SUPPLEMENTARY MATERIAL

Figure S1: Standard curves for IgG, IgG1, IgG2, IgG3 and IgG4 and cross-reactivities. Human IgG and IgG subclass antibodies were coated in serial dilutions and incubated with monoclonal anti-IgG detection antibodies. The standard curves are fitted to a 4-parameter logistic regression model. Monoclonal antibodies directed against one subclass did not cross-react to the other IgG subclasses.



Figure S2: List of gating names. Full names and short names of the gates defined by the gating

strategy.

aating full namé	Gating short name
Ingated	Ungated
ngated/NoBds	NoBds
ngated/NoBds/Sgl	Singlets
igated/NoBds/Sgl/Time	Time
igated/NoBds/Sgl/Time/45+	CD45+
igated/Nobds/Sgl/Time/45/LL	Livel-Rh
igated/NoBds/Sgl/Time/45+/L1/L2	Live2-Pt
gated/NoBds/Sg/Time/45+/L1/L2/66b-45+	CD565-CD45+
gated/NoBas/3g/11me/45+/11/L2/600-45+/3-19-	CD3-CD19-
gated/Nosas/sgl/11me/451/L1/L2/50-451/3-15/55-	CD3-CD19-CD36-
gated/NoBds/Sg/Time/45+/L1/L2/66b-45+/3-19-/56-/14-16-	US4-CD19-CD36-CD14-CD16-
gated/wbgs/sgl/11mt/45+/11/12/xbb/45+/3-15+755+714-16+/127+116-	IC1
gated/wobcs/sg/11me/457/L1/L2/60/457/5-15/367/14-16/12/71L6/11/294-	1.02
gated/woods/sgi/min/sar/Li/L/C/COVMS7/512730/1410/127110/117/2044	1103
gated/NoBds/5gl/Time/451/L1/L2/660-451/3-19-/50- astad/NoBdr/Sal/Time/451/L1/L2/665-451/3-19-/50-	CD3-CD19-DD-
gated/wbs/s/gg/min/457/11/12/cb/457/3127/0K*	CB3-CB19-DR-
gated/wbsb/sg/1mm/457111/12/00/4571515-10/108/5011	Basophile
gated/webs/3g/1111c/457/L1/L2/60/457/312/0K73014/L2/3364	Basophils CD203+CD294+
gated/wobs/sg/11mg/str/12/2664.45/31/27/04/36145/358/2034234544	NK CD56biCD16mid
growy woow, syn mini (451/11/12/000451/3127/07/3011101110 astad/NaDdz/Sal/Tima/A52/11/12/266./62/2.40./00./56mid466i	NK CD56midCD16bi
gated/NoBds/Sal/Time/45+/11/12/55b.45+/2.40-/08-/56mid16mid	NK CD56midCD16mid
gavesy houses say hind (457) L1/L2/000457 (5127) MK Soma Lonna Astad/MAR4/SA/Time/A51/L1/L2/SSA/A51/2.42./DP4	CD2-CD19-DR+
gatesynoodag syn mielydor (L1/L2/0004007) 3129/0K7 astad/NoRds/Sal/Tima/454/L1/L2/6665454/3.19./D2+/14.16.	Dendritic
gategradous/syr/mm/457/L1/L2/66b.451/2.19/DKT/14-16-	DC myelo
gateu/wooda/og/ mmd/457/LL/L2/000457/5-L5/ DKT/L4-L5/L25-L101	DC myelo CD38-DPmid
gated/NoBcs/Sgl/11mt/45+1L1/L2/650-45+73-15-70k+714-16+7L23-116+735-0kmid	DC myelo cost-okinia
gated/Nobg/sg/11me/45+/L1/L2/004-3+/3-13+/DK*/14+10+/L23+11C*/S8+DK*	DC myelo accivated
gated/NoBa3/sgl/11me/45+/L1/L2/bb/45+/3-15+/DR+/14-16-/123+11c-	De prasma Marca a caral Lista and
gated/Nosas/sg/1mm/45/L1/L2/50045/13-15/DR+/14+16+	Mono hondi + Intermed
gated/wbg/s/gi/ime/45/1/1/2/c6A/81/2/10/	Reall CD10+
gated/NoBos/Sgl/Time/45+/L1/L2/60245+/3-15+	Beell (D10+CD20 DD+
gated/wbsg/sg/11me/451/LL/L2/60/451/3-151/20/UK*	Boell plasmablest
gated/Nobas/sgl/11me/45+/11/L2/660-45+/3-19+/20-0.R+/2/+38+	Boeil prasmabilast
gated/Nosas/sg/(Ime/451/L1/L2/50-451/3-191/2010K+	Beell CD23 LeD
igated/Nobas/sg/11me/454/L1/L2/505454/3-134/2040k4/27-igu-	Boell color - Igo-
gated/wbgs/sgl/11me/45+/L1/L2/662-45+/3-15+/20+Uk+/2/-igU+	Boell muitebad erements
gated/violag/sgl/11mc/457/L1/L2/660-457/3-157/2070K7/271gD-	Beall switched memory CCDEL
gated/NoBdS/Sg() (100/45+/11)/L/bDP-45+/3-19+/20+0K+/27+100-/100-0CK6+	Boell switched memory core
gated/Nobas/sgl/11me/45+/11/L2/60-45+/3-15+/20+0K+/27+1g0+	cost costo
