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MHC class II antigen presentation by B cells in health and disease

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Chapter 7

Aberrant MHC class II antigen presentation is linked to expansion of the activated T cell compartment in B-cell chronic lymphocytic leukemia

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Abstract

In patients with B-CLL, the immune dysfunction of T cells towards malignant B cells may contribute to the pathobiology of B-CLL. TCR-dependent oligoclonal/monoclonal expansion of the CD4⁺ T cells however points to antigen-dependent T cell activation. For this reason, we investigated the role of MHC class II antigen presentation in B-CLL. Using flow cytometry, we analyzed the expression levels of HLA-DR, CLIP, HLA-DM and HLA-DO of B cells from 17 B-cell chronic lymphocytic leukemia patients and 10 healthy donors. In addition we analyzed the T cell compartment of patients by expression of CD45RO, CD27, HLA-DR and CD38 and correlated these results to the MHC class II antigen loading pathway results. B-CLL cells showed ubiquitous expression of MHC class II that did not vary from healthy controls. B-CLL cells however showed disturbed expression of HLA-DM and HLA-DO, the editors of the MHC class II antigenic peptide repertoire. The perturbed DM/DO balance altered the peptide repertoire, as it was related to reduced expression of the self-peptide CLIP at the plasma membrane. The T cell compartment in B-CLL patients was significantly decreased in naïve CD4⁺ and CD8⁺ subsets in favor of increased activated effector populations. The percentage of activated T cells inversely correlated with CLIP expression, pointing to improved antigen presentation. Our data show that in B-CLL a relative increase in HLA-DM and a concomitant change in the MHC class II peptide repertoire of the malignant B cells is related to ongoing T cell activation. Altered MHC class II antigen presentation may thus be a new factor in the immune dysfunction and pathobiology of B-CLL.

Introduction

A successful anti-tumor T cell response is achieved by the two effector arms of the immune system. CD8⁺ cytotoxic T lymphocytes (CTLs) ensure specific elimination of tumor cells upon recognition of MHC class I-antigen (Ag) complexes and CD4⁺ T helper cells generate the required T cell help upon activation by MHC class II-Ag complexes (1). The absence of T cell help can lead to abortive induction of CTL responses and subsequent tolerance (2). Moreover, T cell help is required for the maintenance of CTL responses, which is essential in diseases like cancer (3, 4). Indeed, CD4⁺ T cell inclusion in adoptive T cell transfer studies improved tumor clearance by the CD8⁺ T cells (5, 6) and aberrant MHC class II Ag presentation in acute myeloid leukemia was related to poor prognosis (7).

B-cell chronic lymphocytic leukemia (B-CLL) is the most common form of leukemia in adults in the Western world. Typical for the disease is the highly variable clinical course, with survival rates that vary between a few months and two decades (8). B-CLL is characterized by a progressive accumulation of malignant B cells that fail to undergo apoptosis. Notably, B-CLL is characterized by striking immune incompetence and T cell expansion combined with T cell dysfunction (9). It is unclear whether this T cell expansion is indicative for attempted but unsuccessful tumor clearance or contributes in another way to the disease, for instance by creating an environment that supports survival of neoplastic cells (10). Antigen-independent mechanisms have been implicated in the T cell expansion in B-CLL (reviewed in (11)). The TCR-dependent oligoclonal/monoclonal expansion of CD4⁺ T cells in B-CLL however, points to an antigen-driven process. How malignant B cells present antigens via MHC class II molecules to CD4⁺ T cells and whether this may be an explanation for observed T cell expansion in B-CLL is so far unclear.

MHC class II molecules bind exogenous Ags generated in the endosomal/lysosomal pathway. After synthesis, the MHC class II molecule is directed into this pathway by associating to the invariant chain (Ii) (12). During transport to the lysosomal-like MIIC compartments where the majority of Ag loading occurs, the Ii is proteolytically removed, leaving only a small fragment (class II-associated invariant chain peptides (CLIP)) in the class II peptide binding groove (13). Release of CLIP is facilitated by the specialized chaperone HLA-DM (DM). DM catalyses the natural process of peptide dissociation (14). DM thus releases both CLIP and other nonstable binding peptides. Consequently, DM acts as a peptide editor, favoring presentation of stable binding Ags (15-19). HLA-DO (DO), a heterodimer of DO α

and DO β , is expressed in B cells and regulates the action of DM in a pH dependent manner. We and others reported that DO reduces MHC class II-mediated presentation of antigenic peptides in general and modulates the antigenic peptide repertoire by facilitating presentation of particular Ags, while suppressing others. DO therefore both limits and skews the class II-associated antigenic peptide repertoire in B cells (20-22). The relative expression of DO and DM (or the number of DM molecules in association with DO) thus controls Ag presentation in B cells. Not surprisingly, DO and DM expression are very consistent and tightly regulated at different cellular levels in healthy B cells (23) and B cell differentiation status (24). Aberrant expression of DM and/or DO could lead to an altered MHC class II peptide repertoire. This shift in antigen presentation may lead to altered T helper cell activation and subsequent help to CD8⁺ CTLs.

