Public antibodies to malaria antigens generated by two LAIR1 insertion modalities

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In two previously described donors, the extracellular domain of LAIR1, a collagen-binding inhibitory receptor encoded on chromosome 19 (ref. 1), was inserted between the V and DJ segments of an antibody. This insertion generated, through somatic mutations, broadly reactive antibodies against RIFINs, a type of variant antigen expressed on the surface of Plasmodium falciparum-infected erythrocytes2. To investigate how frequently such antibodies are produced in response to malaria infection, we screened plasma from two large cohorts of individuals living in malaria-endemic regions. Here we report that 5–10% of malaria-exposed individuals, but none of the European blood donors tested, have high levels of LAIR1-containing antibodies that dominate the response to infected erythrocytes without conferring enhanced protection against febrile malaria. By analysing the antibody-producing B cell clones at the protein, cDNA and gDNA levels, we characterized additional LAIR1 insertions between the V and DJ segments and discovered a second insertion modality whereby the LAIR1 exon encoding the extracellular domain and flanking intronic sequences are inserted into the switch region. By exon shuffling, this mechanism leads to the production of bispecific antibodies in which the LAIR1 domain is precisely positioned at the elbow between the VH and CH1 domains. Additionally, in one donor the genomic DNA encoding the VH and CH1 domains was deleted, leading to the production of a camel-like LAIR1-containing antibody. Sequencing of the switch regions of memory B cells from European blood donors revealed frequent templated inserts originating from transcribed genes that, in rare cases, comprised exons with orientations and frames compatible with expression. These results reveal different modalities of LAIR1 insertion that lead to public and dominant antibodies against infected erythrocytes and suggest that insertion of templated DNA represents an additional mechanism of antibody diversification that can be selected in the immune response against pathogens and exploited for B cell engineering.

LAIR1-containing antibodies were initially isolated from two Kenyan donors who were selected from a large cohort of more than 500 individuals for their capacity to produce broadly reactive antibodies to P. falciparum-infected erythrocytes2. To establish the prevalence of LAIR1-containing antibodies in malaria-exposed individuals, we screened plasma samples from two large cohorts in Tanzania3 and Mali4. To identify LAIR1-containing antibodies irrespective of their specificity for parasite isolates, we developed a two-determinant immunoassay using beads coated with anti-LAIR1 or control antibodies.

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Six out of 112 Tanzanian donors (5.4%) and 57 out of 656 Malian donors (8.7%) had detectable levels of LAIR1-containing IgG (Fig. 1a). In addition, 2–4% of African donors had LAIR1-containing IgM, with no or variable levels of LAIR1-containing IgG (Fig. 1b, c). By contrast, only 3 and 4 out of 1,043 European blood donors showed a low positive result in the assays for LAIR1-containing IgG and IgM, respectively. The presence of LAIR1-containing antibodies was confirmed by the isolation of 52 immortalized B cell clones from seven East and West African donors (Extended Data Table 1 and Supplementary Table 1), whereas we were not able to isolate LAIR1-containing monoclonal antibodies from four European donors that showed serum reactivity. The finding that 5–10% of individuals living in malaria-endemic regions produce LAIR1-containing antibodies is suggestive of a public antibody response.

To investigate the contribution of LAIR1-containing antibodies to the response to infected erythrocytes, we dissected this response at the polyclonal and monoclonal levels. Staining of infected erythrocytes with plasma from selected individuals with LAIR1-containing antibodies revealed that most infected erythrocytes were recognized by the LAIR1-containing antibodies, whereas only a minority of infected erythrocytes was recognized by conventional IgG (Fig. 1d). Furthermore, when immortalized memory B cell clones from four donors were analysed for reactivity to infected erythrocytes and for the presence of LAIR1, all of the infected erythrocyte-recognizing monoclonal antibodies from three donors and most of such antibodies from the fourth donor contained the LAIR1 insert (Fig. 1e). These findings suggest that, in certain individuals, circulating antibody and memory B cell responses are dominated by LAIR1-containing antibodies, a finding that may be explained both by the breadth of these antibodies’ reactivities and by clonal expansion of the B cells that produce these antibodies.

To investigate the nature of the LAIR1 insertion, we sequenced cDNA and gDNA from B cell clones isolated from different individuals. As reported for the first two Kenyan donors6, the B cell clones isolated from four Malian and Tanzanian donors (E, F, O and Q) contained an insertion of the LAIR1 exon with flanking intronic sequences between the V and DJ segments, positioning the LAIR1 domain in the CDR3 loop (Fig. 2a and Extended Data Fig. 1). The size of the insert and the partial splicing of the upstream intronic region differed between donors, but were identical in the sister clones isolated from each individual, indicating that in each donor the LAIR1-containing antibody response is monoclonal.

