B-cell receptor reactivity against *Rothia mucilaginosa* in nodular lymphocyte-predominant Hodgkin lymphoma

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Supplementary

Supplementary Methods

Laser microdissection of LP cells, IgV region gene PCR and expression cloning

Single, clearly identifiable LP cells were microdissected with an ultraviolet laser (PALM microdissection system, Zeiss Axiovert 200M microscope), pooled in groups of 30 cells and resuspended in 18 µl 1x PCR buffer and digested with 2 µl of proteinase K (Roche, Grenzach, Germany) at 55°C for 4 h, followed by enzyme inactivation at 95°C for 10 min. The LP cell lysates were subjected to two rounds of V_H-, V_κ- and V_λ-specific seminested PCRs (30 and 44 cycles in the first and second round of PCR, respectively) using IgV family-specific primers and J primer mixes, and Expand high fidelity PCR kit (Roche) as described by Küppers *et al.*[1]

Expression of recombinant BCRs and Antigen Screening

The amplified IgV region genes were sequenced and analyzed with IMGT-V-Quest for functionality, V, D and J segment usage and indications for somatic mutations. If both a functional heavy and light chain variable region gene was amplified, the IgV region genes were cloned into TOPO Zero-Blunt vector (Invitrogen Life Technologies, Darmstadt, Germany).[2] IgV gene fragments were re-extended at the 5′ and 3′ ends according to the proper immunoglobulin germline genes. Complete IgV genes were inserted via ApaLI and XhoI for IgV_κ or IgV_λ in front of a κ- or λ-constant region gene, respectively, and via *Nco*I and *BstE*II for IgV_H in front of a γ-1 constant region gene into a modified pCES-1 vector for expression of the Fab fragments.[3] Fabs were expressed and purified.[4]

Generation of DEV cells expressing recombinant BCRs

The only existing NLPHL cell line (DEV) was cultured at 37°C in an atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml), ultraglutamine (2 mM), and 20% fetal calf serum, and transfected with a modified pRTS expression vector with an IgV region heavy chain and constant regions Cγ1-Cγ4, Cµ1-Cµ4, or Cδ1-Cδ4 with membrane coding exons TM1 and TM2 for the transmembrane region and the cytoplasmic tail followed by a 2A sequence and the light chain

variable region and light chain constant region gene. Transfection of DEV cells was performed after three washing steps with RPMI-1640 at a cell density of 2 x 10^7/ml in RPMI-1640 without FCS on ice. Two times 10^6 cells equalizing a volume of 100 µl were transfected with 5 µg plasmid DNA by electroporation using Gene Pulser (Biorad) with a 0.2 cm cuvette, a voltage of 140 V and 30 msec pulses. Subsequently cells were immediately put again on ice and cultured in RPMI-1640 medium supplemented with 20% FCS (Sigma, F2442). Cell lines stably expressing recombinant membranous BCR were selected with hygromycin at 250 µg/ml. Expression of recombinant BCR was induced by addition of doxycycline.[5] Successful transfection was verified by IgV region gene PCRs of transfected cell lines, by Western blot of the FLAG-tagged recombinant BCRs and surface expression of the transfected His-tagged BCR was determined by flow cytometry.

Fab screening in bacterial lysates

To screen for the potential reactivity of Fabs against bacterial antigens, heat-inactivated lysates of two different bacterial strains or patient isolates, including *Rothia aeria* and *Rothia mucilaginosa*, were provided by the Institute of Medical Microbiology and Hygiene of Saarland University, Homburg/Saar, Germany. We subsequently obtained a pool of bacterial cultures from commercially available probiotic capsules (Mikrobiom-Intercell, Intercell Pharma, Höhenkirchen-Siegertsbrunn, Germany), which included *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactococcus lactis, Propionibacterium freudenreichii* and *Streptococcus thermophilus*. Dot blots of bacterial lysates were blocked in 10% (w/v) non-fat dry milk powder in TBST (TBS, 0.1% [v/v] Tween 20) at 4°C overnight, washed twice in TBST, and incubated for 1 hour with the individual Fabs, each at a concentration of 10 µg/ml. Following three 30-minute TBST washes and subsequent incubation with biotinylated goat anti-human heavy and light chain Fab antibody (DIANOVA, 109-065-088) at a dilution of 1:5,000 (v/v) at room temperature for 1 hour, the arrays and blots were incubated for 10 minutes at room temperature with Strep-

POX (1:5,000) in 2% (w/v) milk/TBST. Binding was detected using the ECL system (Amersham Pharmacia, Freiburg, Germany).