We recently showed that increased transcription of *HLA-DOA* mRNA is correlated with shorter survival of B-CLL patients (25). To determine the role of MHC class II-mediated antigen presentation in B-CLL at the protein level, we set out to determine whether anomalies in the MHC class II Ag presentation pathway occurred in patients suffering from B-CLL and whether this is related to the observed expansion of T cell subsets in this disease. Here we show that the relative expression of DO compared to DM is affected in malignant B-CLL cells, improving dissociation of the self-peptide CLIP in MHC class II molecules. This change in the MHC class II peptide repertoire is strongly correlated to a shift from the naïve T cell compartment towards the activated effector T cell compartment.

Materials and methods

Patients and healthy volunteers

Peripheral blood samples from 17 B-CLL patients were obtained after informed consent according to the Declaration of Helsinki and samples were anonymized before analysis. Patients were diagnosed and staged based on standard morphologic and immunophenotypic criteria (26). Cytogenetic data are not available since this was not routinely performed at the time of sample collection. Out of the 17 patients, 14 had not received chemotherapy at the time of sample acquisition and 3 had received prior treatment with chlorambucil. Ten buffy-coats from healthy donors were obtained from the Sanquin Blood Supply Foundation (Sanquin, Amsterdam, The Netherlands).

Isolation of peripheral blood mononuclear cells

Heparinized blood was diluted in PBS and layered on a Ficoll-Hypaque density gradient (Axis-Shield PoC AS, Oslo, Norway). PBMCs were collected from the interphase and washed twice with RPMI 1640 medium w/o phenol red, supplemented with 5% FCS (Bodinco, Alkmaar, the Netherlands), 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-Glutamine, 50 µM 2-mercaptoethanol and 20 µg/ml human apo-transferrin ((Sigma-Aldrich, Munich, Germany), depleted for human IgG with protein G sepharose (Amersham, Uppsala, Sweden)). Cells were cryopreserved and stored in liquid nitrogen until analysis.

Antibodies and Flow Cytometry Analysis

Rapidly thawed mononuclear cell fractions were preincubated with 10% human gammaglobulin (6 mg/ml, Sanquin) and incubated with different combinations of directly labeled antibodies. The following mouse monoclonal antibodies were used: FITC-labeled anti-HLA-DR (BD Biosciences (BD), San Jose, CA, clone L243), anti-HLA-ABC (BD), anti-HLA-DO (BD), -CD8 (BD), -CD45RO (Sanquin), -CD80 (BD); Phycoerythrin-labeled anti-HLA-DM, -CD4, -CD27, -CD38, -CD86 (all BD); Peridinin chlorophyll protein-Cy5.5-labeled anti-CD8 and -CD19 (both BD) and Allophycocyanin-labeled anti-CD4 and -CD5 (both BD). For CLIP detection, cells were incubated with the Cerclip.1 mAb (kindly provided by P. Cresswell)¹⁷ and subsequently stained with PE-conjugated rabbit-anti-mouse immunoglobulin (Dakocytomation, Haverlee, Belgium). A mixture of non-relevant mouse Abs of different isotypes was added to prevent non specific binding of subsequently added directly labeled fluorescent Abs. All plasma membrane stainings were performed at room temperature for 15 minutes. For intracellular staining of HLA-DM and HLA-DO, cells were fixed and permeabilized with a Fix & Perm kit (Caltag Laboratories, Burlingame, CA) and subsequently incubated with antibodies for 20 min at room temperature. After each incubation, cells were washed twice with PBS containing 0.1% bovine serum albumin and 100,000 events were acquired by a FACS Calibur (BD). Lymphocytes were gated by forward and side scatter and analyzed using CellQuest software (BD). B-CLL cells were defined as CD5⁺CD19⁺.

Median fluorescence intensity (MFI) index was defined by the formula:

$$\frac{\text{median fluorescence intensity (total population)} - \text{median fluorescence intensity (isotype control)}}{\text{median fluorescence intensity (isotype control)}}$$

Determination of anti-CMV serology and mutation status

Anti-CMV IgM and IgG was determined by ELISA in plasma samples of 13 patients at the Department of Virus Serology at Sanquin Diagnostics Division, from 4 patients plasma was not available. Determination of the mutational status of the immunoglobulin heavy chain variable (*IGHV*) genes of 20 patients was determined as described (25).

Statistical methods

Statistical analyses were conducted with the SPSS 15.0 software program. Differences between groups were analyzed with the 2-sided Mann-Whitney *U* test. For correlations, the Spearman nonparametric correlation test was used. P values less than 0.05 were considered statistically significant.