Strikingly, B cell clones from three additional donors showed a different insertion modality (Fig. 2b–d). The cDNA of clones isolated from donors M (Malian) and J (Tanzanian) contained only the LAIR1 exon, which was precisely located between the JH and CH1 domains. In both cases, gDNA analysis revealed that a DNA fragment comprising the LAIR1 exon flanked by intronic sequences was inserted into the switch–μ region (Extended Data Fig. 2) and, by alternative splicing, gave rise to two mRNA variants with or without the LAIR1 insert. This was confirmed by the production of antibodies with or without LAIR1 in similar proportions by a single B cell clone (Fig. 2e). Another example of LAIR1 insertion into the switch region was observed in donor B (Kenyan), from whom we isolated a B cell clone (MGB47) producing a truncated LAIR1-containing IgG3 heavy chain without an attached light chain (Fig. 2f, g). In this clone, the gDNA carried multiple deletions that removed most of the VDJ and the entire CH1 region, leading to the production of a camel-like antibody2 (Fig. 2d). Together, these findings highlight a new modality of exon insertion in the switch region that can add an extra domain to an antibody.

The two insertion modalities result in the production of antibodies with non-conventional structures in which an additional domain is inserted into the CDR3 region or into the elbow between VH and CH1 (Fig. 3a). To investigate the effect of the insert position on antibody specificity, we designed different constructs in which unmutated LAIR1, mutated LAIR1 or other Ig-like domains were inserted into the CDR3 or elbow regions of an antibody of known specificity that was used as a scaffold (Fig. 3b). Antibody constructs carrying LAIR1 stained infected erythrocytes and were recognized by an anti-LAIR1 antibody, independent of the position of LAIR1 in the scaffold. While insertion of LAIR1 into the CDR3 region of an antibody specific for granulocyte–macrophage colony-stimulating factor (GM-CSF)5 abolished binding to GM-CSF, insertion of LAIR1, programmed cell death-1 (PD-1), or signalling lymphocytic activation molecule family member (SLAM) Ig-like domains into the elbow region did not affect binding to GM-CSF. This indicates that the VH–CH1 elbow is permissive for insertions of different domains without affecting the original antibody specificity and may therefore be suitable for the generation of bispecific antibodies.

To analyse the role of somatic mutations, we aligned and compared the LAIR1 sequences of 52 antibodies (Extended Data Fig. 3 and Extended Data Table 1). LAIR1 inserts between the V and DJ segments carried several amino acid substitutions clustering at hot spots around
positions 67, 77 and 102 that determined distinct patterns of reactivity with parasite isolates, as well as loss of collagen binding (Fig. 3c). By contrast, LAIR1 inserted into the switch region carried only a few substitutions, which were, however, sufficient to abolish collagen binding. In particular, the camel-like antibody MGB47, which had the highest level of amino acid substitutions among those with inserts in the switch region, showed considerable breadth, staining eight out of the nine parasites tested. Notably, unmutated LAIR1 bound to a few isolates when tested at 1 μg ml⁻¹ and to all parasites when tested at a 100-fold higher concentration (Fig. 3c). We conclude that the unmutated LAIR1 domain binds with low affinity to most parasite isolates and that mutations can increase affinity and modify the spectrum of cross-reactivity. Furthermore, the finding that collagen binding is lost even in cases where the somatic mutation mechanism is less effective, as in the case of insertions into the switch region, suggests that there is strong pressure to ‘redeem’ this B cell receptor from autoreactivity.

The insertion of an extra exon into the switch region represents a new modality of antibody diversification, analogous to exon shuffling, that has the potential to generate a panoply of bind specific antibodies. To investigate how generally and how frequently templated DNA sequences are inserted into the switch region, we isolated gDNA from switched memory B cells of European blood donors, amplified the switch regions and sequenced them using the Illumina platform (Extended Data Fig. 4).

Using a bioinformatics pipeline, we identified templated inserts at a frequency of approximately one in more than 1,000 B cells, with the length of the inserts ranging from below 100 to over 1,000 nucleotides (Fig. 4a, Extended Data Fig. 5 and Supplementary Tables 2–4). Switch region insertions could also be detected using long-read MinION sequencing of intact amplicons, which provided a suitable platform for insert identification, in spite of its high error rate. Using MinION, we confirmed the identity of several inserts using biological replicates and estimated a higher frequency of templated inserts, in the region of one in a few hundred switched memory B cells (Fig. 4c). Most of the inserts were derived from genic regions from all chromosomes and in particular from genes expressed in B cells, such as PAX5 and EBF1 (Fig. 4b, e, f and Extended Data Fig. 7). The genic inserts, which account for 75% of all inserts, were derived from introns and exons, and in some cases comprised an entire exon with preserved splice sites (Fig. 4d).

