Supplementary information to

Lateral Clustering of TLR3:dsRNA Signaling Units Revealed by TLR3ecd:3Fabs Structure

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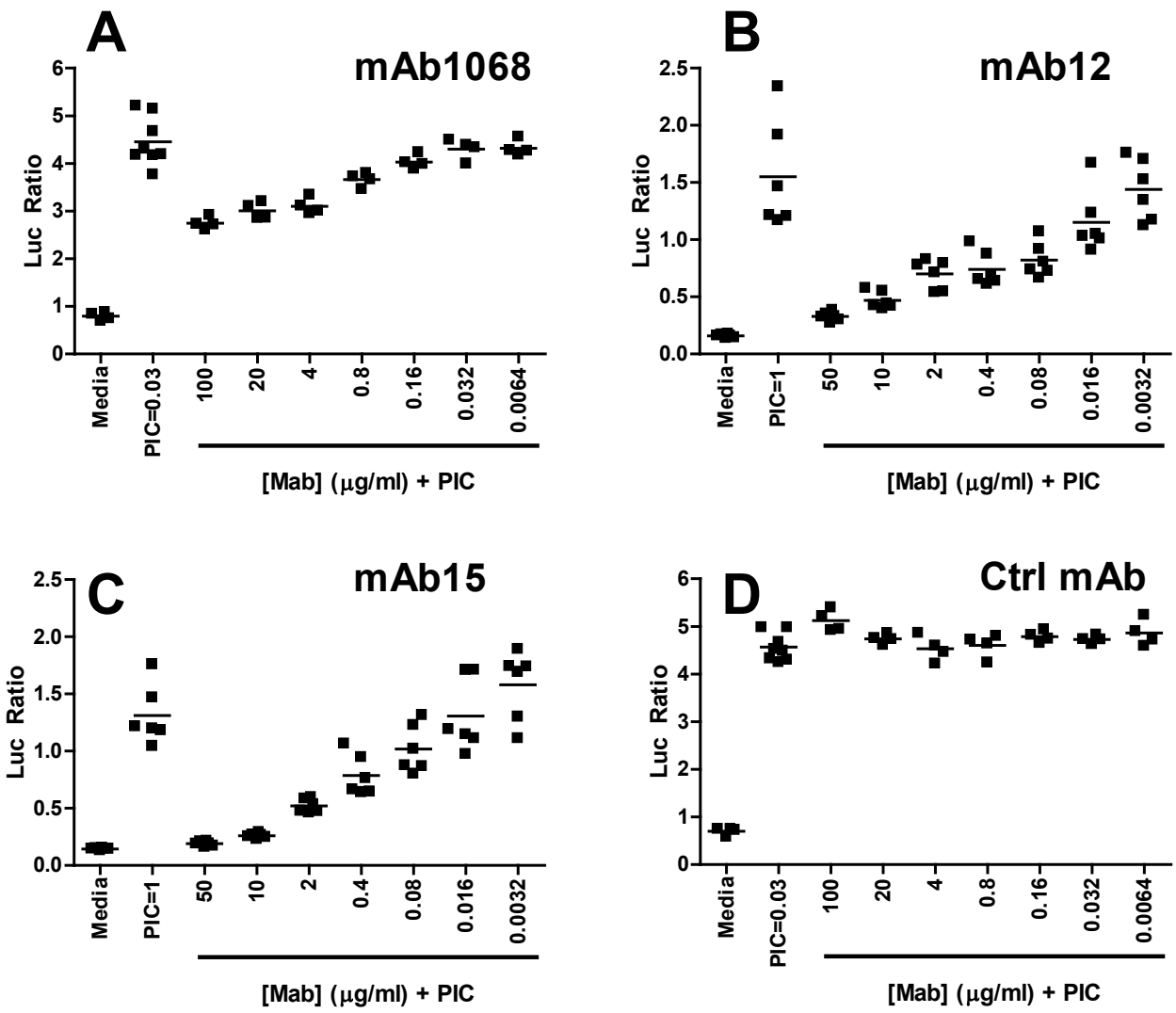

Fig. S1. Monoclonal antibodies inhibit NF-κB driven TLR3 activity induced by poly(I:C) in an NF-κB luciferase reporter assay (RGA). The ratios of luciferase activity induced by poly(I:C) between HEK293T cells transfected with a TLR3 expressing construct and those transfected with a control *Renilla* vector in the presence of antibodies are shown for (**a**) mAb1068, (**b**) mAb12, (**c**) mAb15 and (**d**) a control antibody CNTO3161, respectively. PIC: poly(I:C).