Table 1. Patients' characteristics and expression of different markers on B-CLL cells

Clinical characteristics of patients	
Gender	9 Male / 8 Female
Age at time of sample, years, mean	64 (41-78)
Months from diagnosis, mean (range)	87 (7-217)
Rai stage, number of patients	0 - 8 I - 5 II - 0 III - 2 IV - 2
Mutational status of the <i>IGHV</i> genes	12 mutated, 4 unmutated, 1 ND
CMV serology	5 seronegative, 8 seropositive, 4 ND
Flow cytometric analysis of B-CLL	
Leucocyte count at time of sample,	42 (7-94)
CD4/CD8 ratio (% , mean (range))	1.3 (0.4-2.5)
CD40 (median FI (range))	6.4 (4.1-9.4)
CD80 (median FI (range))	3.4 (0-5.5)
CD86 (median FI (range))	13.8 (6.3-50.0)

Abbreviations: ND, not determined; FI, fluorescence intensity

Results

B-cell chronic lymphocytic leukemia cells express less CLIP in their peptide binding groove

We analyzed cell surface expression of DR and CLIP on the B cells of 17 samples of a random group of B-CLL patients (Table 1) and 10 healthy volunteers by flow cytometry. Expression of MHC class I (HLA-ABC) was not different between healthy

volunteers and B-CLL patients (data not shown). We also found no difference in DR expression on the B cells between control and B-CLL cells (Fig. 1A). B-CLL patients however, have significantly less CLIP ($P < 0.001$) associated to DR (Fig. 1B). The amount of DR molecules still associated to the self-peptide CLIP is indicative for the efficacy of the MHC class II peptide loading process. To analyze the relative occupancy of plasma membrane expressed DR with the self-peptide CLIP, the CLIP level on B cells was related to the DR level. This showed a reduced expression of CLIP associated to DR at the plasma membrane (CLIP/DR) in B-CLL patients compared with healthy controls ($P < 0.001$) (Fig. 1C). Thus, in B-CLL a relative larger proportion of the DR molecules is available for MHC class II mediated antigen presentation to CD4⁺ T cells.

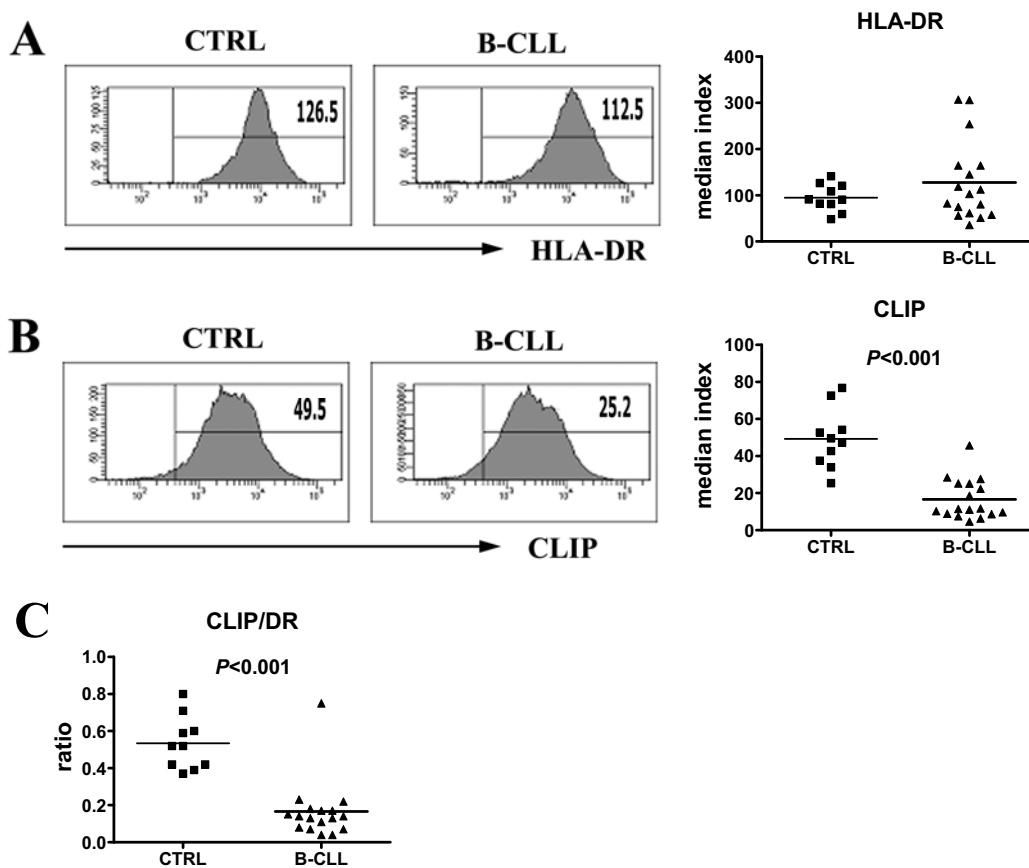


Figure 1. Decreased relative CLIP occupancy of DR in B-CLL.

