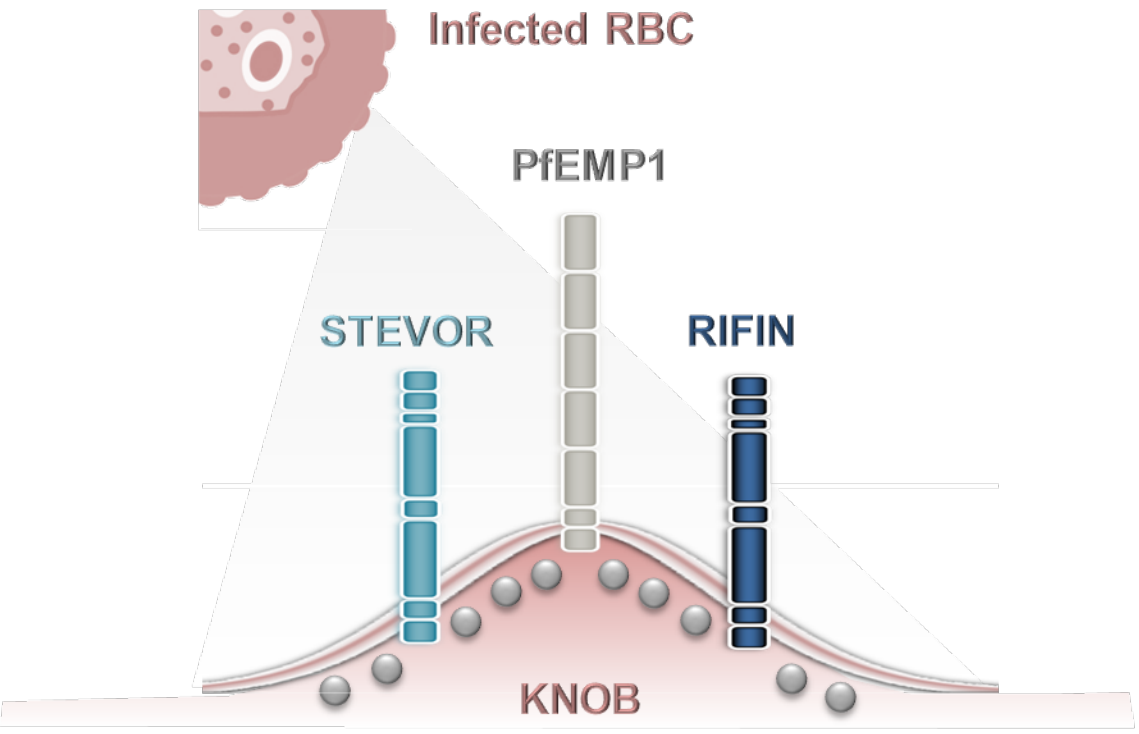
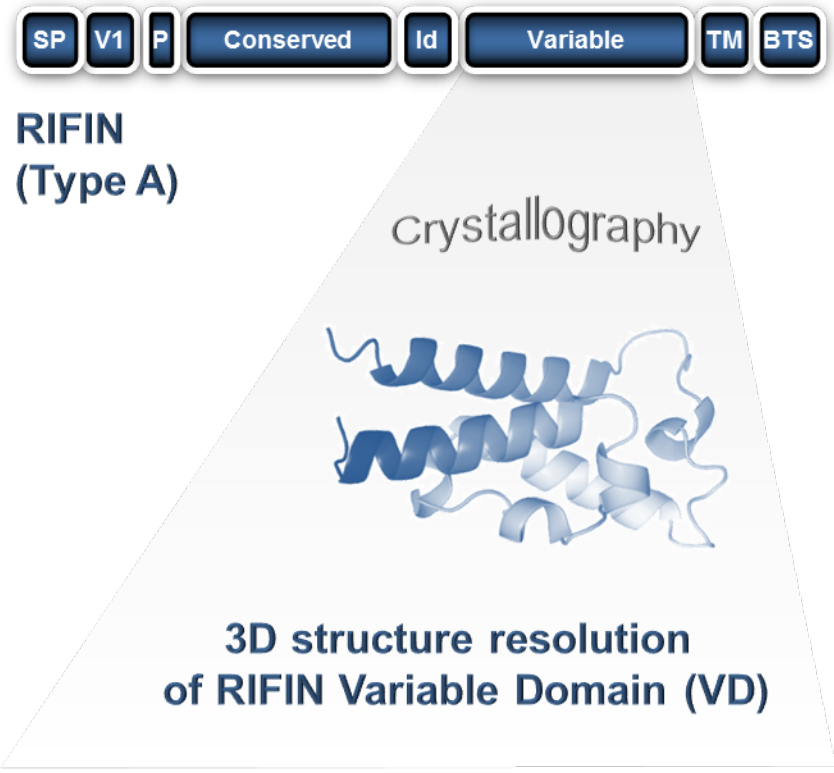
Figure 1. Structural basis for RIFIN-mediated activation of LILRB1 in *falciparum* malaria.
A. Schematic representation of the three main families of adhesive proteins on the surface of infected RBCs during the erythrocytic cycle: the *var* family encoding variant antigens *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), the subtelomeric variant open reading frame family (stevor) encoding STEVOR proteins, and variant polypeptides from the repetitive interspersed family (rif) encoding RIFINs. PfEMP1, STEVOR, and RIFINS form characteristic protuberances on the surface of erythrocytes in association with sub-membranous network of knob proteins. **B.** Gene structure of the *Plasmodium* RIFIN family. This family is composed of approximately 150-200 members and structurally categorized in two subtypes (A and B). The canonical domain architecture of *P. falciparum* type-A RIFINs (the most frequent subtype) consists of a C-terminal signal peptide (SP), a first short variable domain (V1), a Plasmodium export element PEXEL (P), a constant semi-conserved domain, a 25 amino acid-long insertion–deletion sequence indel (Id)), a second large variable domain (VD), a transmembrane region (TM), and a basic terminal sequence (BTS). **C.** Mapping of LILRB1-binding region of RIFINs. This region is composed of a loop linking a couple of helices. The contact surface of LILRB1 with RIFINs is located within the two N-terminal immunoglobulin-like domains I-LD1 and I-LD2. **D.** Mapping of LILRB1-binding region of MHC class I molecules (here HLA-A2). LILRB1 is expressed on various immune cell types, including T cells, B cells, natural killer (NK) cells and monocytes, and cells that are involved in suppressing B cell responses. **E.** Schematic representation of the hypothetical mechanism of the suppression of NK cell activation by antibody-dependent cell-mediated cytotoxicity (ADCC). The 3D similarity between MHC class I molecules and RIFINs is used by the RIFINs at immunological synapse, formed between immune cells and infected RBCs to prevent ADCC.

