Chapter V

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

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Abstract

In patients with B-CLL, T cells show immune dysfunction towards malignant B cells which may contribute to the pathobiology of B-CLL. Oligo/monoclonal expansion of the $CD4^+$ and $CD8^+$ T cells point to an antigen-dependent mechanism. For this reason, we investigated MHC class II antigen presentation in B-CLL. B-CLL cells showed ubiquitous expression of MHC class II that did not vary from healthy controls. B-CLL cells however showed a 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 a reduced expression of the self-peptide CLIP at the plasma membrane. The T cell compartment in the B-CLL patients was significantly decreased in naïve $CD4^+$ and $CD8^+$ subsets in favor of increased effector populations. In addition, the activated T cell populations ($HLA\text{-}DR^+CD38^{+)}$ were increased in both $CD4^+$ and $CD8^+$. The percentage of activated T cells correlated with the amount of CLIP expression. Thus, in B-CLL a relatively 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. Perturbed MHC class II antigen presentation in B-CLL 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) provide the specific kill of tumor cells upon recognition of MHC class I-antigen (Ag) complexes and $CD4^+$ T helper cells generate 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, recently $CD4^+$ T cell inclusion in adoptive T cell transfer studies was demonstrated to be essential for efficacy of treatment[5;6]. It may therefore be likely that aberrant MHC class II Ag presentation by tumor cells may lead to the defective or excessive activation of both $CD4^+$ and $CD8^+$ T cells.

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 may vary between a few months and two decades[7]. B-CLL is characterized by a progressive accumulation of malignant B cells that fail to undergo apoptosis. Interestingly, B-CLL is characterized by striking immune incompetence. It has become increasingly clear that in B-CLL T cell dysfunction is observed[8]. It remains to be solved whether the T cell expansion interferes with successful anti-leukemia immunesurveilance or contributes in another way to the onset and sustainment of the disease, for instance by creating an environment that supports the survival of neoplastic cells. There is also evidence that the ineffective T cell responses may be due to the dysfunctional relationship between the malignant B cells and the T cells[9]. Antigen-independent mechanisms have been implicated in the T cell expansion in B-CLL (reviewed in [10]). In addition, the oligo/monoclonality of the process points to an antigen-driven, TCR-dependent driver for the onset or maintenance of the T cell expansion. To date, little is known about the mechanisms by which 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.

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)[11]. During transport to the lysosomal-like 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[12]. Release of CLIP is facilitated by the specialized chaperone HLA-DM (DM). DM catalyses the natural process of peptide dissociation upon association to MHC class II[13]. DM thus releases both CLIP and other nonstable binding peptides. Consequently, DM acts as a peptide editor, favoring presentation of stable binding Ags[14-18]. HLA-DO (DO), a heterodimer composed of a DOα and DOβ chain, 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 certain Ags, while suppressing others. DO therefore both limits and skews the class II-presented antigenic peptide repertoire in B cells[19-21]. The balance between DO and DM expression thus seems to be key in controlling Ag presentation in B cells, which may explain why in healthy B cells DO and DM expression are very consistent and tightly regulated at different cellular levels[22] and B cell differentiation status[23]. Basically, aberrant expression of DM and/or DO could lead to an altered antigenic peptide repertoire that is presented by MHC class II. This shift in antigen presentation may lead to different T helper cell activation and subsequent help to $CD8^+$ CTLs. To determine the role of MHC class II-mediated antigen presentation in B-CLL, 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 related to the observed expansion of T cell subsets in this disease. Here we show that the malignant cells from patients with B-CLL show a perturbed relative expression of DM and DO and contain relatively less CLIP in the peptide binding groove of MHC class II molecules. In addition, 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.

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 (range)	64 (41-78)
Months from diagnosis, mean (range)	87 (7-217)
Rai stage 0 (number of patients)	8
I	5
II	0
III	2
IV	2
Mutational status of the IgVH genes	12 mutated, 4 unmutated, 1 ND
CMV serology	5 seronegative, 8 seropositive, 4 ND
Flow cytometric analysis of B-CLL patients	
Leucocyte count at time of sample, mean (range)	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)

Patients, Materials and Methods

Patients

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[24]. 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 patients had received prior treatment with chlorambucil. 10 buffy-coats from healthy donors were obtained from the Sanquin Blood Supply Foundation (Sanquin, Amsterdam, The Netherlands).