Interestingly, the somatic mutation mechanism is less effective, as in the case of insertions into the switch region, suggests that there is strong pressure to ‘redeem’ this B cell receptor from autoreactivity.

The finding that unmutated LAIR1 has the inherent ability to bind to infected erythrocytes explains how the insertion of this domain results in the generation of public antibodies with a common specificity. Furthermore, the fact that this domain binds to all parasite isolates tested, albeit with low affinity, suggests a mechanism for the extraordinary clonal expansion and selection of mutated antibody variants with improved affinity and breadth by repeated infections with different Plasmodium parasites. These findings illustrate, in a biologically relevant system, the power of clonal selection driven by both antigen binding and loss of self-reactivity. Furthermore, the binding of infected erythrocytes to LAIR1 suggests the possibility that the parasite might target this inhibitory receptor to modulate the host immune response.
The insertion of LAIR1 into the switch region, resulting in the expression of a new domain in the elbow between the VH and CH1 domains, represents a new and possibly general example of protein engineering by exon shuffling, as suggested by the frequent occurrence of templated inserts. Although insertions in the CDR3 loop result in monospecific antibodies, insertions in the switch region do not affect the specificity of the original antibody but rather add a second specificity that is found in approximately half of the antibodies produced by engineered double-strand breaks in a human cell line and mouse pro-B cells14,15. However, we cannot exclude the possibility that accessible DNA is a primary substrate. The possibility of inducing naive human B cells to switch in vitro offers the opportunity to study the mechanism of templated insertions and to engineer B cells by manipulating the factors involved in DNA repair and by offering different substrates.

The finding of templated inserts in the switch region of switched memory B cells, but not naive B cells, suggests that the insertions occur in germinal centres as a consequence of unconventional repair of activation-induced cytidine deaminase (AID)-induced double-strand DNA breaks. Similarly, repair of recombination activating gene (RAG)-induced double-strand breaks during B cell development in the bone marrow may give rise to insertions in the CDR3 region. Our finding that switch region inserts are derived from transcribed genes suggests the involvement of nascent RNA as an insert template, and recent publications have shown that both nascent12,13 and foreign RNA14,15 can be used to repair double-strand DNA breaks. Furthermore, multiple templated inserts from transcribed genes have been observed in engineered double-strand breaks in a human cell line and mouse pro-B cells14,15. However, we cannot exclude the possibility that accessible DNA is a primary substrate. The possibility of inducing naive human B cells to switch in vitro offers the opportunity to study the mechanism of templated insertions and to engineer B cells by manipulating the factors involved in DNA repair and by offering different substrates.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.
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Supplementary Information is available in the online version of the paper.

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Author Contributions K.P. characterized genomic DNA, analysed the data and wrote the manuscript; J.T. isolated new LAIR1-containing antibodies, analysed the data and wrote the manuscript; L.P. produced mutant antibodies, analysed the data and wrote the manuscript; M.F. performed bioinformatics analysis; Y.C. helped with genomic sequencing; C.S.-F. immortalized memory B cells; T.W. helped with MinION sequencing; D.J. performed cell sorting and analysis; M.A. performed protein analysis; A.A. performed P. falciparum culture; F.M.N., S.J., O.K.D., B.T., I.Z., C.D. and P.C.B. provided cohort samples; T.M.T. and P.D.C. provided cohort samples and analysed the relationship between LAIR1-containing antibodies and malaria risk; F.S. provided supervision; A.L. provided overall supervision, analysed the data and wrote the manuscript.

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

**Serum and plasma samples.** Kenyan plasma samples were obtained from adults living in a malaria-endemic region within Kilifi County. Tanzanian serum and plasma samples were obtained from healthy male volunteers, malaria negative at study enrolment and during peripheral blood mononuclear cell (PBMC) collection. HIV and hepatitis B and C-negative, age 25-40 years (mean ± s.d.)

*The Mali study was conducted in the rural village of Kalifabougou where intense P. falciparum transmission occurs from June to December each year. Six hundred ten individuals (310 males and 300 females) were enrolled, ranging in age from 1 to 26 years (mean 9 years). The cohort has been described in detail elsewhere*.

A smaller number of serum samples was also obtained from adults in the Fulani and Dogon ethnic groups in Mântéourou, Mali. Forty-eight individuals (28 males and 20 females) were enrolled, ranging in age from 21 to 57 years (mean 39.7 years).