(A) and (B) Representative flow-cytometric examples of HLA-DR and CLIP expression (left panels, numbers indicate the median index). DR expression is not significantly different between patients and healthy controls, CLIP expression is significantly decreased ($P < 0.001$) (right panels). (C) The relative CLIP amount in the MHC class II peptide binding groove (CLIP/DR) is significantly decreased ($P < 0.001$). Controls (CTRL) are represented by squares and B-CLL patients by triangles.

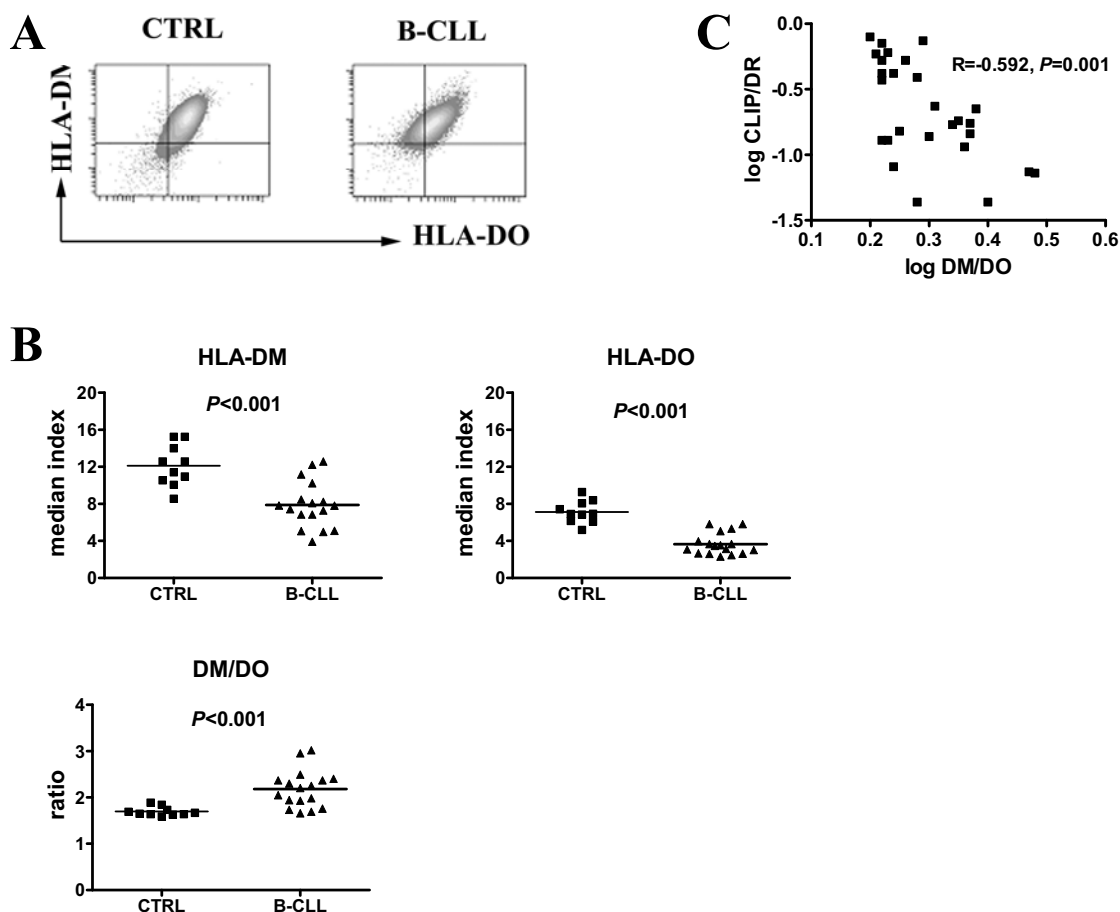


Figure 2. Aberrant expression of DM and DO in B-CLL.

(A) Representative flow-cytometric examples of intracellular HLA-DM and HLA-DO expression. (B) Both DM (upper left panel) and DO (upper right panel) are significantly decreased (both $P < 0.001$) in B-CLL patients. The relative expression of DM and DO (bottom panel) is increased ($P < 0.001$). Controls are represented by squares and B-CLL patients by triangles. (C) Relative CLIP occupancy of DR (CLIP/DR) correlated with the overexpression of DM (DM/DO) ($R = -0.592, P = 0.001$; 10 log values yielding normal distribution).