Isolation of PBMCs

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 in RPMI 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, Amsterdam) 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[16]) and subsequently stained with PE-conjugated rabbit-anti-mouse immunoglobulin (Dakocytomation, Heverlee, Belgium). A mixture of non-relevant mouse Abs of different isotypes was added to avoid aspecific binding of subsequently directly labeled Abs. Next, cells were incubated with directly labeled 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 on 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. The mutational status of the immunoglobulin heavy chain variable (*IgV_H)* genes of 20 patients was determined as described[25]. From patient 16 no material was available for *IgV_H* genes analysis. In brief, *IgV_H* transcripts were amplified using a mixture of forward primers located in the FR1 regions of the *IgV_H* gene families V_H1 to V_H6 or alternatively in the FR3 region of V_H1 to V_H6 in combination with one of the FAM-labeled reverse primers located in the Cμ, Cδ, Cα or Cγ regions. $\geq$ 2% difference from the most homologous germ line gene was considered to be mutated.

Statistical Analysis

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.

Results

B-CLL 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. Expression of HLA-ABC (MHC class I) was not different between B cells from healthy volunteers and the malignant cells from B-CLL patients (data not shown). We also found no difference in DR expression on the B cells between control and B-CLL (Figure 1A). B-CLL patients however, have significantly less CLIP ($P<0.001$) in the peptide binding groove of DR than healthy controls (Figure 1B). When the peptide binding groove of a DR molecule is occupied by the self-peptide CLIP, the

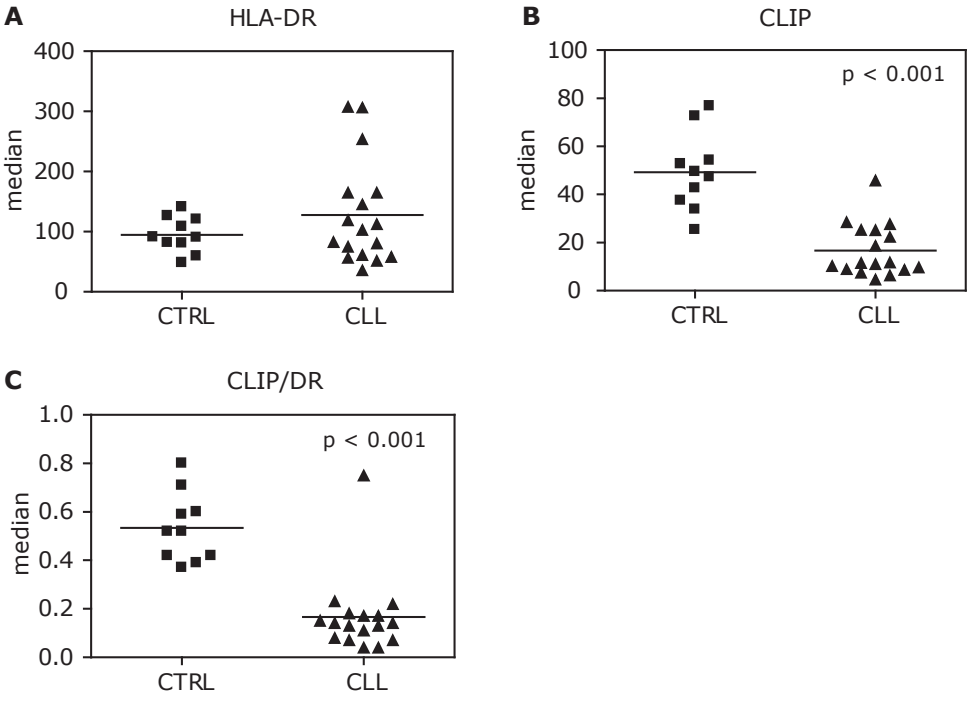


Figure 1. Decreased relative CLIP occupancy of DR in B-CLL. (A) DR expression is not significantly different between patients and healthy controls. (B) CLIP expression is significantly decreased (P < 0.001) and (C) the relative CLIP amount in the MHC class II peptide binding groove (CLIP/DR) is significantly decreased (P < 0.001). Controls are represented by squares and B-CLL patients by triangles.

