B-cell receptor dependent phagocytosis and presentation of particulate antigen by chronic lymphocytic leukemia cells

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Supplementary files

Table S1. Details of samples

| Sample | IGHV status | IGHV usage | % CLL cells | sIgM signal capacity | sIgM MFI |
|--------|-------------|------------|-------------|----------------------|---------|
| 87B    | M           | IGHV2-5*02 F | 95          | 36                   | 12      |
| 132A   | M           | IGHV4-4*07 F | 88          | 18                   | 25      |
| 348C   | M           | IGHV3-15*01 F | 96       | 21                   | 35      |
| 348D   | M           | IGHV3-15*01 F | 98       | 4                    | 53      |
| 351C   | M           | IGHV3-66*01 F, or IGHV3-66*04 F | 96 | 79 | 34 |
| 448    | U           | IGHV3-49*05 F | 93 | nd | 35 |
| 448B   | U           | IGHV3-49*05 F | 83 | 73 | 115 |
| 466    | U           | IGHV4-38-2*02 F | 81 | 12 | 34 |
| 496F   | M           | IGHV3-30*03 F | 75 | 14 | 32 |
| 511A   | U           | IGHV3-72*01 F | 96 | 39 | 32 |
| 523G   | M           | IGHV3-72*01 F | 96 | 13 | 23 |
| 523H   | M           | IGHV3-72*01 F | 92 | 39 | 45 |
| 575C   | M           | IGHV3-15*01 F | 96 | 66 | 117 |
| 604C   | M           | IGHV3-30*03 F, or IGHV3-30*05 F or IGHV3-30*06 F | 85 | 60 | 61 |
IGHV3-30*13 F or IGHV3-30*18 F or IGHV3-30*19 F

| Sample | Gender | IGHV1-3*01     | 95 | 50 | 41 |
|--------|--------|----------------|----|----|----|
| 621 B  | M      | IGHV3-7*02 F, or Homsap IGHV3-7*03 F | 98 | 27 | 116 |
| 720 U  | U      | IGHV1-69*01 F | 92 | 11 | 63 |
| 732 U  | U      | IGHV1-46*01 F, or Homsap IGHV1-46*03 F | 91 | 47 | 85 |
| 774A U | U      | IGHV1-69*01 F | 97 | 25 | 40 |
| 780 U  | U      | IGHV3-21*01 F, or Homsap IGHV3-21*02 F | 99 | 81 | 65 |
| 888 U  | U      | IGHV3-21*01 F, or Homsap IGHV3-21*02 F | 94 | 65 | 139 |
| 929 U  | U      | Homsap IGHV1-69*01 F, or Homsap IGHV1-69D*01 F | 90 | 58 | 134 |
| 1384 M | M      | IGHV3-33*01 F, or IGHV3-33*06 F | 84 | 21 | 82 |
| 1427 U | U      | IGHV1-8*01 F  | 95 | 83 | 1268 |
| 1431 U | U      | IGHV3-21*01 F, or IGHV3-21*02 | 97 | 76 | 50 |

Diagnosis of CLL was according to the IWCLL-NCl 2008 criteria and the monoclonal B-cell population in the peripheral blood had a typical IgM⁺IgD⁺ CLL phenotype in all cases. Where treatment for CLL had taken place, this was at least 6 months prior to sample collection.

aWhere suffix is not shown, this is the first sample obtained from that patient, typically obtained shortly after diagnosis. A, B, C etc. indicate subsequent samples from the same patient.
bIGHV mutation status (M, mutated; U, unmutated) and cgene usage.
dPercentage of CLL cells. eMaximal percentage of cells with increased intracellular calcium following treatment with soluble anti-IgM. fMean fluorescence intensity. nd, not determined. Samples 496F, 511A, 348C and 448B were the HLA-DRB1-*04:01 positive samples used for antigen presentation experiments and samples 87B, 132A, 523G, 1384, 1427 and 1431 were used for RNA-seq.
### Table S2. Top-10 IPA canonical pathways enriched in the anti-IgM-induced transcriptional response at 6 hours