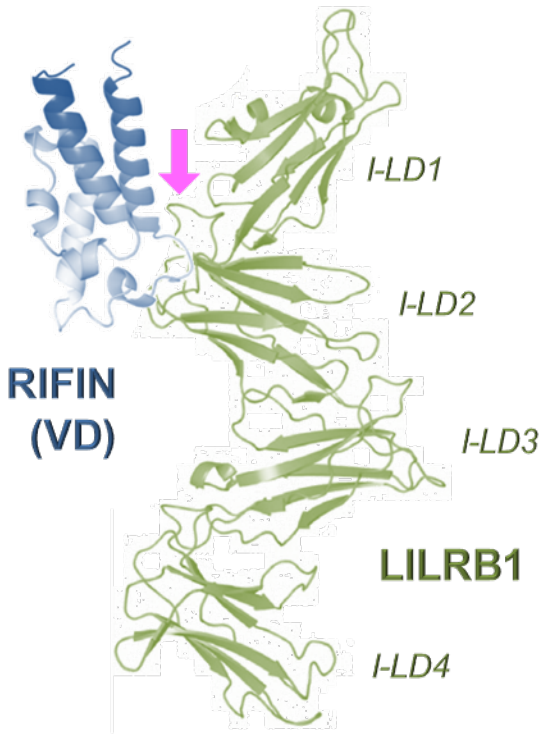
A


Infected RBC
PfEMP1
STEVOR
RIFIN
KNOB

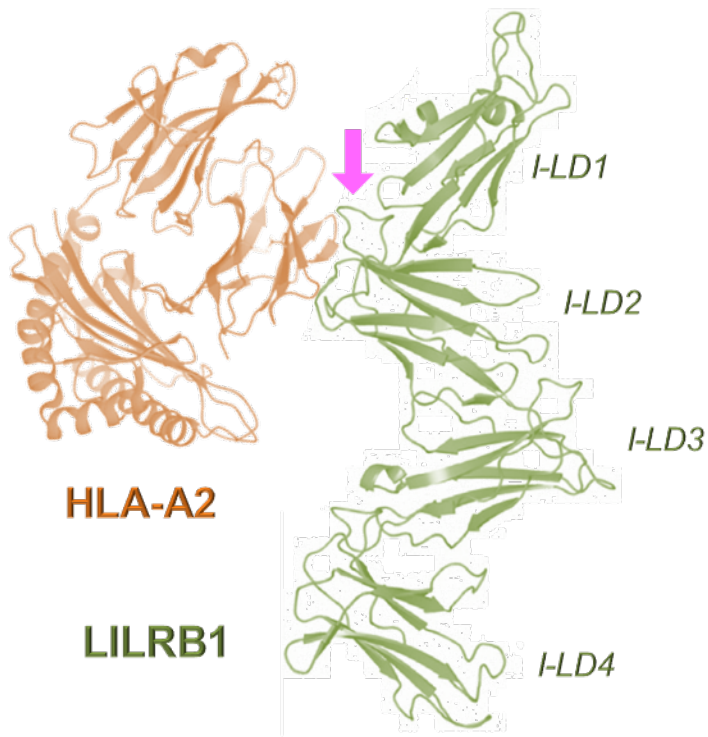
B


SP
V1
P
Conserved
Id
Variable
TM
BTS
RIFIN
(Type A)
Crystallography
3D structure resolution
of RIFIN Variable Domain (VD)

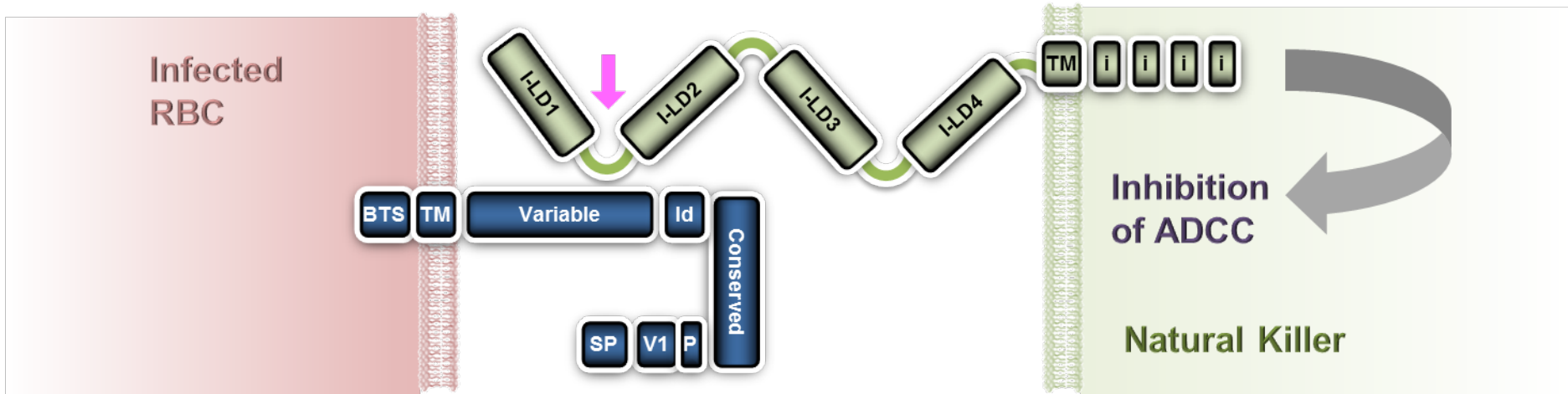
C


I-LD1
I-LD2
I-LD3
I-LD4
RIFIN
(VD)
LILRB1

D


I-LD1
I-LD2
I-LD3
I-LD4
HLA-A2
LILRB1

E


Infected
RBC
I-LD1
I-LD2
I-LD3
I-LD4
TM
i
i
i
i
BTS
TM
Variable
Id
Conserved
SP
V1
P
Inhibition
of ADCC
Natural Killer