peptide loading process of tumor or other antigenic peptides has not occurred efficiently and MHC class II responses will be poorer. To analyze the relative occupancy of the plasma membrane expressed DR with CLIP, the CLIP level on B cells was related to the DR level to yield a relative amount of CLIP in the peptide binding groove of DR. This showed a reduced expression of CLIP at the plasma membrane (CLIP/DR) in B-CLL patients compared with healthy controls (P<0.001) (Figure 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.

Reduced DM and DO expression in B-CLL 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. High cell surface expression of CLIP correlates with high levels of DO relative to DM and may thus indicate low effectiveness of antigen presentation. As in B-CLL the DR molecules expressed

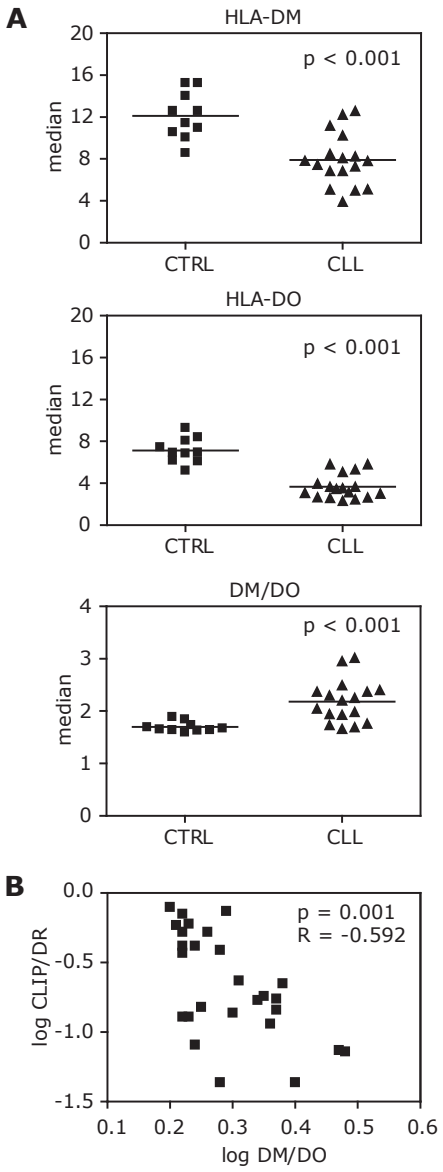


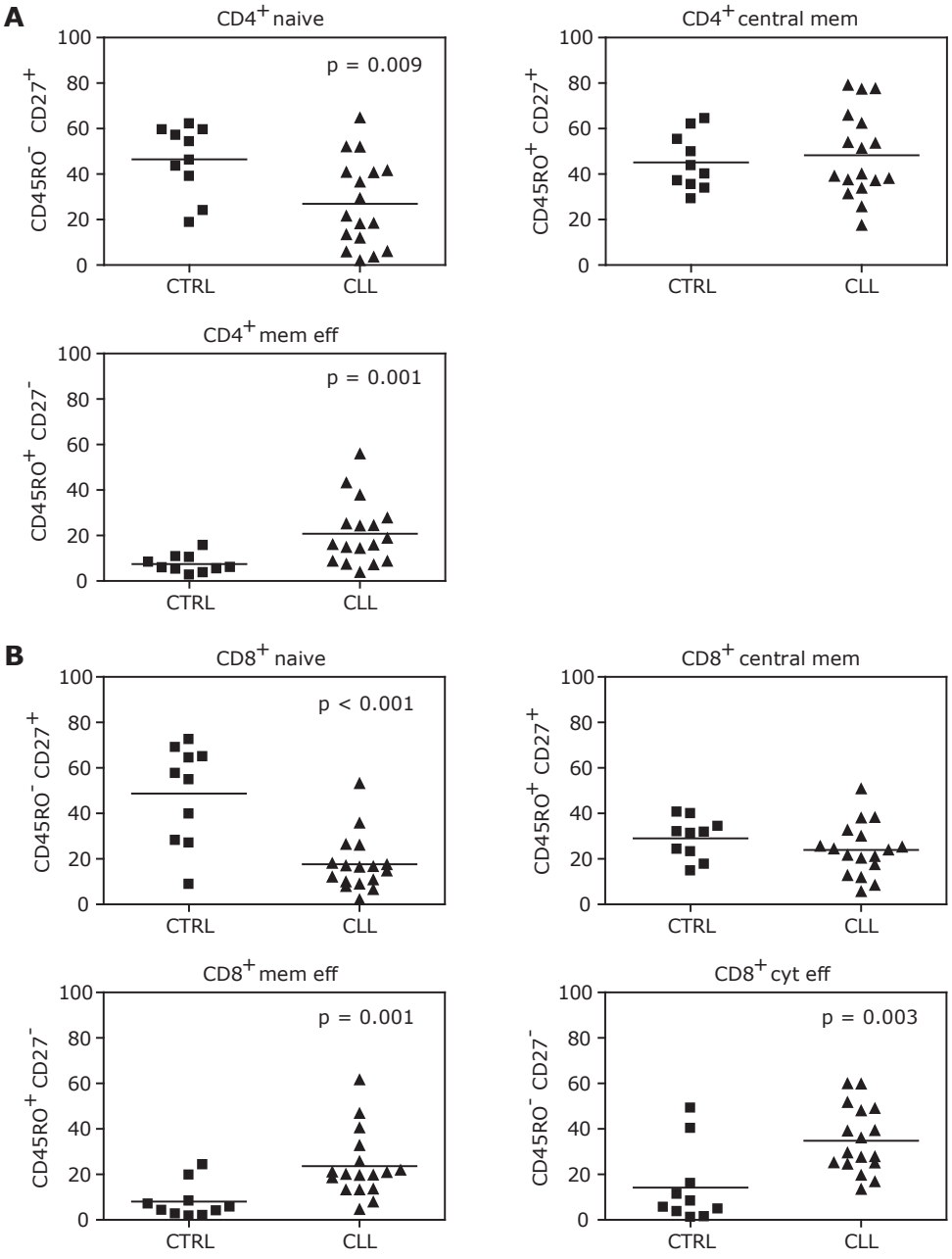
Figure 2. Aberrant expression of DM and DO in B-CLL. (A) Both DM (upper left panel) and DO (middle panel) are significantly decreased (both P < 0.001) in B-CLL patients. The relative expression of DM and DO (bottom panel) is increased (P < 0.001). Controls are represented by squares and B-CLL patients by triangles. (B) 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).

relatively reduced CLIP levels, we investigated the intracellular expression levels of DM and DO. We indeed found a significantly disturbed expression of DM and DO in B-CLL compared to healthy controls, both DM and DO being significantly reduced (P<0.001 for both) (Figure 2A, upper panels). When we compared DM to DO, a relative overexpression of DM was observed in B-CLL (P<0.001) (Figure 2A, bottom panel). This relative overexpression of DM (DM/DO) correlated with the efficiency of CLIP removal from DR (CLIP/DR) (R= -0.592, P=0.001) (Figure 2B). Both relative expression of DM over DO and the relative CLIP occupancy of DR did not correlate to B-CLL mutational status (as measured by IgV_H gene analysis), CMV status, costimulatory markers (CD40, CD80 and CD86), Rai stage of disease or treatment regimen (data not shown). Together, these data show a perturbed balance of the MHC class II peptide editors in B-CLL in combination with a reduced expression of CLIP in plasma membrane deposited DR, suggesting improved MHC class II antigen presentation in B-CLL.