To characterize antigens detected in the *R. mucilaginosa* lysate, polyacrylamide gel electrophoresis (PAGE) separation of *R. mucilaginosa* lysates was performed, and the lysates were Western blotted under reducing and non-reducing conditions with *R. mucilaginosa*-reactive LP cell-derived recombinant Fabs as primary antibodies. To identify the specific *R. mucilaginosa* antigens of the two bands detected by Western blot at 75 kDa and 35–40 kDa in the *R. mucilaginosa* lysate, the lysate was separated in two-dimensional gels in the lab of Proteome Factory. The gels then underwent silver staining and Western blotting with *R. mucilaginosa*-reactive LP cell-derived recombinant Fabs as primary antibodies followed by isolation and mass spectrometry of the identified spots (Proteome Factory AG, Berlin, Germany). In brief, NCBIprot database version 20161128 was used (106,762,850 sequences, 39,119,668,168 residues) with the following parameters: enzyme: trypsin; fixed modifications: carbamidomethyl (C); variable modifications: deamidated (NQ), oxidation (M); mass values: monoisotopic; protein mass: unrestricted; peptide mass tolerance: ± 3 ppm; fragment mass tolerance: ± 0.6 Da; maximum missed cleavages; instrument type: ESI-Trap. Candidate genes from *R. mucilaginosa* were amplified from bacterial DNA and cloned with a C-terminal FLAG-tag into a pSFI vector and expressed in HEK293 cells.

Nunc MaxiSorp enzyme-linked immunosorbent assay (ELISA) plates were coated overnight at 4°C with murine anti-FLAG antibody (Sigma, F3165, Munich) at 1:2500 v/v dilution. After blocking with 1.5% (w/v) gelatin in TBS and washing with TBS with Triton-X, recombinant NLPHL-derived Fabs were added at 10 µg/ml or patient sera at 1:100 dilution at room temperature for 1 hour. After a washing step with TBS, biotinylated goat anti-human IgG (heavy and light chain) (Dianova) at 1:2500 (v/v), sheep anti-human IgG1, IgG2, IgG3 and IgG4 (Binding Site, AU006, AU007, AU008 and AU009, Birmingham, United Kingdom) at 1:5000 (v/v), or rabbit anti-human IgM (Dianova, 109-476-129) at 1:2500 (v/v) were added for 1 hour at room temperature followed by a washing step with TBS. For the determination of IgG subclasses and IgM, corresponding biotinylated secondary antibodies were applied followed by peroxidase-labeled streptavidin (Roche) at 1:50,000.

To verify the specificity of the reactivities of NLPHL-derived Fabs against the components of *R. mucilaginosa*, recombinant Fabs derived from primary central nervous system lymphoma (PCNSL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), primary mediastinal B-cell lymphoma (PMBCL) and chronic lymphocytic leukemia (CLL) were screened against these recombinant FLAG-tagged antigens of *R. mucilaginosa* by ELISA.[4,6,7]

Fab screening on virome array

To extend the screening for infectious antigens, the recombinant NLPHL-Fabs were screened

at concentrations of 1 µg/ml, 10 µg/ml, and 20 µg/ml on a custom-made VIROME microarray representing 2,206 viral genes chosen from 158 viruses using nucleic acid programmable protein array technology (ASU, Biodesign Institute, Arizona State University, USA). Screening and analysis of the arrays was performed by ASU. Briefly, Array Pro 6.3 software was used with normalization to the median of all samples, and normalized values ≥2 were considered candidate antigens. The protein arrays were incubated for 1 hour with pooled Fabs, each at a concentration of 10 µg/ml. Expression clones of candidate antigens were obtained from the DNASU plasmid repository (ASU, Biodesign Institute).

Generation of DEV cells expressing *Rothia* spp.-reactive BCRs followed by activation, proliferation, and apoptosis assays after stimulation with *Rothia* antigens and immunotoxins

For Western blot analysis of the BCR pathway activation of transfected DEV cells either expressing a BCR with reactivity against *Rothia mucilaginosa* Gltf or *Moraxella catarrhalis* RpoC, respectively, and of IgD or IgG class, $1x10^6$ cells were incubated with no antigen, recombinant RpoC at 5 µg/ml, Gltf or Bdh at 5µg/ml or anti-IgM/IgD at 1 µg/ml. As primary antibodies rabbit antibodies against pTyr525/526 SYK diluted 1:2000, pTyr759 PLCγ2 diluted 1:1000, pTyr223 BTK diluted 1:1000 and pTyr96 BLNK diluted 1:1000 (B cell signaling sampler kit, 9768, CST, Massachusetts, USA), rabbit antibody against actin diluted 1:2000 (Sigma, A5060) and murine antibody against MYC at a concentration of 1 µg/ml (Santa Cruz) were utilized, followed by washing steps and incubation with POX-conjugated anti rabbit or anti mouse antibodies diluted at 1:3000.

EZ4U, a non-radioactive proliferation assay (Biomedica, BI-5000), was performed according to the manufacturer's instructions with DEV cells (IgD^-), and the DEV cell line transfected to express RpoC- or Gltf-reactive surface BCR of the IgD or IgG class. The IgV genes were derived from case #2 and case #3, and the IgD constant region gene from PBMCs of a healthy blood donor.[8,9] *Rothia mucilaginosa* Gltf and Bdh, *Moraxella catarrhalis* RpoC and as a control recombinant human SLP2, which is a frequent antigenic target of paraproteins from patients with multiple myeloma, were added at 10 µg/ml. Adsorbance of Formazan at 450 nm was determined after 3 days at 37°C. Statistical significance was calculated by multiple t-tests (Prism9, graphpad).