**Ethics approval.** In all cases, written informed consent was obtained from the participants (or guardians of participating children) before inclusion in the study. The acquisition and use of the Kenyan plasma samples were approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (protocol number: KEMRI-ERU 3149), as well as the Oxford Tropical Research Ethics Committee. The Tanzanian samples were obtained with informed consent from the trial participants. The clinical trial was conducted according to good clinical practice and with authorization from the Institutional Review Board of the Ifakara Health Institute, the National Institute for Medical Research Tanzania, the Tanzanian Food and Drugs Authority and the Commission cantonale d’éthique de la recherche sur l’être humain du canton de Vaud, Switzerland. The trial is registered at https://clinicaltrials.gov/ with identifier: NCT01949909. The Mali study was approved by The Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry at the University of Sciences, Technique and Technology of Bamako, and the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Written informed consent was obtained from participants or parents or guardians of participating children before inclusion in the Mali study.

**Parasite culture.** *P. falciparum* parasites were initially obtained from children who were diagnosed with malaria in Kilifi County, Kenya. The parasites were adapted to *in vitro* culture and cultivated using standard protocols. The *P. falciparum* laboratory line 3D7 was also cultured under the same conditions. 3D7D21 was derived by enrichment of 3D7 parasites reactive with the LAIR1-containing monoclonal antibody MGD21. Parasites were cryopreserved at the late trophozoite stage in small aliquots for subsequent use in assays.

**Screening of serum or plasma with bead-based immunoassay.** Serum and plasma were tested for the presence of LAIR1-containing antibodies using a two-determinant bead-based immunoassay. Anti-goat IgG microbeads (Spherotech) were coated with goat anti-human LAIR1 (R&D Systems, AF2664) and 40 μg SYBR Green I (ThermoFisher Scientific) or goat anti-human EGF (R&D Systems, AF-259-NA) without SYBR Green I for 20 min at room temperature. The beads were washed, mixed, and incubated with the serum at a 1/30 dilution for 1 h at room temperature under shaking conditions. Beads coated with anti-LAIR1 were differentiated from control beads coated with anti-EGF based on SYBR green staining. Serum antibody binding was detected using 2.5 μg ml⁻¹ Alexa Fluor 647-conjugated goat anti-human IgG (Jackson ImmunoResearch, 709-066-098) or Alexa Fluor 647-conjugated donkey anti-human IgM (Jackson ImmunoResearch, 709-066-073). FACS Diva (version 6.2) was used for acquisition of serum and FlowJo (version 10.1) was used for flow cytometry analysis. ΔMFI was calculated by subtracting the MFI of the anti-EGF control beads from that of the anti-LAIR1 beads in the IgG or IgM channel.

**B-cell immortalization and isolation of monoclonal antibodies.** IgM or IgG memory B cells were isolated from buffy coats of malaria endemic donors (in the UK, from Amp Taq Polymerase [England Biologs]) in 50 ml reaction volumes with incubation for 3 min at 95 °C, followed by 30 cycles of 95 °C for 40 s, 60 °C for 30 s, 65 °C for 3 min and a final extension for 10 min at 65 °C. The upstream switch-μ forward primer S-μ-FW (CCTGGCTCTAGGGGCTGAGCAGGCTCC), donO/Q_FW (ATGGAAGTCAAGCGAGGAGATAGATAGAT), donM_FW2 (GGAAGCAGTGCAGAAAACTGTGATGTTG), donM_W2 (GTGTCGAAAGTGACGCAAGAAACACTGTGATGTTG), donO/Q_FW (ATGGAAGTCAAGCGAGGAGATAGATAGAT) were used for PCR amplification of V region insert and V-D-J inserts were analysed by PCR amplification of gDNA and Sanger sequencing.

**Amplification of antibody gDNA.** Genomic DNA was isolated from B cell clones with a commercial kit (Qiagen). For amplification of the κ chain, the heavy chain constant region was used as a template. The heavy chain constant region was used as a template for amplification of the heavy chain constant region.

**Production of recombinant antibodies, antibody variants and fusion proteins.** Antibody heavy and light chains were cloned into human IgG1, Igk and Igλ expression vectors and expressed by transient transfection of Expi293F cells (ThermoFisher Scientific) using polyethyleneimine (PEI). Cell lines were routinely tested for mycoplasma contamination. The antibodies were affinity purified by protein A chromatography (GE Healthcare). Variants of the GCE536, MGD21, MGMT1 and MGMT5 antibodies were produced by inserting or exchanging the mutated or unmutated LAIR1 sequences or by substituting these sequences with the Ig-like domains of PD1 and SLAM genes (sequences from Ensembl genome database: ENSG00000188389 and ENSG0000017090, respectively). The antibody constructs were tested for binding of 9,215 infected erythrocytes and binding values (%) at 1 μg ml⁻¹ antibody concentration were calculated by interpolation of binding curves fitted to a sigmoidal curve model (Graphpad Prism 7).