Expansion of CD4$^+$ and CD8$^+$ effector T cell compartments in B-CLL

Does the altered CLIP expression on B-CLL cells result in differences in CD4$^+$ T cell differentiation in these patients? In line with previous observations[8], the CD4$^+$/CD8$^+$ ratio in B-CLL patients was inverted compared to healthy controls. No correlation was observed between the relative CLIP occupancy

of DR and the $CD4^+/CD8^+$ ratio. Next, 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. Patients with B-CLL showed a lower percentage of naïve $CD4^+$ T cells compared with healthy controls (P=0.009) (Figure 3A, upper left panel), an unchanged central memory $CD4^+$ T cell compartment (Figure 3A, upper right panel) and an expansion of the



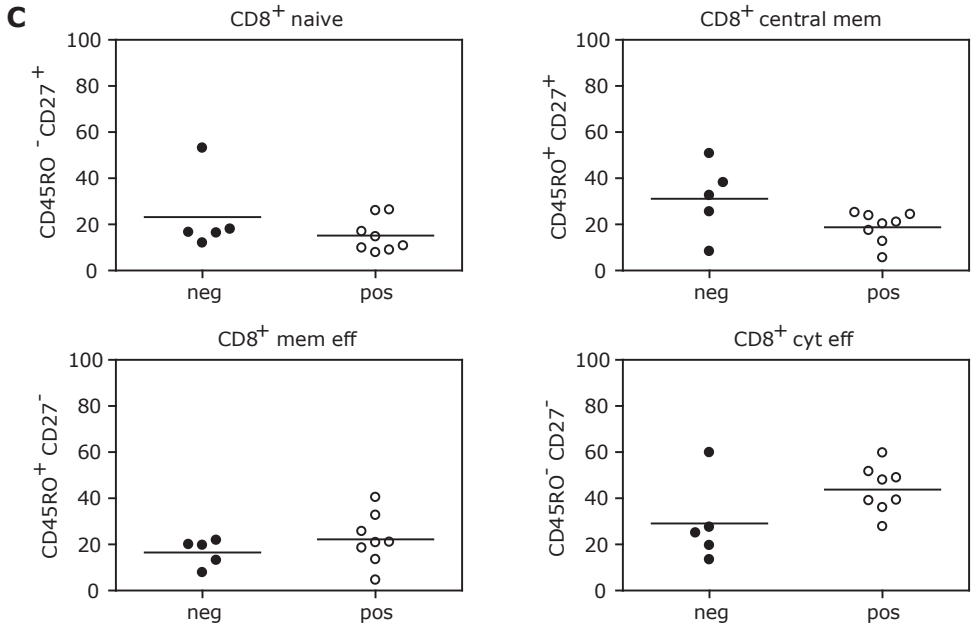


Figure 3. Expansion of effector type $CD4^+$ and $CD8^+$ T cells in B-CLL. (A) The naïve $CD4^+$ T cell compartment (upper left panel) is significantly decreased (P = 0.009) in B-CLL patients. The $CD4^+$ central memory T cells (upper right panel) are not significantly different between patients and 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. (B) 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. (C) No significant differences in $CD8^+$ T cell subsets were found between CMV seronegative (filled circles) and CMV seropositive (open circles) B-CLL patients.

memory effector $CD4^+$ T cell compartment (P=0.001) (Figure 3A, bottom panel). This points to an ongoing activation of $CD4^+$ T cells in B-CLL. When assessing antigen presentation indicators, the relative CLIP occupancy of DR was found to correlate with the percentage of $CD4^+CD45RO^-CD27^+$ naïve T cells (R=0.386, P=0.047).

In addition, 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. Patients with B-CLL showed a decrease in the percentage of naïve $CD8^+$ T cells (P<0.001) (Figure 3B, upper left panel) and no difference in percentages of $CD8^+$ central memory T cells (Figure 3B, upper right panel). The percentage of $CD8^+$ memory effector T cells was increased (P=0.001) (Fig. 3B, bottom left panel) as well as the $CD8^+$ cytotoxic effector T cells (P=0.003) (Fig. 3B, bottom right panel).

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

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

Because the increase in cytotoxic effector T cells in B-CLL patients has been related to CMV infection[26], we tested patients for CMV infection. Due to limited availability of patient material we could test only 13 patients for CMV serology. None of the B-CLL patients tested seropositive for IgM and 8 patients tested seropositive for IgG against CMV, indicating prior exposure of those patients to CMV. In our patient cohort, we did not observe a significant difference in $CD8^+$ T cell populations between CMV seropositive and CMV seronegative B-CLL patients (Figure 3C).