For the analysis of apoptosis, a 5×10^5 cells/ml suspension of DEV cells stably transfected to express either a BCR (#2) with reactivity against *R. mucilaginosa* Gltf, a BCR (#3) with reactivity against *M. catarrhalis* RpoC, or a different antigen were treated by adding the recombinant conjugate of the epitope of *R. mucilaginosa* Gltf with ETA' or of *M. catarrhalis* RpoC with ETA' (both at 0.5 µg/ml) for 24 hours at 37 °C in 5% CO_2. Following incubation, the cells were washed twice with PBS and resuspended in 500 µl of binding buffer. Then, 5 µl of AnnexinV-FITC (SIGMA, APOAF) and 10 µl of propidium iodide (SIGMA) were added to each cell suspension and incubated for 10 minutes at room temperature followed by analysis using FACSCanto. In addition, the effects of the immunotoxins were measured by trypan blue assays at 0, 24, and 48 hours.

HLA genotyping

HLA-DRB1 and HLA-DQ typing was performed for all patients via sequencing (Labor Thiele, Kaiserslautern, Germany).

Statistical analysis

Normality was evaluated using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Statistical significance was calculated using unpaired, two-tailed t-tests for comparison of parametric distributions, without adaptation for multiple comparisons (Prism 9, GraphPad).

Supplementary Results

The median age of patients with successfully amplified IgV genes was 28 years. Eleven patients had IgD+ LP cells (Supplementary Figure 1), and seven of these patients were adolescents (Table 1). Two IgD+ NLPHL samples were obtained from inguinal lymph nodes, but these were relapses. A male predominance was observed among the NLPHL cases (18 of 22, 82%). All cases had mutated IgV genes, with mutation frequencies for heavy and light chain IgV genes (VH and VL, respectively) ranging between 0 and 18.0% (average: 8.3% for VH and 4.8% for VL gene segments; Table 2, Suppl. Table 2). The complementarity determining region (CDR) 3 of VH region genes isolated from IgD+ LP cells was significantly longer (median: 28 amino acids; n = 11), compared with the CDR3 of VH region genes isolated from IgD- LP cells (median: 19 amino acids; n = 11; non-parametric distributed; p=0.0094, Mann-Whitney-test). Seven of the eleven IgD+ NLPHL cases expressed a member of the VH3 family, compared with two of the twelve IgD- NLPHL cases.

Supplementary Table 1: Characteristics of the NLPHL patients included in this study

Case	sex	Age (y)	primary disagnosis /relapse	Initial Stage	Localization	LP cells IgD^+	HLA-DRB1	Pattern	Reactivity against *M. cat.* RpoC or *M. osl.* Succinate-CoA ligase sub α	Reactivity against *R. mucilaginosa*
1	m	52	relapse	na	axillary	no	DRB1*01:01:01/DRB1*11:01:01	A	no	no
2	m	15	relapse	IIA	supraclavicular	no	DRB1*01:01:01/DRB1*11:02:01	A	no	yes
3	m	14	2nd relapse	IIA	cervical	yes	DRB1*04:01:01/DRB1*11:01:01	A	yes	no
4	m	41	first manifestation	IIA	cervical	no	DRB1*04:01:01/DRB1*15:01:01	na	no	no
5	f	37	first manifestation	IIIB	retroperitoneal	no	DRB1*11:01:01/DRB1*11:01:01	na	no	no
6	m	15	relapse	IIB	cervical	yes	DRB1*04:07:01/DRB1*07:01:01	C	yes	no
7	m	51	2nd relapse	IA	axillary	no	DRB1*11:01:01/DRB1*15:01:01	A+D	no	no
8	m	40	primary progressive	IVB	abdominal	no	DRB1*04:01:01/DRB1*08:01:02	D+E	no	no
9	m	42	relapse	na	inguinal	yes	DRB1*07/DRB1*07	na	yes	no
10	m	12	2nd relapse	IVA	inguinal	yes	DRB1*01:01:01/DRB1*04:01:01	na	yes	no
11	m	15	first manifestation	na	cervical	yes	DRB1*03:01:01/DRB1*07:01:01	C	yes	no
12	m	31	first manifestation	IA	cervical	no	DRB1*07:01:01/DRB1*13:02:01	A	no	yes
13	m	18	first manifestation	IIA	cervical	yes	DRB1*04:01:01/DRB1*07:01:01	D	yes	no
14	m	30	first manifestation	I E	parotid	yes	DRB1*01:01:01/DRB1*11:01:01	E, B + D	yes	no
15	f	16	first manifestation	I	axillary	yes	DRB1*01:01:01/DRB1*11:02:01	A	no	yes
16	m	44	relapse	IV B	axillary	no	DRB1*09:01:02G/ 16:01:01G	E	no	yes

17	m	30	first manifestation	IV B	abdominal	yes	DRB1*04:01:01G/ 11:01:01G	D	yes	no
18	m	26	first manifestation	IIA	cervical	yes	DRB1*07:01:01G	C	yes	no
19	m	11	first manifestation	na	inguinal	no	DRB1*03:01:01G/ 04:05:01G	E	no	yes
20	f	15	relapse	II A	cervical	yes	DRB1*03:01:01G/ 04:01:01G	C	no	no
21	f	18	relapse	II A	infraclavicular	no	DRB1*13:01:01G/ 15:03:01G	A	yes	no
22	m	9	first manifestation	I A	inguinal	no	DRB1*10:01:01G/ 14:01:01G	A	no	no