Reduced DM and DO expression in B-cell chronic lymphocytic leukemia cells

Antigen binding to newly synthesized MHC class II molecules is modulated by the expression of the peptide editors DM and DO. A high expression of DM compared to DO favors exchange of CLIP for antigenic peptides (20). Since DR molecules expressed reduced CLIP levels in B-CLL, we investigated the intracellular expression levels of DM and DO. Representative examples are shown in Figure 2A. The relative expression levels of DM and DO in B-CLL were markedly different from healthy controls, with both DM and DO being significantly reduced ($P < 0.001$ for both) (Fig. 2B, upper panels). When DM was compared to DO, a relative overexpression of DM

was observed in B-CLL ($P < 0.001$) (Fig. 2B, *bottom panel*). This correlated with the efficiency of CLIP removal from DR (CLIP/DR) ($R = -0.592$, $P = 0.001$) (Fig. 2C). Both relative overexpression of DM and the relative CLIP occupancy of DR did not correlate to B-CLL mutational status (as measured by *IGHV* gene analysis), CMV status, costimulatory markers (CD40, CD80 and CD86), Rai stage of disease or treatment regimen (data not shown). Collectively, these data show differential expression of the MHC class II peptide editors in B-CLL in combination with a reduced expression of CLIP in plasma membrane deposited DR.

Expansion of CD4⁺ and CD8⁺ effector T cell compartments in B-cell chronic lymphocytic leukemia

How does the altered CLIP expression on B-CLL cells relate to differences in CD4⁺ T cell differentiation between CLL patients and healthy controls? In line with previous observations (9), the CD4⁺/CD8⁺ ratio in B-CLL patients was lower compared to healthy controls (Table 1). No correlation was observed between the relative CLIP occupancy of DR and the CD4⁺/CD8⁺ ratio. We analyzed the peripheral T cells for the CD4⁺CD45RO⁻CD27⁺ naïve T cells, CD4⁺CD45RO⁺CD27⁺ central memory T cells and the CD4⁺CD45RO⁺CD27⁻ memory effector cells. Representative examples are shown in Figure 3A. Patients with B-CLL showed a lower percentage of naïve CD4⁺ T cells compared to healthy controls ($P = 0.009$) (Fig. 3B, *upper left panel*), an unvaried central memory CD4⁺ T cell compartment (Fig. 3B, *upper right panel*) and an expansion of the memory effector CD4⁺ T cell compartment ($P = 0.001$) (Fig. 3B, *bottom panel*). This points to ongoing activation of CD4⁺ T cells in B-CLL.

We analyzed the percentages of CD8⁺CD45RO⁻CD27⁺ naïve T cells, CD8⁺CD45RO⁺CD27⁺ central memory T cells, CD8⁺CD45RO⁺CD27⁻ memory effector T cells and CD8⁺CD45RO⁻CD27⁻ cytotoxic effector T cells. Representative examples are shown in Figure 3C. Patients with B-CLL showed a lower the percentage of naïve CD8⁺ T cells ($P < 0.001$) (Fig. 3D, *upper left panel*) and no difference in CD8⁺ central memory T cells (Fig. 3D, *upper right panel*). The percentage of CD8⁺ memory effector T cells was increased ($P = 0.001$) (Fig. 3D, *bottom left panel*), as well as the CD8⁺ cytotoxic effector T cells ($P = 0.003$) (Fig. 3D, *bottom right panel*). Because the increase in cytotoxic effector T cells in B-CLL patients has been related to CMV infection (27), we tested patients for CMV infection. In our patient cohort, no significant difference in CD8⁺ T cell populations was observed between CMV seropositive and CMV seronegative B-CLL patients (data not shown).