$CD4^+$ 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? Therefore we analyzed the relation between the shifts in $CD4^+$ and $CD8^+$ T cell compartments. The expansion of the $CD4^+$ memory effector T cell compartment correlated with the observed shifts in $CD8^+$ compartment in B-CLL, with the strongest correlation between the $CD4^+$ memory effector and $CD8^+$ memory effector compartments (Table 2).

Increased T cell activation in B-CLL

As a marker for ongoing T lymphocyte activation, we analyzed the expression of HLA-DR and CD38 on T cells. 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) (Figure 4A). 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) (Figure 4B). No differences in HLA-DR^+CD38^+ T cells were found between CMV seronegative patients and seropositive patients (Figure 4C). Thus, patients with B-CLL show a higher percentage of activated $CD4^+$ and $CD8^+$ T cells than healthy controls.

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 amount in the MHC class II peptide binding groove (CLIP/DR) and the percentage of $CD4^+$HLA-DR^+CD38^+ (R=-0.750, P<0.001) (Figure 4D, left panel). To a lesser extent, the relative CLIP occupancy of DR correlated to the percentage of $CD8^+$HLA-DR^+CD38^+ T cells (R=-0.617, P=0.001) (Figure 4D, right panel).

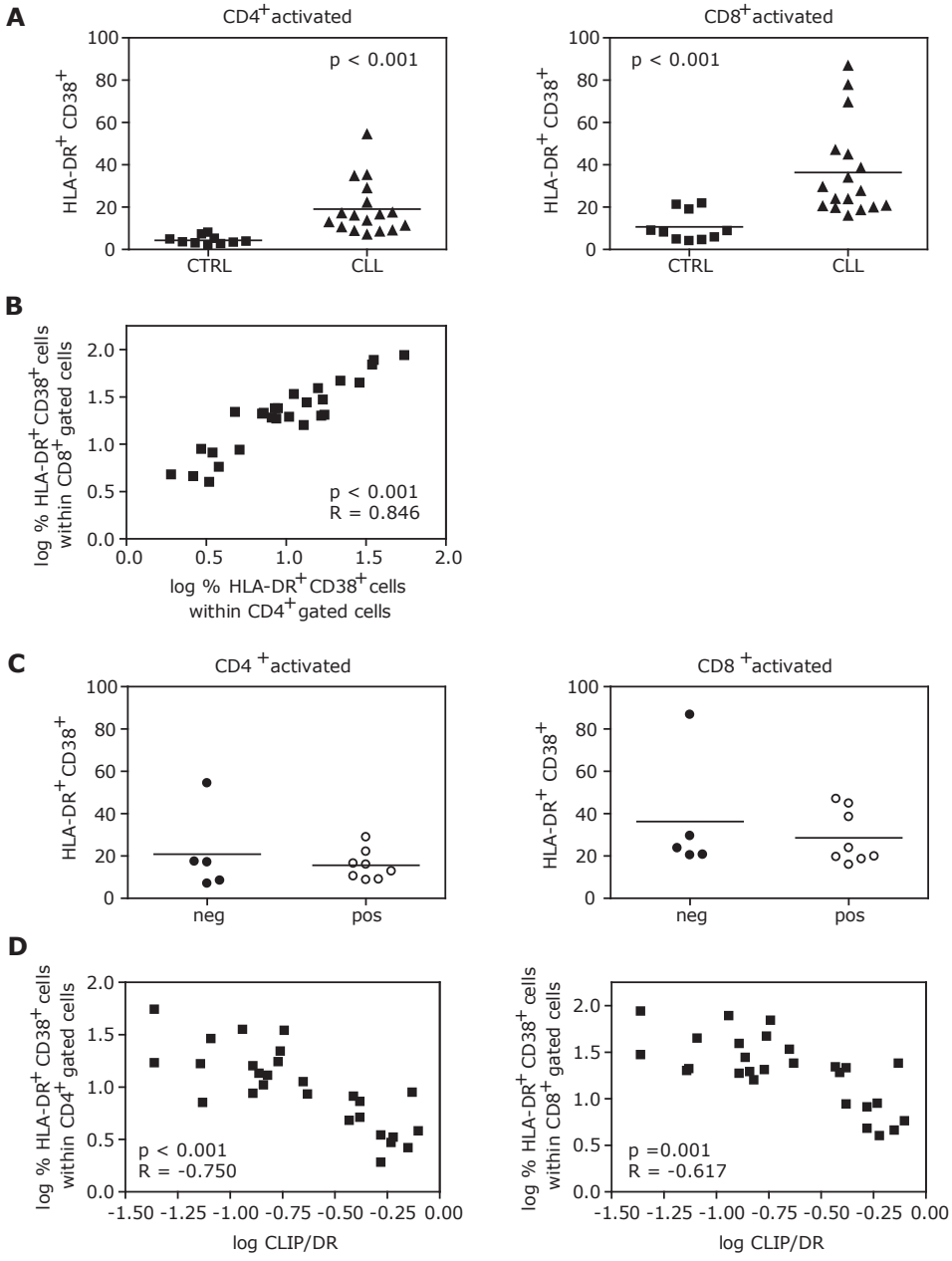


Figure 4. Increase in subsets of activated T cells correlates with the relative CLIP occupancy of DR. (A) Both in the $CD4^+$ (left panel) and $CD8^+$ (right panel) T cell compartment more of the T cells have an activated phenotype (both $P < 0.001$). (B) The percentage of activated $CD4^+$ T cells correlated with the percentage of activated $CD8^+$ T cells ($R = 0.846$, $P < 0.001$). (C) No significant differences in percentage activated $CD4^+$ and $CD8^+$ T cells were found between CMV seronegative (filled circles) and CMV seropositive (open circles) B-CLL patients. (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 extend CLIP/DR correlated with the percentage of $CD8^+$ activated T cells ($R = -0.617$, $P = 0.001$).

Thus, a lower occupancy of the MHC class II peptide binding groove with CLIP strongly correlates with an increase in activated $CD4^+$ T cells, as observed in B-CLL patients.

Discussion

Deficiencies in components of the MHC class I Ag processing pathway have been shown in a variety of human cancers[27;28], and some studies have correlated the presence of these deficiencies with tumor progression[29;30]. Here we identify aberrancies in the MHC class II Ag processing machinery in B-CLL and demonstrate that abnormal expression of the MHC class II chaperones 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, the occurrence of tumor immune escape due to genetic alterations in the MHC class II genes in the malignant cells does not apply here. This in contrast to poor prognosis correlated to the overall loss of MHC class II expression in diffuse large B cell lymphomas[31;32].