Supplementary Table 2: IgV region gene analysis of LP cells and identified antigenic targets

Case	VH/VL gene	Homology (%)	DH gene	JH/JL gene	length CDR3 (AA)	Junction	Identified Antigen
1	VH1-3-01	91.3	D3-10*01	JH4*02	20	CAREVRPPRIIMIWGVGLLDFW	Human RPS27a (autoantigen)
	VK1-27*01	97.5	-	JK4*01	9	CQKYNSAPLTF	
2	VH1-46*01	87.5[1]	D3-16*02	JH2*01	15	YYCARDEGDIRRYFDLW	*R. mucilaginosa* glycosyltransferase Gltf
	VK3-15*01	92.8	- D3-3*01	JH5*01 -	10	CQQYNYWPPVTF	
3	VH3-11*01	95.1	D3-3*01	JH6*03	26	CARVAGAAGRNYNYWSGYWEDYYFMDVW	*M. catarrhalis* RpoC
	VK2D30*01	99.3	-	JK4*01	9	CMQGTHWPR	
4	VH4-31*01	99.2	D3-22	JH3*02	19	CARGPPPYDSSGYYSHGLDIW	∅
	VK1-37	99.3	-	JK3*01	8	GQRTYNAPRF	
5	VH4-39*01	100	D6-19*01	JH6*04	15	CASMGAVAGMMFGMDVW	Human pyruvate carbocxylase 3
	VK1-5*03	96	-	JK1*01	8	CQEYNSYWTF	
6	VH3-07*01	95.5	D3-3*01	JH6*04	26	CAREVLRWGGSYDFWSNYYEDYFALDVW	*M. catarrhalis* RpoC
	VK-1D43*01	98.5	-	JK3*01	10	CQQYYSTPPFTF	
7	VH1-69	86.4	D6-19*01	JH5*02	17	CARDYSRGVCGPRYGMDVW	∅
	VK3-11*01	93.8	-	JK4*01	9	CQQRSNWPPAF	
8a	VH3-30	89.9	D2-15*01	JH6*02	22	CARKGGDPVLALFVPNFAMDVW	∅
	VK1-33*01	91.6	-	JK4*01	9	CQQYNSLPITF	
9	VH3-48*03	82.0	D3-3*01	JH6*02	30	CAKSVLTAKSGKSYKFWNNYHEDYHYYLMDVW	*M. catarrhalis* RpoC
	VK1-27*01	87.1	-	JK3*01	10	CQNYNTVPLTF	
10	VH4-59*01	86.4[2]	D3-3*01	JH6*03	33	CATVDPTVVEGRVKYYDFWSGYYGTDQRYYYMDVW	*M. catarrhalis* RpoC