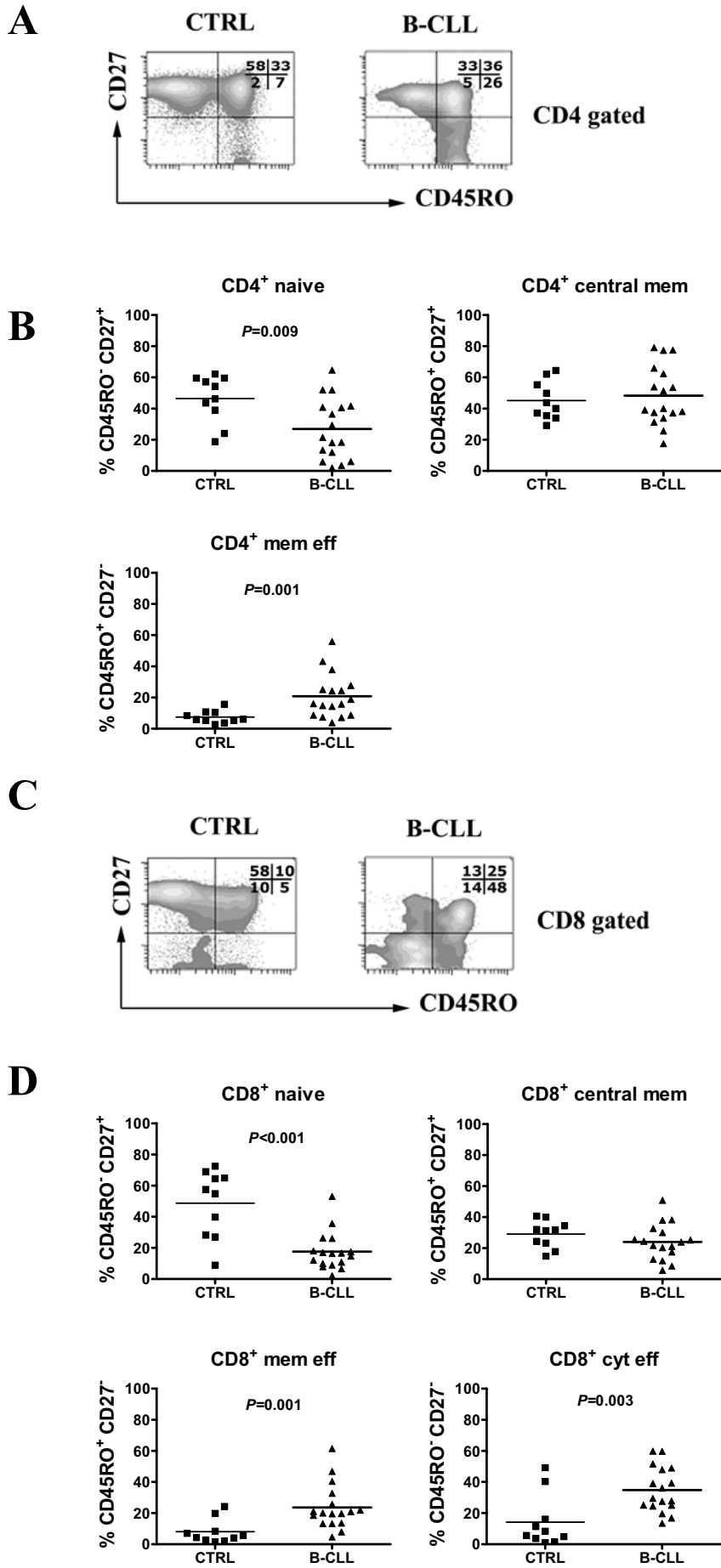


Figure 3. Expansion of effector type CD4⁺ and CD8⁺ T cells in B-CLL.

(A) Representative flow-cytometric examples of CD45RO and CD27 expression on CD4 gated cells. Percentages of cells in each quadrant are given. (B) The naïve CD4⁺ T cell compartment (upper left panel) is significantly decreased ($P = 0.009$) in B-CLL patients. The CD4⁺ central memory T cells (upper right panel) are not significantly different between patients and healthy controls and the CD4⁺ memory effector subset (bottom panel) is significantly increased ($P = 0.001$). Controls are represented by squares and B-CLL patients by triangles. (C) Representative flow-cytometric examples of CD45RO and CD27 expression on CD8 gated cells. Percentages of cells in each quadrant are given. (D) The naïve CD8⁺ T cell compartment (upper left panel) is significantly decreased ($P < 0.001$) in B-CLL patients. The CD8⁺ central memory T cells (upper right panel) are not significantly different between patients and healthy controls. The CD8⁺ memory effector subset (bottom left panel) is significantly increased ($P = 0.001$) as well as the CD8⁺ cytotoxic effector subset (bottom right panel) ($P = 0.003$). Controls are represented by squares and B-CLL patients by triangles.

CD4⁺ T cells provide help to the effector function of CD8⁺ T cells. Is there a relationship between the expanded CD4⁺ effector and CD8⁺ compartments in B-CLL? Indeed, the expansion of the CD4⁺ memory effector T cell compartment correlated with the observed expansions in CD8⁺ compartment in B-CLL, with the strongest correlation between the CD4⁺ memory effector and CD8⁺ memory effector compartments (Table 2).

Table 2. Correlations between the CD4⁺ memory effector and CD8⁺ T cell compartments

	CD4 ⁺ CD45RO ⁺ CD27 ⁻ memory
CD8 ⁺ CD45RO ⁻ CD27 ⁺ naive	R = -0.835, $P < 0.001$
CD8 ⁺ CD45RO ⁺ CD27 ⁻ memory	R = 0.881, $P < 0.001$
CD8 ⁺ CD45RO ⁻ CD27 ⁻ cytotoxic	R = 0.584, $P = 0.001$

Increased T cell activation in B-cell chronic lymphocytic leukemia

As a marker for ongoing T lymphocyte activation, we analyzed the expression of HLA-DR and CD38 on T cells. Representative examples are shown in Figure 4A. Patients with B-CLL showed increased levels of CD4⁺HLA-DR⁺CD38⁺ T cells and CD8⁺HLA-DR⁺CD38⁺ T cells compared to healthy controls (both $P < 0.001$) (Fig. 4B). The percentage of activated CD4⁺ T cells showed a positive correlation with the percentage of activated CD8⁺ T cells ($R = 0.846$, $P < 0.001$) (Fig. 4C). Thus, patients with B-CLL show a higher percentage of activated CD4⁺ and CD8⁺ T cells than healthy controls.