gated/Nosas/sg/11me/45+/L1/L2/66-45+/3+13+	CUSTOUS-
gated/wbgs/sgl/time/45+/LL/LZ/bb245+/3+13+26-	T-cell TCDad
gatad/Noba3/5gl/11mc/457/L1/L2/60/457/3713719/56/10.8ga-	CDR+Tcell
gated/wobds/sg/11mm/45+114/12/co0+45+/5+15+25+17(0)/46+1	CDS+FU
gated/wbds/sgl/11me/4571L1/L2/60/457/37137137071CKgl/4-67/45K4CK7-	CD9+CM
gatudi Nubusi yaji Timu (457) LLI L2/60/457/3719730/TCR3U/4467/45RALCR77	CDB+IE
gated/wbgs/sg/11me/45+111/12/60/45+/5+15+15+06/10xg/4+6+/45K4+00K7+	CDOTTE
gated/wbgs/sgl/time/45+/LL/L2/60/45+/3+19+36+/CKgi/4-8+/45K4+CCK7+	CDitTeal
gategravous/syrrine/454/11/12/666.451/3113-/56/10094/07/000-457/3113-/56/10090/416-	CD4+TCEI CD4+CD25-
gateg/hvoda/jog/imid/451/L1/L2/000451/5115//50/itckg0/416/25-	CD4+CD23*
gateg/woods/bg//time/45+/L1/L2/666-45+/3+19-/56-/TCK90-/4+8-/25-/45KA-CCK/-	
gateg/1006us/5g//1111e/4547/L1/L2/000434/34154/50//1CKg0/4467/25/45KA-UCK/4 astad/beBds/6g//Time/AEL/14/10/66b-AEL/3410/F6/TCDgd//446/05/46D8-CCD7+AEDA-CCD7	CD4 Teall Roolean CD4CD4
gateg/Nobles/bg//time/45+/L1/L2/000-45+/b+1/5+/50+/TCKg0/4+6-/25-/45KA-UCK/+ 0745KA-UCK/+	CD4 Tcell DOUBAL CD45RA-
gateg/woods/bg// http://bi/litic/000-407/5119-/00/ 1 CKg0-/416-/25-/45KA-CCK/ T 0F45KA-CCK/ -/CCR4-CXCR5- asted/NoRdr/Sa/Time/A6L/11/12/66b-A6L/2110-/66_/TCRad-/418-/26_/46DA-CCR7+ 46DA-CCR7-/CCR4-CXCR5-	CD4+Tb1
galed/hvodus/bg//inite/Mot/LL/LL/DOD/MOt/201/201/CKGU/HTDY/20/40KA-ULK/+ 0140KA-ULK/+/LLK4+(XLK2)/LXLK3+(LK6+	CD4 Troll CCR4+CYCP5
yateg/wobds/by/ime/45t/Lt/Lc/660-45t/3t15-/56/TCKg0-/4t8-/25-/45KA-UCK/t of 45KA-UCK/-/CCK4tCXCK5-	CD4+TC8+CACK3+ CD4+TC9
gateg/woods/bg//time/45+/L1/L2/000+45+/5+/55/10kg0/4+6-/25-/45KA-ULK/+ 0/45KA-ULK/-/CLK4+LXLK5-/CXCR3-CCR6-	CD4+ TH2 CD4+ Th17
gateg/nobds/bg//time/45+/L1/L2/660-45+/3+19-/56-/TCRg0-/4+8-/25-/45KA-UCR/+ of 45KA-UCR/-/CCR4+CXCR5-/CXCR3-CCR6+	CD4Trall CVCDEt
gateg/Nobas/Sg//Time/45+/L1/L2/66b-45+/3+19-/S6-/TCKgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CXCR5+	CD4 TCEII CACKOT
guteg/nobds/bg//time/45+/L1/L2/660-45+/3+19-/56-/TCKg0-/4+8-/25-/45RA+CCR/-	CD4+TE CD4+ anim
gated/NoBds/5gi/Time/45+/L1/L2/660-45+/3+19-/56-/TCRgd-/4+8-/25-/45RA+CCR/+	CD4+CD25+CD127
gateg/NoBds/Sgi/Time/45+/L1/L2/666-45+/3+19-/56-/TCRgd-/4+8-/25+127-	CD4+CD25+CD127-
gated/NOBds/Sgl/TIme/45+/L1/L2/666-45+/3+19-/56-/TCRgd-/4+8-/25+127-/CCR4+45RA-	CD4+1Peg
gated/Nobds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56-/TCRgd+	Tell Topel (DSC)
gatea/NoBas/Sgi/Time/45+/L1/L2/666+45+/3+19-/56-/TCRgd+/gdCD16+	CBRITCKG0+CD16+
gated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56+	CD3+CD19-CD56+
gated/NoBds/SgI/Time/45+/L1/L2/66b-45+/3+19-/56+/TCRgd+56+	Tcell-TCRgd+CDS6+
gated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56+/TCRgd+56+/gdCD16+	Icell-TCRgd+CD56+CD16+
gated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56+/56+TCRgd-	NKT
gated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56+/56+TCRgd-/4-8-	NKT CD4-CD8-
gated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56+/56+TCRgd-/4-8+	NKT CD8+
gated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3+19-/56+/56+TCRgd-/4+8-	NKT CD4+
gated/NoBds/Sgl/Time/45+/L1/L2/66b+45mid	CD66b+CD45mid
gated/NoBds/Sgl/Time/45+/L1/L2/66b+45mid/16-294+	Eosinophils
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Figure S3: Graphical overview of the gating strategy (illustrated on one representative sample).