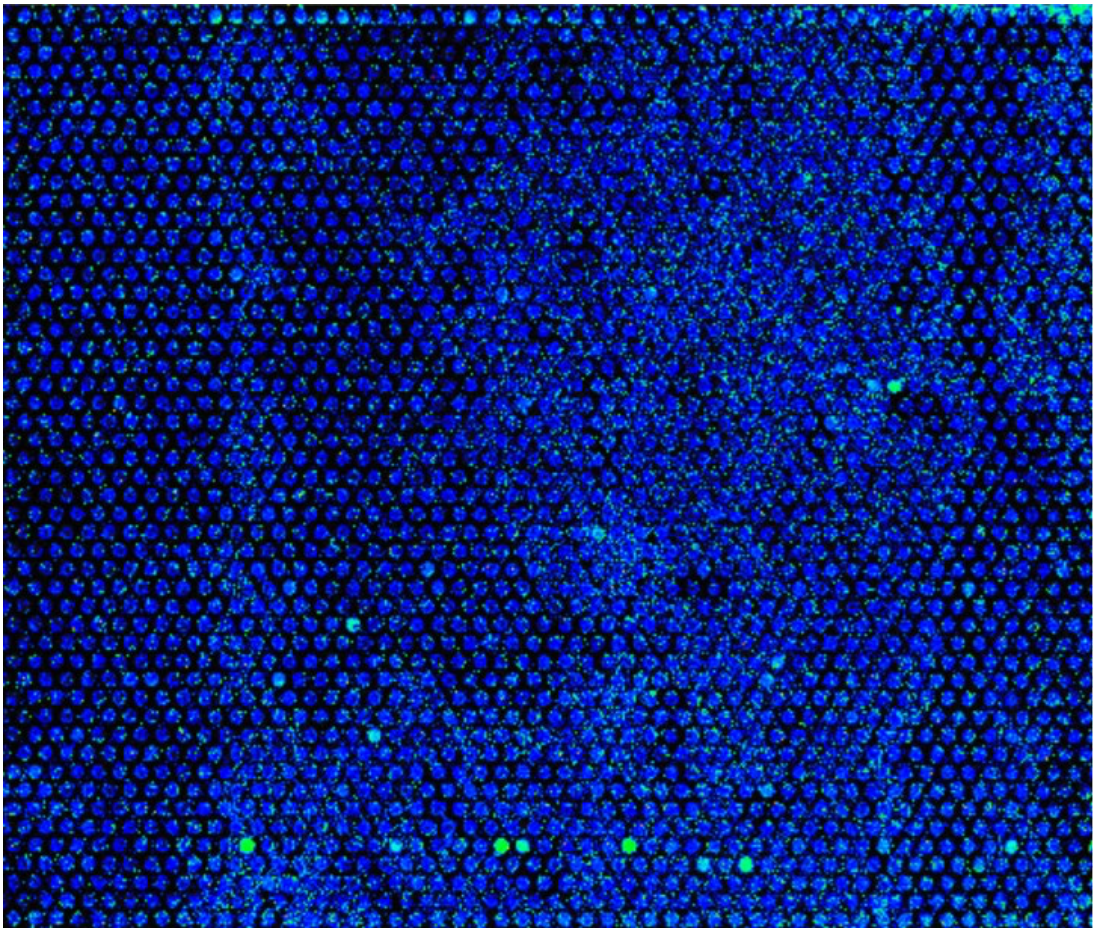
	VL3-21*01	92.8	-	JL2*01	11	CQVWDSSSDHPVF	
11	VH3-11*01	95.5	D3-3*01	JH6*02	30	CARLLTSEGSRKYYDFWSNYWEGYQYYTMDVW	*M. osloensis* SUCLG1
	VK2-28	99.2	-	JK3*03	9	CMQGLQTVFTF	
12	VH4-34	100	D3-10*01	JH5*02	16	CARGPYLWFGERGWFDPW	*R. mucilaginosa* Bdh
	VK1-5*01	94	-	JK2*03	9	CQQYNSYPYCF	
13	VH3-11*01	83.0	D3-3*01	JH6*03	26	CARLCAAGGRSYDFWSGYYENYFYMEVW	*M. catarrhalis* RpoC
	VK1-5*01	86.7	-	JK4*01	10	CQQYSGSSRVTF	
14	VH3-11*01	87.6	D3-3*01	JH6*02	30	CARLIEAGGVGKHYDFWSGYYTVDYYYGMDVW	*M. catarrhalis* RpoC
	VK1-43*01	94.3	-	JK4*01	10	CQQYYSTPPLTF	
15	VH3-30*04	89.8	D3-3*01	JH6*02	33	CARTTWVGVVGRIKYYDFWSGYHGTGMEYYTMDVW	*R. mucilaginosa* Gltf
	VL9-49*01	98.1	-	JL3*02	12	CGADHGSGSNFWVF	
	VK1-17*01	94.3	-	JK4*01	10	CLQHNSYPRLTF	
16	VH23*04	90.3	DH6-19*01	JH2*02	19	CAKLPLRPQWLYRGYFDLW	*R. mucilaginosa* Gltf
	VK3-15*01	98.6		JK4*01	11	CQQYNNWPLTF	
17	VH3-11*01	87.5	??	JH4*02	28	CARDIHHQWLNPVINPHWVDPVDYFDYW	*M. catarrhalis* RpoC
18	VK1-5*01	94.6		JK3*01	11	CQQYSHFSITF	
18	VH1-69*13	99.6	DH5-12*01	JH3*02	18	CASPGRYSGYGYDAFDIW	*M. catarrhalis* RpoC
	VL9-49*01	100		JL3*02	17	CGADHGSGSNFGPGWVF	
19	VH3-7*01	94.4	DH3-3*01	JH6*03	35	CATIVLDSIKGSVRYYDFWSGHHGLSYYYYYMDVW	*R. mucilaginosa* Gltf
	VK3-20*01	99.6		JK5*01	12	CQQYGSSPPITF	
20	VH2-5*02	96.6	DH3-9*01	JH3*02	18	CAHTHEDILTGSDALDIW	∅
	VL1-47*01	95.8		JH3*02	13	CAAWDESLRGRMF	
	VL2-14*03	98.3		JL3*02	12	CSSYTSSSTLVF	
21	VH1-2*02	88.9	DH3-22*01	JH4*02	18	CARDRIEDFFDSSGYVYW	*M. catarrhalis* RpoC

	VK2-49*03	99		JK3*01	12	CMQGIHLPPFTF	(both combinations of IGVH and both light chains bound RpoC)
	VL3-21*02	95		JL2*01	13	CQVWDSTSDHVIF	
22	VH1-18*01	97.6	DH2-15*01	JH3*02	23	CARVPWFGLCSGGSCYEDAFDIW	∅
	VH5-10-1*01	99.6	DH3-10*01	JH5*02	16	CARSLYYGSGPRFDPW	
	VL1-44*01	98.5		JL2*01	13	CAAWDDSLNGPLF	
	VL2-23*02	96.5		JL1*01	12	CCSYAGIRGGVF	
	VL3-10*01	98.7		JL1*01 F	13	CYSTDSSGNHRVF	
	VL3-25*03	99.6		JLJ2*01	13	CQSADSSGTYVVF	