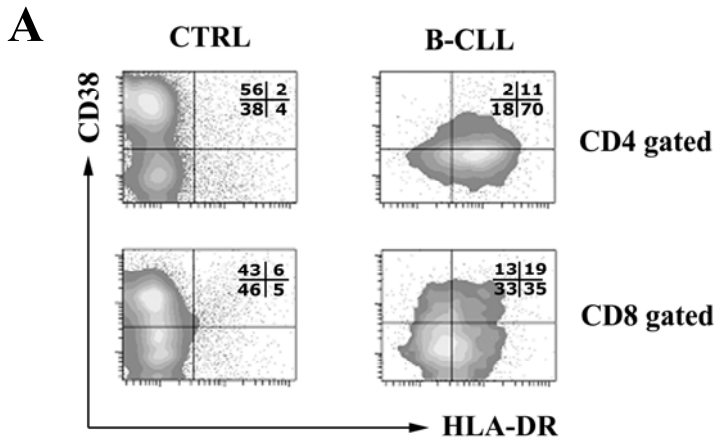
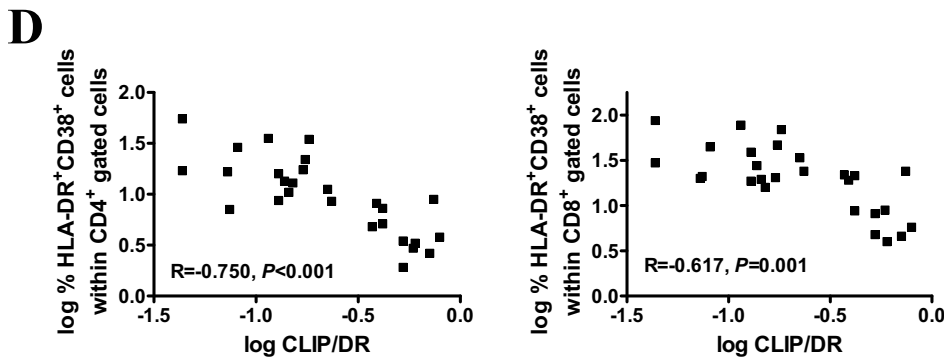
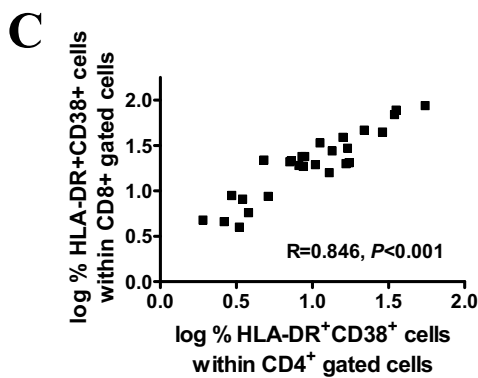
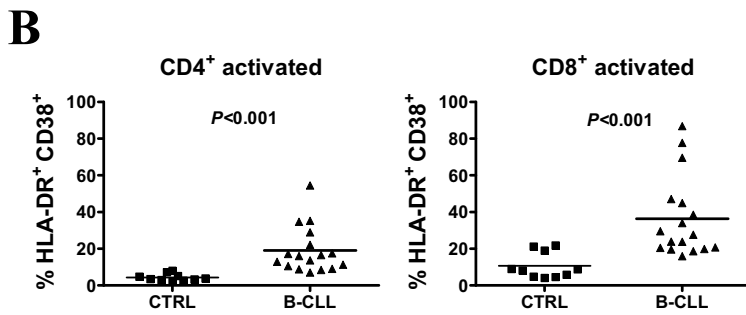


Figure 4. Increase in subsets of activated T cells correlates with the relative CLIP occupancy of DR.

(A) Representative flow-cytometric examples of HLA-DR and CD38 expression on CD4 (upper panels) and CD8 (lower panels) gated cells. Percentages of cells in each quadrant are given. (B) Both in the CD4⁺ (left panel) and CD8⁺ (right panel) T cell compartment more of the T cells have an activated phenotype (both $P < 0.001$). (C) The percentage of activated CD4⁺ T cells correlated with the percentage of activated CD8⁺ T cells ($R = 0.846$, $P < 0.001$). (D) Relative CLIP occupancy of DR (CLIP/DR) correlated with the percentage of CD4⁺ activated T cells ($R = -0.750$, $P < 0.001$) and to a lesser extent CLIP/DR correlated with the percentage of CD8⁺ activated T cells ($R = -0.617$, $P = 0.001$).



Correlation of the T cell parameters with the parameters involved in antigen presentation in our samples demonstrated a strong negative correlation between the relative CLIP levels associated to DR and the percentage of activated CD4⁺ T cells ($R=-0.750$, $P<0.001$) (Fig. 4D, *left panel*). To a lesser extent, the relative CLIP expression correlated to the percentage of activated CD8⁺ T cells ($R=-0.617$, $P=0.001$) (Fig. 4D, *right panel*).

