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SYMPOSIUM IN WRITING

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T and B cells in B-chronic lymphocytic leukaemia: Faust, Mephistopheles and the pact with the Devil

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Abstract A large number of human malignancies are associated with decreased numbers of circulating T cells. B-CLL, in this regard, represents an anomaly since there is not only high numbers of circulating B cells, characteristic of the malignancy, but also a massive expansion of both CD4 and CD8 T cells. These T cells for the most part may probably not represent a leukaemia-specific TCR-dependent expansion. On the contrary, these T cells, especially the CD4 subset, might support a ''microenvironment'' sustaining the growth of the leukaemic B cell clone. Conversely, the leukaemic B cells may produce membrane-bound as well as soluble factors that stimulate the proliferation of these T cells in an antigen independent manner. In addition to these T cells lacking anti-leukaemic reactivity, there exist spontaneously occurring leukaemia-specific T cells recognizing several leukaemia-associated antigens, e.g. the tumour derived idiotype, survivin and telomerase. Both CD4 and CD8 leukaemia-specific T cells have been identified using proliferation and γ-IFN assays. These reactive T cells can lyse autologous tumour cells in an MHC class I and II restricted manner. Spontaneously occurring leukaemia-specific T cells are more frequently noted at an indolent stage rather than in progressive disease. Preliminary results from vaccination trials using whole tumour cell preparations as vaccine have demonstrated that vaccination may induce a leukaemia-specific T cell response, which might be associated with clinical benefits. Extended clinical trials are required to establish the therapeutic effects of vaccination in B-CLL. Studies in our laboratory as well as those of others indicate that whole tumour cell antigen in the form of apoptotic bodies or RNA loaded on to dendritic cells may be a suitable vaccine candidate. Patients with low stage disease may maximally benefit from this form of therapy.

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Introduction

Chronic lymphocytic leukaemia of the B cell type (B-CLL) is one of the most common leukaemias in the elderly and is manifest as a clonal proliferation of $CD5^+$ cells expressing CD19, CD23 and dim surface IgM. The occurrence of multiple T cell abnormalities in B-CLL patients is well established and the changes in numbers and functions of T cells appear to be different compared to other cancers and even other chronic B cell malignancies. T cell dysfunctions account for pathological conditions like hypogammaglobulinemia, autoimmune hemolytic anaemia, increased susceptibility to infections etc, commonly noted in B-CLL patients. These aberrantly functioning T cells may actually contribute to creating and supporting the ''microenvironment'' that sustains the malignant clone of B cells and impede their apoptosis [25]. In the midst of this expanded T cell population that are largely devoid of anti-leukaemic activity, there exist a small but measurable population of T cells that represent a natural specific T cell response against the leukaemic cell clone, which can be expanded by vaccination and possess the ability to mediate a therapeutic response against the leukaemic cells [27, 28, 89].

The expanded T-cell population noted in B-CLL represent a paradox in which the T cells may actually contribute to the onset, sustenance and exacerbation of the disease rather than the anti-neoplastic immunosurveillance function normally associated with T cells. Likewise, detailed knowledge about the leukaemia cell reactive T cells is of great importance to better

understand the pathobiology of CLL and how such T cells may be expanded and used in a therapeutic approach.

Phenotypic characterization of different T cell subsets

Elevated absolute numbers of total circulating T cells have frequently been found in B-CLL patients, mainly due to an increase of $CD8^+$ T cells, which resulted in a low CD4/CD8 ratio. Total number of $CD4^+$ T cells is also increased. Compared to healthy individuals, total T lymphocyte count, $CD8^+$ cells and $CD4^+$ cells in CLL patients have been reported to be increased about 3-fold, 4-fold and 2.5-fold, respectively [7, 24, 46, 94].

CD54 is an adhesion molecule constitutively expressed on T cells and upregulated upon activation. An increased frequency of $CD4^+/CD54^+$T cells has been observed with advancing stage of disease [74]. A reduced expression of the CD28 co-stimulatory molecule correlating with advancing disease stage was noted on CD4 as well as on CD8 T cells [73, 74]. The reduction in expression was more pronounced on CD8 compared to CD4 T cells. Surface-bound as well as cytoplasmic CTLA-4 molecules showed a reversed pattern compared to CD28. The expression of CTLA-4 was increased compared to healthy controls and with advancing stage of disease [74].

Within the CD4 T cell population a subset expressing CD57 but not CD28 and containing perforin (PF) was detected, suggesting that these cells may have cytolytic potential [65]. An increase of perforin-containing cells was not identified within CD8 and NK cell populations. The $CD4^+/PF^+$ population expressed high levels of CD45RO compared to CD45 RA indicating prior antigen exposure. The function of the $CD4^+/PF^+$ T cells in CLL is not clear. They may be cytolytic against the autologous leukaemic cells [16, 65]. They may also play a role in immune regulation of the B-CLL clone [31]. Since these cells are known to produce IL-4, they may be part of an anti-apoptotic environment for the tumour cells [67]. Support for $CD4^+$ T cell activation can also be derived from the observation that high levels of soluble CD4 molecules can be found in sera of progressive patients compared to non-progressive patients or normal individuals, indicating a selective increase of T helper cell activity [2].