Supplementary Table 3: Significant Candidate Antigens of Virome Array Sreening

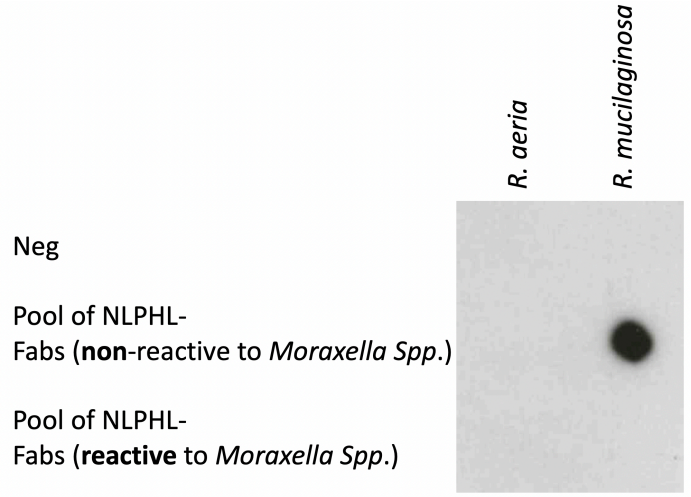
					Sample	NLPHL
SpotNo	Row	Column	Protein	Gene symbol	Species	111_17-3
2237	47	29	H1N1-NP	NP	NAPPA control	17,03
2219	47	11	EBNA1	EBNA-1	NAPPA control	15,26
2231	47	23	BFRF3	BFRF3	NAPPA control	13,17
1049	22	41	CbCD00959563	NA	Coxsackievirus B1	5,82
2290	48	34	MsCD00595052	NA	Measles, strain Ichinose WT	5,55
2232	47	24	CagA_884_2.1	HP0547	Helicobacter pylori 26695	3,72
1648	35	16	HrCD00959476	NA	Human rhinovirus A1	3,34
2255	47	47	H3N2-M1	M1	NAPPA control	3,17
1937	41	17	HsCD00959820	M1	H1N1 subtype	3,00
2226	47	18	CagA_FL_2.1	HP0547	Helicobacter pylori 26695	2,73
2288	48	32	CbCD00594880	NA	Coxsackievirus B4 (strain E2)	2,63
1419	30	27	CaCD00959623	NA	Coxsackievirus A22	2,31
1048	22	40	CaCD00959834	NA	Coxsackievirus A9	2,26
1764	37	36	HrCD00956402	NP	Human rubulavirus 2	2,25
2099	44	35	HcCD00959896	Nucleocapsid protein	Human coronavirus NL63	2,19
2249	47	41	EBNA2	EBNA2	NAPPA control	2,11
1788	38	12	HsCD00959755	M1	H3N2 subtype	2,08
1811	38	35	HsCD00959751	NP	H1N1 subtype	2,07
1507	32	19	EeCD00959632	NA	Echovirus E25	1,89

Supplementary Figure 1:



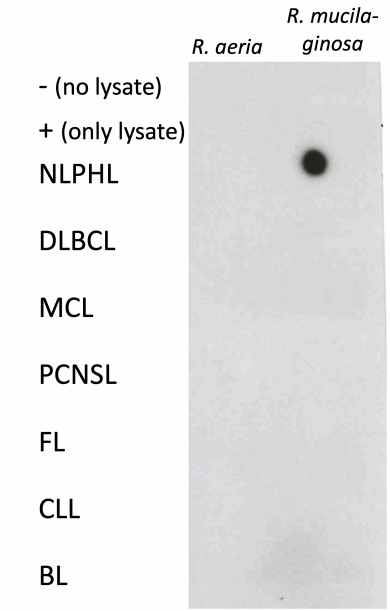
Supplementary Figure 1: NAPPA microarray analysis of a pool of recombinant NLPHL Fabs.

Supplementary Figure 2:



Supplementary Figure 2: Representative Immuno-dot blots of bacterial lysates of *R. aeria* and of *R. mucilaginosa*, shows reactivity against lysate of *R. mucilaginosa* from a pool of Fabs, which had previously fount to be not reactive to lysates of Moraxella spp.