Thus, in B-CLL a lower occupancy of the MHC class II peptide binding groove with CLIP strongly correlates with an increase in activated CD4⁺ and CD8⁺ T cell compartments.

Discussion

Deficiencies in components of the MHC class I Ag processing pathway have been shown in a variety of human cancers (28, 29), and some studies have correlated these deficiencies with tumor progression (30, 31). Here we identify aberrancies in the MHC class II Ag processing machinery in B-CLL and demonstrate that this is accompanied with increased T cell activation in B-CLL patients. B-CLL cells always express DR and the class II chaperones DM and DO. Thus, tumor immune escape due to genetic silencing of the MHC class II genes does not seem to occur in B-CLL. This in contrast to poor prognosis correlated to the overall loss of MHC class II expression in diffuse large B cell lymphomas (32, 33).

It is under debate whether B-CLL disease is a homogenous entity. Based on the mutational status of the immunoglobulin heavy-chain variable-region (*IGHV*) genes, B-CLL cases can be divided into two subgroups, resembling either a resting or a germinal center-experienced phenotype. DO expression is reported to vary during B cell development (24, 34), but in our cohort we could not demonstrate a difference in DO expression between patients with mutated and unmutated *IGHV* genes.

In B-CLL patients the presence of T cells with an anti-tumor specificity declines during disease progression (35). A lower CD4/CD8 ratio is observed in patients with progressive disease together with a concomitant Th1 to Th2 shift, which is detrimental for an effective anti-tumor response. These observations point to perturbed MHC class II-mediated CD4⁺ and CD8⁺ activation in B-CLL. Since the malignant B cells are poor APCs and DR cell surface expression is not altered, Dazzi and colleagues described that poor Ag presentation is due to a low B7 molecule expression (36). Although reduced expression of the costimulatory markers CD80 and CD86 is an established phenomenon in B-CLL (and confirmed in this study, see

Table 1), we now show that additional aberrancies in antigen presentation are present in the MHC class II antigen loading pathway itself. In order to get stable binding peptides in the peptide binding groove of a class II molecule, DR associates with DM which results in the release of CLIP and the preferential binding of Ags with an optimal binding motif to the class II backbone. In normal B cells about 50% of DM is associated to DO which then fails to properly support MHC class II peptide loading (24), whereas the other 50% is free for peptide editing of the class II Ag repertoire. The expression of DM in B-CLL shows that CLIP on newly synthesized class II molecules can be exchanged with antigenic peptides through the editing function of DM. The result that DM is relatively higher expressed than DO in B-CLL implies that more free DM is available for the generation of MHC class II complexes with antigens after removal of CLIP. Indeed, a decreased level of CLIP associated to DR at the plasma membrane in B-CLL patients is observed. Thus, the peptide repertoire presented by MHC class II molecules is modulated by DM and DO in healthy controls as well as in B-CLL patients. In addition, the composition of the MHC class II peptide repertoire is different in B-CLL compared to healthy controls. The amount of CLIP associated to DR apparently varies between different types of leukemias. In acute myeloid leukemia we recently observed a relative overexpression of MHC class II complexes still containing CLIP at the plasma membrane in patients with poor prognosis (7). For acute myeloid leukemia, CLIP may constitute a form of tumor immunoediting or tumor immune escape. In B-CLL, reduced CLIP levels may serve another function. There is ample evidence that in B-CLL aggressive and non-aggressive forms arise due to the intrinsic properties of the B-CLL cells themselves and therefore the relative contribution of failed immune surveillance is under debate. The strong clinical manifestations of immune dysfunction and the expanded circulating T cell compartment have led to the hypothesis that T cells may be involved in the pathobiology of B-CLL through the creation of a "leukemia-supportive" environment (37). Still, the mechanisms underlying the onset and sustainment of the expansion of these T cell populations in B-CLL were poorly defined. Our observations suggest that altered MHC class II antigen presentation by the malignant B cells may be involved; T cells of B-CLL patients are more differentiated towards effector and immune activated T cells and these findings correlate with parameters of improved MHC class II antigen presentation (a reduced CLIP expression and a relative overexpression of DM). In spite of the lack of strong costimulation, this correlation suggests that T cell

activation is still antigen-driven, in line with the observation that T cell expansion in B-CLL is oligoclonal or monoclonal (38). Whether initiation of T cell activation and maintenance of T cell activation are both antigen-driven remains to be established, but the observed correlations propose a contribution of MHC class II antigen presentation at certain stages in the pathobiology of B-CLL. A perturbed MHC class II antigen presentation pathway in B-CLL may thus be a new factor in the immune dysfunction and pathobiology of B-CLL.

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Chapter 7

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