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for 4 min followed by 30 cycles of 98 °C for 40 s, 58 °C for 30 s and 72 °C for 4 min, with a final extension for 10 min at 72 °C. Size-selected, purified switch amplicons were sent to GATC Biotech (Germany) for library preparation, barcoding, and Illumina MiSeq sequencing.

MINION sequencing. Oxford Nanopore Technology (ONT) was used to generate biological and technical replicates of Illumina MiSeq sequencing runs. For biological replicates, barcodes were introduced by the addition of recommended BC-sequences to S2- and S− primers and PCR amplification. The sequencing library was sequenced using the Nanopore 2D sequencing kit SQK-LSK207, followed by loading onto Nanopore flow cells FLO-MIN106 and sequencing with the MINION Mk1B sequencer for up to 20 h.

Infected erythrocyte binding assays. Recombinant monoclonal antibodies that could bind to infected erythrocytes. A mixture of four parasite isolates (3D7-MGD21*, 9106, 9605 and 11019) was stained with 10 μg ml−1 DAPI for 30 min at room temperature and incubated with test sera at a 1/30 dilution for 20 min at room temperature. The infected erythrocytes were then incubated with goat anti-human LAIR1 (R&D Systems, AF2664) for 20 min at room temperature. The binding of LAIR1-containing antibodies to the infected erythrocyte surface was detected by the simultaneous addition of Alexa Fluor 488-conjugated donkey anti-goat IgG (Jackson ImmunoResearch, 705-546-147) and Alexa Fluor 647-conjugated donkey anti-human IgG (Jackson ImmunoResearch, 709-666-098).

ELISA. Total IgGs were quantified using 96-well MaxiSorp plates (Nunc) coated with goat anti-human IgG (Southern Biotech, 2040-08) using Certified Reference Material 470 (ERMs-DA470, Sigma-Aldrich) as a standard. To test specific binding of antibody constructs, ELISA plates were coated with 2 μg ml−1 of type I recombinant human collagen (Millipore, CC050), 2 μg ml−1 of an anti-human LAIR1 antibody (clone DX26, BD Biosciences 550810), 1 μg ml−1 of recombinant human GM-CSF (Genentech), 2 μg ml−1 of an anti-PD1 or an anti-SLAM antibody (R&D Systems, AF1086 and AF164). Plates were blocked with 1% bovine serum albumin (BSA) and incubated with titrated antibodies, followed by AP-conjugated goat anti-human IgG, FC fragment specific (Jackson Immuno Research, 109-056-098). Plates were then washed, substrate (p-NPP, Sigma) was added and plates were read at 405 nm.

Western blots. B cell supernatants containing secreted antibodies were diluted in H2O, 4× sample loading buffer (Life Technologies) and 10× sample reducing agent (Life Technologies) and loaded onto precast gels with a 4–12% acrylamide gradient (Invitrogen). The iBlot2 apparatus (Life Technologies) was used for protein transfer to PVDF membranes followed by blocking for 1 h at room temperature with 3% BSA in TBS. The membrane was incubated with different combinations of primary and secondary antibodies diluted in TBS/1% BSA for 1 h at room temperature with 2× sodium thiosulfate incubations to wash the membrane between incubations. For detection of IgG, anti-human IgG-biotinylated antibody (Southern Biotech, 2040-08) was used at 1 μg ml−1, followed by 25 ng ml−1 streptavidin-horseradish peroxidase (HRP) (Jackson ImmunoResearch, 016-030-084). IgM isotypes were stained with 10 μg ml−1 unlabeled goat anti-human IgM (Southern Biotech, 2020-01) and 8 ng ml−1 donkey anti-goat HRP (Jackson ImmunoResearch, 705-036-147). To detect LAIR1-containing antibodies, a polyclonal goat anti-human LAIR1 antibody (R&D) at 2 μg ml−1 was combined with secondary donkey anti-goat HRP. Membranes were developed with ECL-substrate on a Las4000 imager (General Electric Company).

Surface staining of B cell lines. EBV immortalized B cells were stained with different fluorochromes that label antibodies to detect surface immunoglobulin expression and LAIR1 co-staining (Alexa Fluor 647-conjugated anti-human IgG, Jackson ImmunoResearch, 109-606-170; FITC-conjugated anti-human kappa, DAKO, F0434, PE-conjugated anti-human lambda, DAKO, R0437; PE-conjugated anti-human LAIR1 clone DX26, BD Bioscience, 550811). Cells were analysed by flow cytometry and FlowJo software. Dead cells were excluded from the analysis by 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) staining. The time point of the analysis was selected for optimal BCR expression levels and for down-regulation of the original LAIR1 receptor, because EBV cell lines downregulate the inhibitory receptor LAIR1 at an early time point after immortalization but may also decrease surface-Ig levels after certain passages.