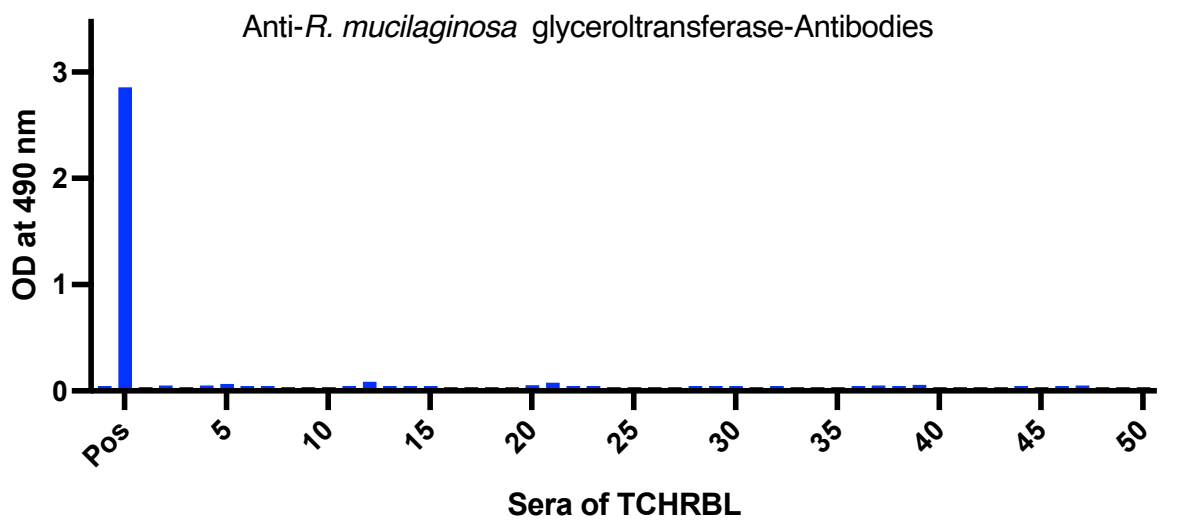
Supplementary Figure 3:

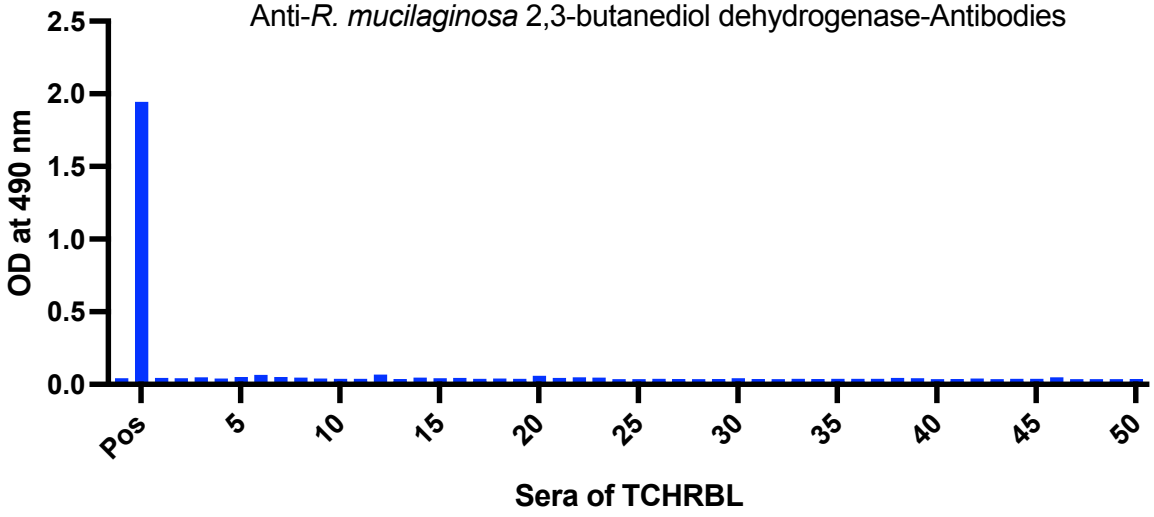


Supplementary Figure 3: Representative Immuno-dot blots of bacterial lysates of *R. aeria* and of *R. mucilaginosa* with pools of recombinant Fabs of NLPHL and different B-NHLs.

Supplementary Figure 4:

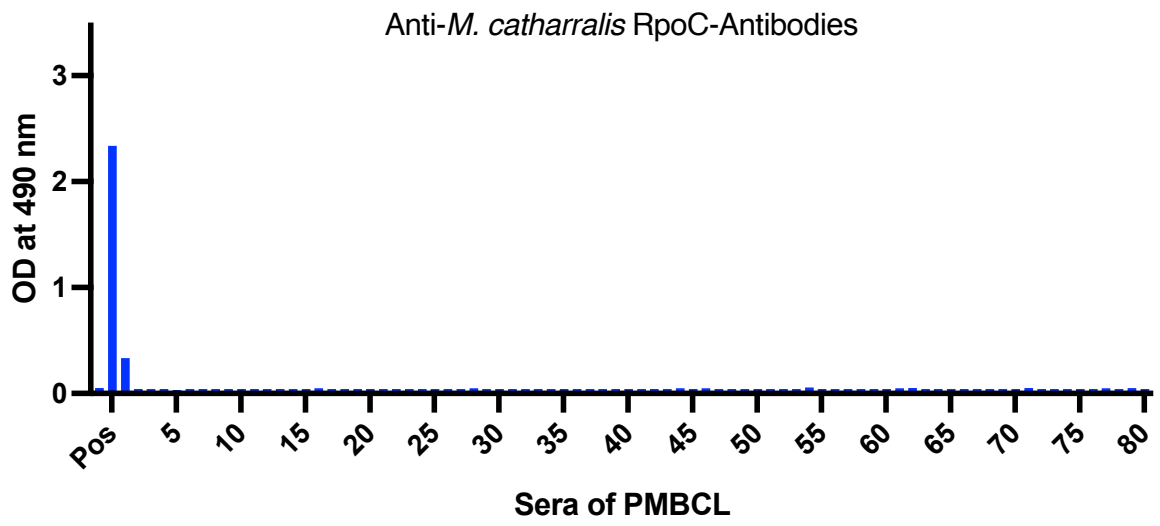
A)