The concept that B-CLL disease is a homogenous entity is under debate. It is argued that based on the mutational status of the immunoglobulin heavy-chain variable-region (IgV_H) 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[23;33], but in our cohort we could not demonstrate a difference in DO expression between patients with mutated and unmutated IgV_H genes.

In B-CLL patients the presence of T cells with an anti-tumor specificity declines during disease progression[34]. An inversed 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[35]. Although reduced expression of the costimulatory markers CD80 and CD86 is by now well established in B-CLL (as 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[23], whereas the other 50% is free for unrestricted 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 finding that the relative expression of DM over DO is elevated in B-CLL implies that in these patients more free DM is available for the generation of MHC class II complexes with antigens. In this way, the relative overexpression of DM has shifted the composition of the antigen repertoire towards truly stably binding antigens and efficient removal of CLIP from MHC class II. Indeed, we did observe that the relative overexpression of DM is correlated with a decrease in the amount of CLIP in the peptide binding groove of DR at the plasma membrane in B-CLL patients. 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/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[36]. In the situation of AML, CLIP may constitute a form of tumor immunoediting or tumor immune escape. In B-CLL, reduced CLIP 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 lead to the hypothesis that T cells maybe 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 various T cell populations in B-CLL were poorly defined. The observations in this paper may point to one of the mechanisms involved in why this occurs; 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). Although the lack of strong costimulation may prevent the induction of an effective anti-leukemia immune response, the changes in the MHC class II antigen repertoire may lead to ongoing T helper cell activation. Cytokine-mediated pathways may subsequently lead to the observed ongoing cytotoxic T cell activation, but remains to be investigated. Whether initiation of T cell activation and maintenance of T cell activation are both antigen-driven remains to be established but the observed correlations strongly imply the 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.

Acknowledgments

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