Normalized CyTOF data were cleaned with several steps like excluding normalization beads, gating on single and live cells. The gate L2 (in red) was used as reference to calculate cell frequencies of single live CD45+ (indicated as number in percent below the gate name), leading to the identification of 66 cell populations within the main immune cell families, such as B cells and plasmacells, innate lymphoid cells, natural killer cells (NK), basophils, monocytes and dendritic cells (DC), NKT-like cells, CD8 and CD4 T cells, granulocytes.



Figure S4: List of samples available and analyzed per subject and time-point.

Open symbols could not be included for flow cytometry analysis, either because the acquired sample did not fulfill the inclusion criteria (< 50% mortality rate and/or CD45+ cells >50000 events, open circle), or because the PBMC sample was not available (open diamond). Serum was available for open symbols to measure immunoglobulin levels and cytokine concentrations.

	Timepoint				
Subject	VO	V1	V2	V3	V4
Ctrl1	٠				
Ctrl2	•				
Ctrl3	•				
Ctrl4	•				
Ctrl5	•				
Ctrl6				•	•
Ctrl7	0	•	•	•	
Ctrl8		\diamond		•	
Ctrl9	•		\diamond	•	•
Pat1	•	•	•	•	•
Pat2	٠	۲	٠	•	•
Pat3	۲	۲	0	•	٠
Pat4	۲	٠	٠	•	•
Pat5		0	٠	•	•
Pat6		•	•	•	•
Pat7		•	•	•	•
Pat8		•	•	•	•
Pat9		•	•	•	•
Pat10		•	•	•	•
Pat11	•				
Pat12	۲				
Pat13	٠				
Pat14	•				
Pat15	•				
Pat16	۲				

Figure S5: Longitudinal anti- α -Gal immunoglobulin profiles showing individual kinetic curves. IgE (right Y-axis), IgG and IgG subclasses (left Y-axis) were plotted for each individual subject. Sera were diluted 1/50 for IgG and 1/20 for all IgG subclasses.



Figure S6: Proportions of slgG1 to slgG2 is higher in patients compared to controls. (A): The weight of slgG1, resp. slgG2 is expressed as percent (%) of all 4 lgG subclasses (median with interquartile range) for patients and controls. Multiple Mann-Whitney tests between patients and controls at each time-point showed only 1 significant difference (** p-value < 0.01). (B): The ratio of slgG1 to slgG2 is higher for patients than controls, all samples combined. The line represents the median for each group, Mann-Whitney test shows a significant difference between groups (**** p-value < 0.0001).



Figure S7: Longitudinal analysis of blood cell frequencies shows no significant variations.

Main cell types from total blood count, expressed as frequencies of total leucocyte counts for patient (blue line) and control group (grey line), are plotted as median with interquartile range. Friedman tests were not significant.



Figure S8: Plasmacytoid dendritic cell (pDC) frequencies are higher in controls regardless of the time of blood withdrawal. Frequencies of pDC cell are expressed as percent of single live CD45+ cells and divided into 2 categories of blood withdrawal before (am) or after noon (pm) for control (grey) and patient (blue) group. Boxes are delimited by interquartile range with median line and whiskers from minimal to maximal value. Pair-wise comparisons with Mann-Whitney tests show significant differences (* p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001).