Another indication for a persistent activation of T cells in B-CLL patients is the cytokine pattern of CLL T cells in vivo. Spontaneous production of IL-4 and IL-2 has been shown in CD4 T cells, while GM-CSF and TNF-α is known to be produced by both CD4 and CD8 T cells [75]. Higher levels of cytokine production were noted in patients with progressive disease. Moreover, T cells from patients with progressive disease were more prone to produce cytokines as compared to controls and patients with indolent disease. Other studies have also reported an increased production of IL-4, but the production of IL-2 as well as of TNF-α varied [55, 78]. Additionally, IL-6 is known to be produced by T cells in indolent disease [2, 39]. IL-6 has been suggested to be a growth factor for myeloma cells produced by the microenvironment [36]. The reason for the ongoing cytokine production is unclear but it is interesting to note that these cytokines have been suggested to be growth factors for the leukaemic cells [18, 54, 80, 85]. As the cytokine production seemed to increase by advancing stage, it might be speculated that increasing number of tumour cells lead to release of higher levels of tumour-derived soluble factors which activate polyclonal T cells.

Another interesting finding is that CD4 and CD8 T cells of B-CLL patients with indolent disease exhibit a dominance of a type 1 (γ-interferon) over type 2 (IL-4) cytokine production after a short incubation in vitro. In contrast, T cells from progressive patients continued to be predominantly type 2 [64]. The impact of type 1 and 2 T cells on the regulation of cancer immunity is still unclear but a shift from a type 1 to a type 2 cytokine pattern has been described in various tumours both in animals and man. This shift may contribute to the ability of cancer cells to escape immune surveillance.

The collusion of T and B cells in CLL: anatomy of the immune dysregulation

The overt clinical manifestations of immune dysfunction in CLL patients taken together with the increased numbers of circulating T cells has led to the long-standing hypothesis that T cells are involved in the pathobiology of B-CLL. The cause for the initiation and expansion of various T cell populations in CLL is not clear. Are the T cells activated following a TCR engagement or is it a TCR independent function? It seems unlikely that the huge expansion of T cells should be antigen driven and TCR dependent. In healthy donors the average total number of T cells is 1.8×10^9/l while in CLL patients the corresponding figure is 5.5×10^9/l [46]. It is unlikely that this profound change in T cell numbers is antigen driven. Recent studies have revealed some of the possible mechanisms by which the T cell dysregulation may occur and their implication in the pathobiology of CLL. A subpopulation of the activated $CD4^+$ T cells in B-CLL is known to express the CD40 ligand (CD154). Ligation of the CD40 receptor on B-CLL cells induces the secretion of the chemoattractant cytokine CCL22 that in turn increases the migration capability of $CD4^+/CD40L^+$ T cells expressing the receptor for CCL 22, CCR4. The chemoattracted CD4/CD154 T cells may migrate towards CLL cells, bind to CD40 on CLL cells and induce chemokine/cytokine production by the leukaemic clone, which may lead to progressive accumulation of neoplastic cells [26]. Several mechanisms may be involved in this circuit contributing to the growth of CLL cells. CD40 cross-linking can trigger rescue from apoptosis by upregulating the expression of anti-apoptotic genes like survivin

and facilitate proliferation of CLL cells [23, 29]. Activated CD4 T cells secrete several growth factors (see above), which might support the growth of the CLL clone including IL-2, IL-4, TNF-α, GM-CSF, IL-6, which may also support the growth of normal B cells [2, 75].

B-CLL cells from a subset of patients are also known to aberrantly express the B-lymphocyte stimulator, BLyS, also known as B cell activating factor (BAFF) [61]. In addition to stimulating the leukaemic clone in an autocrine fashion, the production of BLyS may also affect T cell numbers and function. It is known that activated T cells express one of the receptors for BLyS, called transmembrane activator and CAML interactor (TACI) [91]. In vitro studies have indicated that BLyS can stimulate T cell activation and proliferation [38]. The essential requirement for TACI-ligand interaction for T cell stimulation and proliferation has also been demonstrated in an animal model of arthritis [86]. BLyS–TACI interactions may thus be one of the possible pathways through which the T cells are activated and maintained in a state of chronic stimulation seen in B-CLL. It is most likely that polyclonal expansion of T cells is induced by various factors released by the malignant cells as well as by surrounding non-tumour cells engaged in the disease process. These factors also maintain the T cells in a state of chronic activation facilitating in turn the growth of B-CLL cells [12]. These activated T cells may continuously release cytokines, which may have an anti-apoptotic effect on the activated T cells and act in an autocrine or paracrine mode resulting in a polyclonal expansion [3, 4]. However, there is also TCR dependent oligoclonal/monoclonal expansion of T cells in B-CLL indicating involvement of CLL-specific antigens. A hypothetical model for T cells reacting against native CLL cells is presented in Fig 1.