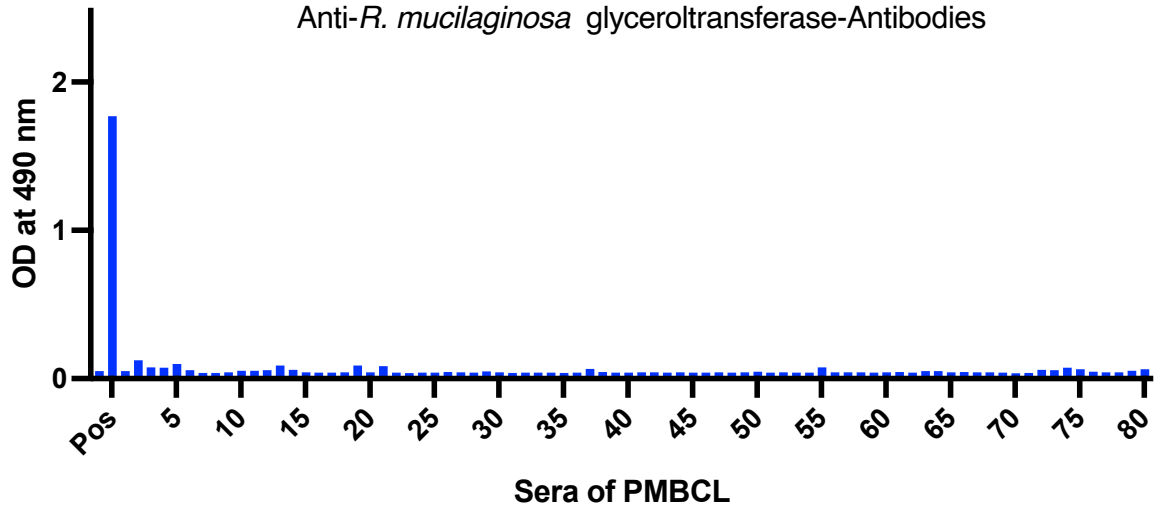


B)

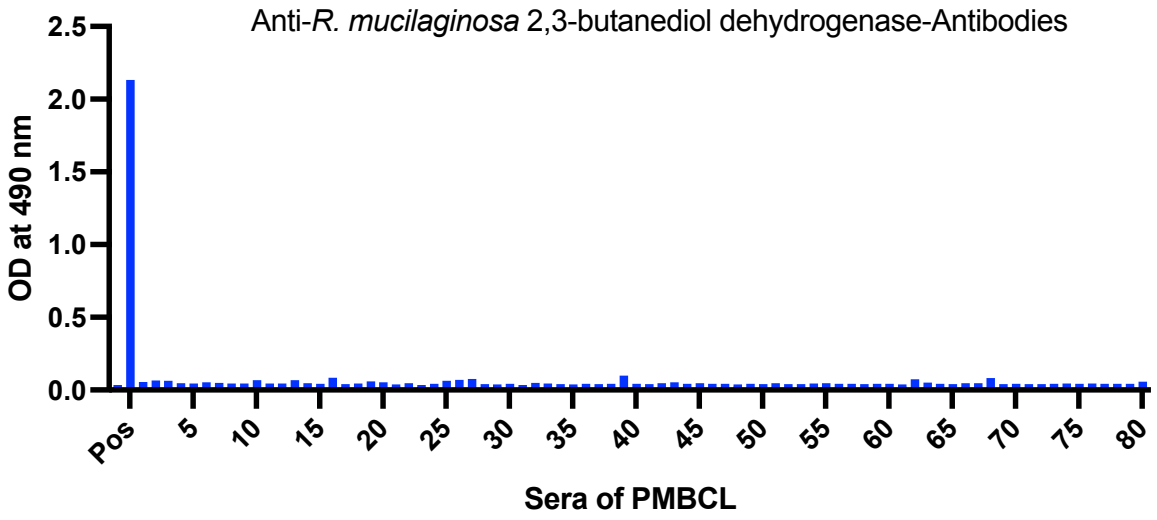
C)


Anti-*M. catharralis* RpoC-Antibodies
OD at 490 nm
0
1
2
3
Pos
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Sera of PMBCL

D)


Anti-*R. mucilaginosa* glyceroltransferase-Antibodies
OD at 490 nm
0
1
2
Pos
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Sera of PMBCL

E)



Supplementary Figure 4: Sera of patients with THRBCL (diluted 1:100) were tested for antibodies against A) glycosyl transferase and B) 2,3-butanediol dehydrogenase of *R. mucilaginosa*. All THRBCL sera were negative for anti-glycosyl transferase and for anti-2,3-butanediol dehydrogenase-antibodies. Plasma of patients with PMBCL (diluted 1:100) were tested for antibodies against C) *M. catarrhalis* RpoC, D) against *R. mucilaginosa* glycosyl transferase and E) 2,3-butanediol dehydrogenase.

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