Genomic insertion analysis after Illumina sequencing. We generated a computational pipeline to analyse targeted amplicons of 300 bp paired-end (PE) reads obtained by MiSeq Illumina sequencing methodology (Extended Data Fig. 5). Raw sequences reads were trimmed to remove adaptor contamination and poor-quality base calls using Trim Galore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/, v0.4.2, parameters–Illumina–paired -q 20-length 99). In addition, assessment of the PE reads was performed by average quality score per base position, using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimmed reads were aligned in paired-end mode to the GRCh37 human genome assembly using Burrows–Wheeler Alignment tool (v0.7.12, parameters: bwa mem)24. The switch region of the IgH locus was defined on GRCh37, with the following coordinates: chr14:106050000–106337000. To find insertions in the selected genomic region when switch coverage was above 2 reads and/or above 40 reads, thus, generating two respective and separate workflows, with a sequence length comprises between 50 bp and 2,000 bp. Genome coverage was processed using Bedtools (http://bedtools.readthedocs.io/en/latest/index.html, v2.26.0) and a dedicated python script using pysam (https://github.com/pysam-developers/pysam) was written to identify potential insert. In both workflows (2 and 4 reads), potential inserts were assigned if they fulfilled the following criteria: (i) one mapping read in 5′ end is chimaeric with the switch region; (ii) two mapping reads in 3′ end is chimaeric with the switch region; (iii) two discordant reads have their mates read mapped in the switch region. Afterwards, insertions coming from 2 and 40 minimum reads coverage were merged according to the following rule: if the difference between two inserts that overlap was equal or below 10 bp, we kept the shortest one, otherwise the longest one was kept.

The list of potential inserts was annotated using GENCODE v1925 and BEDOPS tools26 and individually validated by a de novo assembly of the contig sequence. Non-chimaeric, but properly paired reads mapping the insert coordinates and chimaeric reads corresponding to the encompassing mate pairs and spanning mate pairs, were extracted using SAMTools27. Reads mapping to the switch region were extracted only if they were spanning mate pairs related to the insert coordinates. Selected reads were uniquely mapped to the region of interest (no XA tag), with a minimum mapping quality of 5. Original read sequences were retrieved, pulled together and used as input files to perform a de novo assembly using the Trinity software28. Finally, to validate a contig sequence for each insert, we used BLAST29 (command-line version, v2.5.0+). The consensus insert sequences were 'blasted' against the switch region and we removed the inserts that had an alignment with at least half of their sequence length at a minimum 80% identity (parameters: -task megablast -mask no -perc_identity 80). Then, we blasted each contig sequence against the switch region sequence and the consensus insert sequence (parameters: -task blastn –mask_no –perc_identity 70) to confirm whether the contig was made of the complete insert sequence and contained two flanking sequences of at least 50 bp that matched the switch region. The shortest contig that fulfilled the criteria mentioned above was selected for each insert.

Bioinformatic analysis of MINION sequencing. To analyse targeted amplicon 2–4 kb reads obtained by ONT sequencing methodology, we developed a pipeline (Extended Data Fig. 6). Raw sequences reads were quality-filtered using Metrichor baselcacher (https://metrichor.com/s/). 2D reads with a sequence length above 1,000 bp (or 2,000 bp depending on the primers used for the amplification) were aligned against GRCh37 human genome assembly with LAST software30 (parameters: last−train and lastal −p ONT_fasta_sequences.par last−split −m1e−6). Then we parsed 1 AST output and selected reads that contain an insert (minimum 50 bp length), two flanking regions of minimum 100 bp mapping to the switch region (switch locus defined as chr14:106050000–106337000) and allowing a gap of 100 bp maximum between the insert and the switch region. Finally, we merged the insert coordinates of the overlapping inserts with bedtools and annotated the inserts list with GENCODE v1925 using BEDOPS tool26. Scripts are available at MinION and Illumina inserts coordinates were merged with Bedtools (merge command with default parameter). The circular genomic representation of the inserts has been generated using the Circos software31. Insert switches with genic or non-genic regions were subjected to an enrichment analysis using Top scoring genes and the gene-set library32,33. Statistical analysis. The number n described in the figure legends refers to the number of independent experiments. The analysis of the relationship between the presence of LAIR1-containing antibodies and protection from malaria was performed in the R statistical environment (v.3.2.5). Two-tailed Fisher’s exact tests were performed to investigate the association between LAIR1-containing antibodies and protection from febrile malaria.

Code availability. Scripts for Illumina and MinION sequence analysis are available at https://bitbucket.org/mathildefog/switchilluminawrapper and https://bitbucket.org/mathildefog/switchminion, respectively.