| Rank | Ingenuity Canonical Pathway^a | -log p-value | Ratio | z-score^b | Molecules |
|------|--------------------------------|--------------|-------|-----------|-----------|
| 1    | Antigen Presentation Pathway   | 10.7         | 0.359 | NaN       | CALR, CANX, CIITA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB5, PDIA3 |
| 2    | PD-1, PD-L1 Cancer Immunotherapy | 8.47       | 0.179 | -1.604    | BCL2L1, CD274, CD80, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB5, IL2RB, PDCD1, STAT5A, TGFβ2, TNF, TNFRSF1B |
| 3    | MSP-RON Signalling in Macrophages | 7.73       | 0.162 | -1.886    | CIITA, CREB5, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB5, KLK1, KLK2, NFKB1, REL, SOCS3, STAT3, TNF |
| 4    | Sirtuin Signalling Pathway      | 7.51       | 0.106 | 0.447     | DOT1L, ESRRα, GADD45α, GADD45β, GADD45G, LDHA, MAPK12, MAPK6, MYC, MYCN, NAMPT, NFKB1, PCK2, PGAM1, POLR1A, POLR1B, POLR1C, PPIF, REL, RRP9, STAT3, TIMM13, TIMM23, TIMM8A, TIMM9, TNF, TOMM20L, TOMM40, TUBA1B, TUBA1C, VDAC1 |
| 5    | tRNA Charging                   | 7.28       | 0.282 | 3.317     | AARS1, EARS2, FARSβ, GARS1, IARS1, NARS2, SARS1, TARS1, VARS1, WARS1, YARS1 |
| 6    | Th1 and Th2 Activation Pathway  | 6.91       | 0.128 | NaN       | BHLHE41, CD274, CD80, GF11, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB5, IL2RB, IL6R, NFIŁ3, NFKB1, SOCS3, STAT3, STAT5A, TNFRSF4 |
| 7    | B cell Development              | 6.8        | 0.256 | NaN       | CD80, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB5 |
| 8    | Purine Nucleotides De Novo Biosynthesis II | 6.19 | 0.545 | 2.449     | ADSL, ATIC, GART, PAICS, PFAS, PPAT |
| 9    | Th1 Pathway                     | 6.01       | 0.139 | 3.051     | CD274, CD80, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB5, IL6R, NFIŁ3, NFKB1, SOCS3, STAT3 |
| 10   | Role of PKR in Interferon Induction and Antiviral Response | 5.99 | 0.132 | -0.500    | CYCS, DNAJC3, HSP90AA1, HSP90AB1, HSP90B1, HSPA5, HSPA8, HSPA9, MAP2K3, MAPK12, NFKB1, NFKBIE, NLRP6, NPM1, PDGFA, REL, STAT3, TNF |

^aIPA canonical pathway analysis was performed using genes that were significantly up-regulated (log2FC > 1.0; FDR < 0.05) at 6 hours in anti-IgM-treated cells. ^bNaN, no activity pattern predicted
Table S3. Top-10 IPA canonical pathways enriched in the anti-IgM-induced transcriptional response at 24 hours

| Rank | Ingenuity Canonical Pathwaya | \(-\log P\)-value | Ratio | z-scoreb | Molecules |
|------|-------------------------------|-------------------|-------|----------|-----------|
| 1    | BAG2 Signaling Pathway        | 11.2              | 0.25  | 1.667    | CASP3, CDKN1A, HSP90AA1, HSPA2, HSPA5, HSPA8, HSPA9, MYC, NFKB1, PSMB2, PSMB3, PSMB5, PSMB6, PSMB7, PSMC1, PSMC2, PSMC3, PSMD1, PSMD11, PSMD14, REL |
| 2    | Superpathway of Cholesterol Biosynthesis | 10.8          | 0.448 | 3.606    | ACAT2, CYP51A1, DHCR24, DHCR7, FDT1, HMGCR, HMGCS1, IDI1, MSMO1, MVD, MVK, NSDHL, SQLE |
| 3    | Inhibition of ARE-Mediated mRNA Degradation Pathway | 8.5         | 0.155 | 2.673    | EXOSC4, EXOSC5, LTA, MAPK12, MAPK6, PRRKAR1B, PRRKAR2B, PSMB2, PSMB3, PSMB5, PSMB6, PSMB7, PSMC1, PSMC2, PSMC3, PSMD1, PSMD11, PSMD14, TNF, TNFRSF1B, TNFSF14, TNFSF15, TNFSF4, YWHAE, YWHAG |
| 4    | Sirtuin Signaling Pathway      | 8.48             | 0.12  | 0        | ATP5F1B, ATP5MC1, ATP5PF, GADD45B, GADD45G, LDHA, MAPK12, MAPK6, MYC, MYCN, NAMPT, NDUFA4L2, NDUFA8, NDUFA1, NDUFB3, NFKB1, NQO1, PCK2, PGAM1, PGK1, PP1, REL, RRP9, SLC25A5, SOD1, SREBF1, TIMM13, TIMM23, TNF, TOMM22, TOMM40, TOMM40L, TP73, TUBA1B, TUBA1C, VDAC1 |
| 5    | Antigen Presentation Pathway   | 7.81             | 0.308 | NaN      | CALR, CANX, CD74, HLA-DPA1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB5, PDIA3, PSMB5, PSMB6 |
| 6    | Crosstalk between Dendritic Cells and Natural Killer Cells | 7.18          | 0.187 | 3.464    | ACTB, ACTG1, CAMK2B, CCR7, CD80, CD83, CSF2, FSCN1, HLA-DRA, HLA-DRB1, HLA-DRB5, IL2RB, LTA, NFKB1, REL, TNF, TNFRSF1B |
| 7    | FAT10 Signaling Pathway        | 6.79             | 0.232 | NaN      | PSMB2, PSMB3, PSMB5, PSMB6, PSMB7, PSMC1, PSMC2, PSMC3, PSMD1, PSMD11, PSMD14, SQSTM1, TNF |
| 8    | Cholesterol Biosynthesis I     | 6.75             | 0.538 | 2.646    | CYP51A1, DHCR24, DHCR7, FDT1, MSMO1, NSDHL, SQLE |
| 9    | Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol) | 6.75          | 0.538 | 2.646    | CYP51A1, DHCR24, DHCR7, FDT1, MSMO1, NSDHL, SQLE |
| 10   | Cholesterol Biosynthesis III (via Desmosterol) | 6.75          | 0.538 | 2.646    | CYP51A1, DHCR24, DHCR7, FDT1, MSMO1, NSDHL, SQLE |