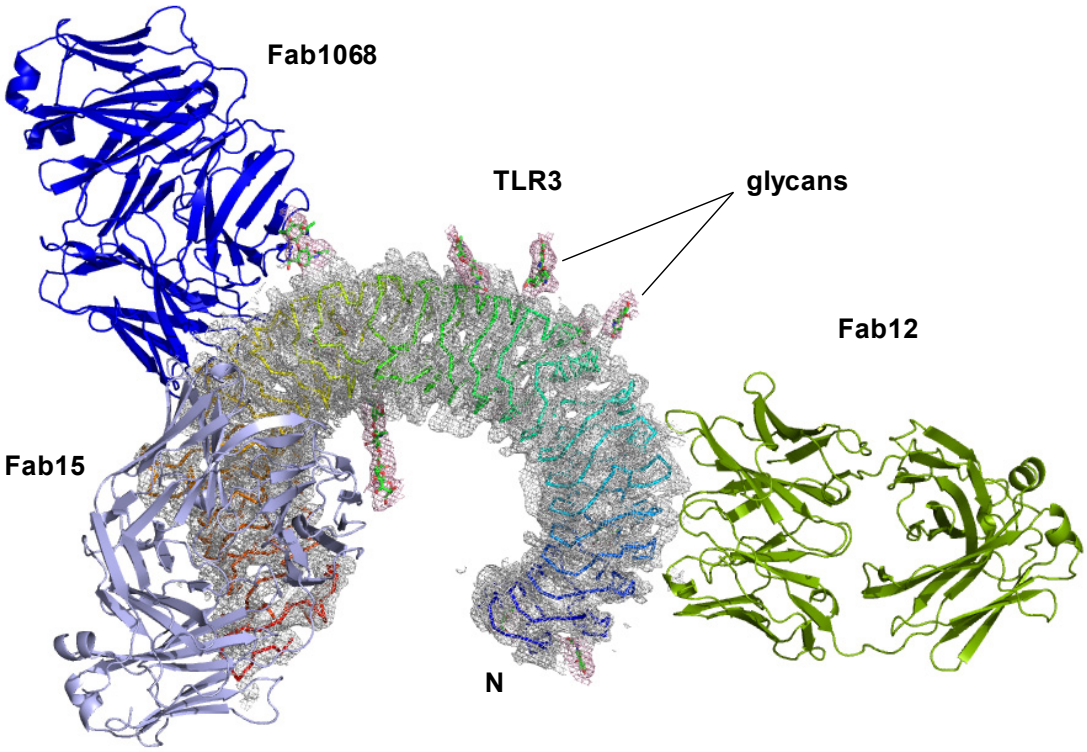

Fig. S2. The electron density map (2Fo-Fc map contoured at 1σ) of the quaternary complex (around TLR3 only) of TLR3:Fab12:Fab15:Fab1068. TLR3 is shown as Cα trace in a rainbow coloring scheme. Fab12, Fab15 and Fab1068 are shown as ribbons in split-pea, light-blue and blue, respectively. The 11 glycans are displayed as stick models within the pink electron density envelope.

Supplemental results

Fab12 Structure

Two Fab molecules occupy the asymmetric unit. The final atomic model contains residues 1-212 of the light chain (LC) and 1-224 of the heavy chain (HC) in molecule 1, which includes 4 out of 6 C-terminal His residues. Molecule 2 has a break in the electron density between residues 137 and 142 of HC. The inter-chain disulfide bridge Cys^{212}(LC)-Cys^{136}(HC) is clearly defined in molecule 1 but not in molecule 2. The two Fab molecules have an elbow angle of 230.6° and 228.8° , respectively, which are outside the observed range for the λ-containing Fabs [1].

The V_L and V_H domains in the two Fv's individually superimpose with an rmsd of < 0.35 Å. There are several local structural differences in the CDR-L1s and -L3s in the two independent Fabs. In the crystal, these two loops are packed against C_{H1} of a symmetry related Fab molecule with several inter-molecular H-bonds. There is also a relative rotation of about 7° between the V_L and V_H domains when the two Fv structures are superimposed on V_H or V_L. This is larger than the values typically observed for the multiple copies of a Fab in the same crystal [2]. There are no significant differences in the rotamers of the V_L/V_H interface residues. Presumably the crystal packing interactions that caused the CDR-L1 and -L3 conformational changes are also responsible for this relative V_L/V_H movement. This also suggests an unusually large degree of flexibility in V_L/V_H interactions.

The CDR surface is concave due to the long CDR-H2 loop. Aromatic residues (mostly Tyr) dominate the central part of the CDR surface. There is a pocket in the middle, between CDR-L1, -L3, and -H3. Another feature of Fab12 is a polarized distribution of charges over the CDR surface. A negatively charged cluster is located at CDR-L2, whereas a positively charged cluster

is at CDR-H2. While acidic residues are not uncommon in CDR-L2, the presence of three basic residues in CDR-H2 is unusual, and suggests a role in antigen recognition.

Fab1068 structure

There are 4 independent Fab molecules in the asymmetric unit of the C1068 crystal. The final model includes all amino acid residues of the light chains and heavy chains except for residues 135-137 of the heavy chains. For one of the heavy chains, the 6 histidine residues of the His tag were clearly visible, whereas only the first three of the His tag were defined for the other three heavy chains. The light chain elbow has an inserted glycine residue, G108a, which is well defined in the electron density. This glycine residue was the result of RNA splicing of the genomic DNA construct. All CDR residues of all four Fab molecules are well ordered in the electron density. The three light chain CDR loops CDR-L1, -L2 and -L3 and the heavy chain CDR loops CDR-H1 and -H2 adopt well-characterized canonical structures (2-1-1-1-2, respectively) based on the backbone torsion angles and structural superposition with representative antibody structures [3; 4]. Three of the elbow angles between the V and C domains of the Fab molecules are ~170º and the fourth is 153º. The four Fv's have nearly identical structures. The rmsd of V_L ranges from 0.19 to 0.34 Å, and the rmsd for V_H ranges from 0.19- to 0.29 Å. There is a small degree of variation in the V_L/V_H pairing. When the V_L domains are superimposed, the rmsd for V_H ranges from 0.41 to 1.72 Å. This rmsd corresponds to a maximum rigid body rotation of about 2.5º for V_H with respect to V_L in the four Fv structures, which is typical for multicopy Fab structures [2].

References

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