Data availability. Sequence data for the monoclonal antibodies isolated in this study have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the accession codes indicated in Supplementary Table 1. The NGS data for switch region sequencing are deposited in NCBI Sequence Read Archive (SRA) with the accession code PRJNA382214. The analyses of these sequences are provided in Supplementary Tables 2–4.
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Extended Data Figure 1 | Alignment of gDNA and cDNA sequences of LAIR1-containing antibodies. Shown is one representative antibody from each donor. a, MGE9 (donor E); b, MGF21 (donor F); c, MGO3 (donor O); d, MQ4 (donor Q).
Extended Data Figure 2 | Genomic sequences of switch regions containing LAIR1 inserts. Shown is one representative antibody for each donor; a, MMJ5 (donor J); b, MGM5 (donor M); c, MGB47 (donor B). The chromosome coordinates of the insertion sites are indicated in blue and green.

**a** MMJ5

| Position in reference genome (Chr 14, GRCh37.p13 assembly): |
|---|
| chr14:166325639-166325639 |

**b** MGM5

| Position in reference genome (Chr 14, GRCh37.p13 assembly): |
|---|
| chr14:166325696-166325696 |

**c** MGB47

| Position in reference genome (Chr 14, GRCh37.p13 assembly): |
|---|
| chr14:166325856-166325856 |
Extended Data Figure 3 | Somatically mutated and conserved regions in the LAIR1 domains inserted in the VDJ or in the switch region. a, Amino acid substitutions in antibodies isolated from different donors and mean R/S ratios at each residue. The mutational analysis takes into consideration the germline LAIR1 alleles found in each donor. In donor C, the P98L substitution is uncoloured because it may arise from polymorphism, since the donor is heterozygous at this position. The number of nucleotide mutations and amino acid substitutions are reported in brackets next to the antibody names. b, Graphic representation of mutational hot spots (red) and of most conserved regions (blue).
Extended Data Figure 4 | Validation of switch region inserts combining Illumina and MinION technologies. a, Illumina and MinION workflows. Switch regions of polyclonal naive or IgG/IgA switched B cells were amplified by PCR. For Illumina sequencing, PCR amplicons were fragmented, re-amplified during library preparation and sequenced using the 2 × 300 bp MiSeq system. The bioinformatic analysis included the assembly of contiguous, chimaeric reads. For insert confirmation, independently generated PCR-barcoded primary products were sequenced with MinION technology and analysed with a different bioinformatic approach for long, error-prone MinION reads. b, Multiple identical switch inserts for donor 6 were confirmed in biological replicate experiments with independent technical and analytical setups. Shown are the experimental designs, shared and unique reads in a Venn diagram and an alignment of Illumina and MinION sequences covering the switch insertion sites for two examples (LCP1, RAVER1). c, Shared and unique switch inserts in technical and biological replicate experiments of donor 5.
**FASTQ (raw) = targeted amplicon reads (300 bp PE)**

a. Quality-based trimming and adapter removal (*Trim Galore, FastQC*). Remove reads < 100bp

**FASTQ (trimmed)**

b. Align reads to human genome GRCh37 (*BWA mem*)

| Chr14 | insert |
|-------|--------|
| Chr?   |        |

Switch region
chr14:106050000-106337000

**BAM**

c. Select genomic ranges where coverage >= 2 (or 40) reads and 50bp <= length < 2000bp

| Chr14 |
|-------|
| insert |

One read in 3' is chimaeric with Switch region

One read in 5' is chimaeric with Switch region

Two discordant reads have their mates in Switch region

**FASTQ**

d. Identify potential inserts like the following: (*pysam*)

| Chr14 |
|-------|
| insert |

One read in 3’ is chimaeric with Switch region

Two discordant reads have their mates in Switch region (proper paired with a chimaeric read)

**FASTA**

e. Merge inserts coming from 2 and 40 reads coverage and annotate them with GENCODE v19

*For overlapping inserts, if difference between 2 inserts <= 10bp, we keep the shortest one, otherwise we keep the longest one.*

(*Java, BEDOPS*)

f. *De novo* assembly for each potential insert (*samtools, pysam*)

| Chr14 |
|-------|
| insert |

Filter in paired reads containing the insert (chimaeric or non-chimaeric ones): encompassing mate pairs and spanning mate pairs

Filter in the spanning mate pairs in the Switch region (proper paired with a chimaeric read)

**De novo assembly (Trinity)**

Validate insert (*BLAST, Java*)

- insert sequence not homologous with Switch region
- insert sequence complete in the contig
- two flanking regions of minimum 50bp that match with Switch
  → select the shortest contig sequence that fulfills the criteria