aIPA canonical pathway analysis was performed using genes that were significantly up-regulated (log2FC > 1.0; FDR < 0.05) at 24 hours in anti-IgM-treated cells. bNaN, no activity pattern predicted.
Figure S1. Anti-IgM-regulated pathways. IPA canonical pathway analysis for anti-IgM-induced genes following sIgM stimulation for 6 (upper) or 24 hours (lower). The Ag presentation pathway is highlighted.
**Figure S2.** CLL samples were incubated with goat anti-IgM-coated latex beads for 3 hours at 37°C before analysis of bead internalization by confocal microscopy. Graph shows the distribution of the number of internalized beads per cell (mean ± range). Data are from analysis of 2 samples each with 10 representative confocal fields analyzed (mean of 33 ± 15 (SD) cells per field).

**Figure S3.** Analysis of anti-IgM bead internalization by flow cytometry. Histogram shows gating strategy to identify the beads-and-cells population based on FSC and SSC. FITC-SSC dot plots divided (vertical line) into Q1 (cells with internalized beads/low FITC) and Q2 (cells with external beads/high FITC) for anti-IgM beads at 37°C, control beads at 37°C or anti-IgM beads at 4°C. The percentage of events in Q1 and Q2 is shown. Results shown are from 4 CLL samples.
Figure S4. Anti-IgM bead internalization, IGHV mutation status and sIgM expression. Graphs show the percentage of cells with internalized beads for all samples analyzed (n = 10) for (A) U-CLL and M-CLL samples (with mean ± SD), (B) sIgM expression and (C) sIgM signalling capacity (anti-IgM-induced calcium mobilization). Results of statistical analysis using (A) Student’s t-test or (B,C) Pearson’s analysis are shown.

Figure S5. Characterization of SKW3-T18 cells. (A) Flow cytometric analysis of SKW3-T18 cells (green) stained for TCR expression using anti-TCRβ H57-597 antibody (left) or tested for DR4/mCk-tetramer binding (right). Controls were either parental TCR-negative SKW3 cells (grey) or SKW3 cells expressing an irrelevant TCR (purple); (B) flow cytometric analysis of CD69 expression (percent positive cells) by SKW3-T18 cells or SKW3-HA1.7.
cells (expressing the HA1.7 TCR [1]) following incubation with BOLETH cells (ECACC 88052031) loaded with mCk-derived peptide (NVKWKIDGSRNGV) or \( \text{HA}^{306-318} \) peptide (PKYVKONTKLAT). PMA was used as a positive control for T-cell activation and CD69 expression was quantified on CD19-negative T cells; (C) peptide sensitivity of SKW-T18 cells assessed using serial dilution of mCk-derived peptide loaded on BOLETH cells. Graph shows representative results for cell surface CD69 expression (flow cytometry) and IL-2 secretion (ELISA)

Supplementary Reference

1. Hennecke J, Carfi A, Wiley DC. Structure of a covalently stabilized complex of a human alphabeta T-cell receptor, influenza HA peptide and MHC class II molecule, HLA-DR1. EMBO J. 2000;19:5611–24.