Extended Data Figure 5 | Pipeline for data analysis using the Illumina platform. Shown is the scheme of the bioinformatics workflow used for the analysis of Illumina sequences.
FAST5 (raw) = targeted amplicon reads 2-4 kb

a. Quality-based filtering: 2D reads (Metrichor)

b. Remove reads < 1000bp (or 2000bp) and convert to FASTA format

c. Reads alignment to human genome GRCh37 (LAST)

- Insert with length >= 50bp
- Two flanking regions mapping to the Switch (length >= 100bp)
- Gap <= 100bp between the insert and the Switch flanking region(s)

d. Parse LAST output and select reads with an insert (Java)

- Insert with length >= 50bp
- Two flanking regions mapping to the Switch (length >= 100bp)
- Gap <= 100bp between the insert and the Switch flanking region(s)

e. Merge inserts’ coordinates (for overlapping inserts) and annotate them with GENCODE v19

(bedtools, BEDOPS)

Extended Data Figure 6 | Pipeline for data analysis using the MinION technology. Shown is the scheme of the bioinformatics workflow used for the analysis.
Extended Data Figure 7 | Examples of genes that donate multiple inserts. Shown is the original position of the inserts donated by PAX5 and EBF1 as well as a list of genes that donated two or more inserts.
Extended Data Figure 8 | Examples of potentially functional inserts. Shown is the alignment of the contig sequence and the genomic region from which the insert was derived, as well as the potential amino acid sequence inserted between the VH and CH1.
Extended Data Figure 9 | Relationship between LAIR1-IgG or LAIR1-IgM status and protection from febrile malaria. Shown is the clinical status of 551 members of the Malian cohort, stratified by LAIR1-containing IgG (a) or LAIR1-containing IgM (b) status, over the years 2012 and 2013. Febrile malaria is defined as parasite density ≥ 2,500 asexual parasites per microlitre of blood and an axillary temperature of ≥ 37.5 °C.
## Extended Data Table 1 | V gene and insert usage of LAIR1-containing antibodies

| Donor | mAb    | Isotype | Heavy chain VDJ genes (% identity to GL) | Light chain VJ genes (% identity to GL) | LAIR1 mutations (% identity to GL) |
|-------|--------|---------|----------------------------------------|----------------------------------------|-----------------------------------|
| M     | MGJ1   | IgG1    | VK1-5 (83.0) D3-16 JH4 (89.4) VL2-10 (88.4) JK3 (91.5) | VK1-5 (89.3) JK3 (91.9) | JK3 (99.3) |
| M     | MGJ2   | IgG3    | VK1-5 (90.6) D5-19 JH3 (91.8) VL2-14 (93.4) JK3 (91.9) | VK1-5 (96.1) JK3 (91.9) | JK3 (99.7) |
| M     | MGJ3   | IgG1    | VK1-5 (85.4) D3-21 JH3 (89.8) VL2-14 (94.4) JK3 (91.9) | VK1-5 (86.5) JK3 (91.9) | JK3 (99.7) |
| M     | MGJ5   | IgG1    | VK1-5 (87.5) D3-16 JH4 (89.8) VL2-14 (98.9) JK3 (97.4) | VK1-5 (86.5) JK3 (97.4) | JK3 (99.3) |
| M     | MMJ1   | IgG1    | VK1-5 (88.2) D5-19 JH3 (85.7) VL2-14 (90.0) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ2   | IgM     | VK1-5 (90.6) D6-19 JH3 (81.6) VL2-14 (93.2) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ5   | IgM     | VK1-5 (91.7) D6-19 JH3 (83.7) VL2-14 (92.5) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ6   | IgM     | VK1-5 (91.0) D6-19 JH3 (79.6) VL2-14 (91.4) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ7   | IgM     | VK1-5 (90.6) D6-19 JH3 (81.6) VL2-14 (92.8) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ8   | IgM     | VK1-5 (90.6) D6-19 JH3 (81.6) VL2-14 (93.2) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ10  | IgM    | VK1-5 (90.6) D6-19 JH3 (81.6) VL2-14 (93.2) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ16  | IgM    | VK1-5 (90.6) D6-19 JH3 (81.6) VL2-14 (93.2) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ23  | IgM    | VK1-5 (91.7) D6-19 JH3 (83.7) VL2-14 (92.5) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |
| J     | MMJ25  | IgM    | VK1-5 (90.6) D6-19 JH3 (83.7) VL2-14 (92.5) JK3 (86.5) | VK1-5 (86.5) JK3 (86.5) | JK3 (99.3) |

Isotype and V(D)J gene usage of heavy chain and light chain of mAbs containing LAIR1 in the switch or in the VDJ region. Genes of the monoclonal antibodies containing a V(DJ) insert cannot always be properly predicted by IMGT. Mutations of the LAIR1 insert are shown as % of identity to the genomic unmutated LAIR1 exon. GL, germline; nd, not determined.