T cell receptor (TCR) usage in B-CLL

The first study showing the presence of clonal T cell populations in B-CLL was published in 1990 [87]. Clonal re-arrangement of the Vβ chain was noted in three of five patients with stage 0 disease, but not in eight patients with advanced disease. This study indicated that the presence of clonal T cells may represent a host response directed against leukaemia-related antigens or reflect a specific T–B-cell interaction.

In an extended study, analysing the usage of 20 TCR-BV subsets in B-CLL ($n = 10$), a statistically significant over-expression of four TCR-BV subsets within the CD4 T cell population was found, while only one such subset was detected within the CD8 population. However, an examination of individual patients for overexpression of a particular BV family (defined as > mean + 3 SD of healthy controls) revealed that $CD4^+$ cells of seven of ten patients and $CD8^+$ cells of six of ten patients demonstrated skewing of the BV repertoire. The number of overexpressed BV subsets for an individual patient varied from one to seven. Analyses of the CDR3 length polymorphism showed a significantly higher degree of restriction within the CD4 and CD8 T cells of B-CLL patients when compared to healthy individuals [69]. Nevertheless, these analyses did not conclusively demonstrate that the overexpression of a particular BV subset or the occurrence of an oligoclonal/monoclonal TCR-BV type was associated with a T cell clone that was reactive against leukaemic cells. To further substantiate these findings, T cells were stimulated in vitro with autologous leukaemic B cells. Following stimulation, CDR3 length analyses showed that in expanded TCR-BV populations, polyclonal patterns shifted towards a monoclonal/oligoclonal profile, whereas monoclonal patterns intrinsically present in the peripheral blood

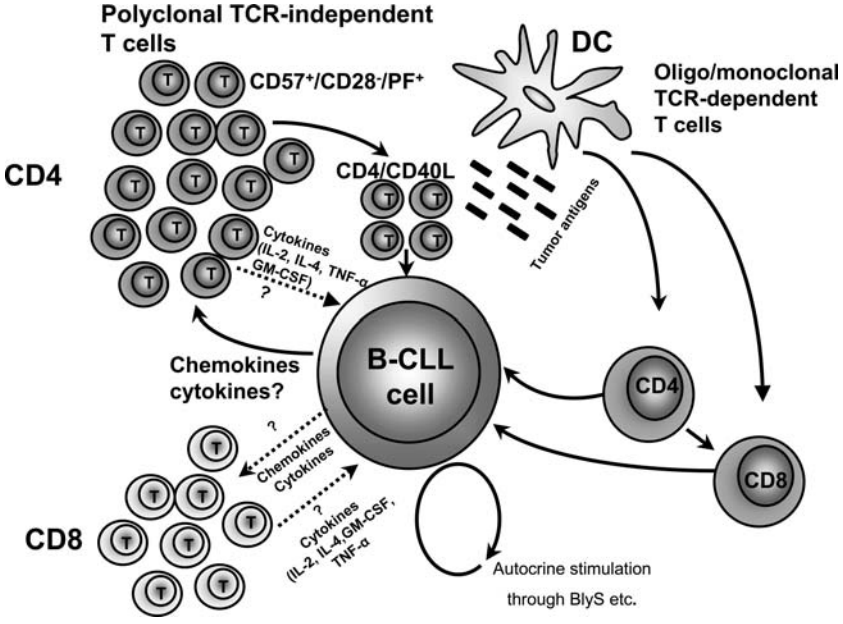

Fig. 1 Hypothetical model for T cell reactivity against native B-CLL cells. *DC* dendritic cells; *BlyS* B lymphocyte stimulator

remained monoclonal. The specific TCR-BV subset also expanded further upon in vitro stimulation. The data suggested that leukaemia cell induced specific memory CD4 and CD8 T cells are present in vivo in CLL patients and that several leukaemia cell associated antigens were recognized by the patient immune system [22, 72]. However, additional overexpression/expansion of polyclonal TCR-BV families, which did not recognize the leukaemia cells in a TCR-dependent manner, could also be detected.

Dendritic cells in CLL patients

Normal B cells can act as antigen presenting cells, but are much less potent than professional APC like the dendritic cell (DC). Although the leukaemic B cells exhibit normal levels of MHC class I and II molecules, they are intrinsically defective in the expression of co-stimulatory molecules like CD80/86 and cell adhesion molecules like CD54 [10, 70, 79, 83]. These molecules are critical for the induction of an immune response (afferent phase) but are much less important for the efferent phase, i.e., recognition of tumour cells by effector cells [84]. Although the leukaemic cells themselves might be able to induce an immune response, their capability is impaired. It is more likely that remnants of apoptotic or necrotic leukaemic cells are endocytized by DC, which then initiate the leukaemia-specific immune response.

There seems to be a clear difference in DC function of CLL patients at various stages of the disease. At an indolent stage, DC generated from monocytes have the same expression profile of CD54, 80, 83 and 86 as DC from normal individuals. The intensity of CD80 and MHC class I molecules was increased. An augmented expression of IL-10, IL-1β and IL-12 p^{35} was also noted. DC of indolent CLL patients had a similar capacity to stimulate in MLR as well as to present a recall antigen as normal DC. The reason behind the increase in some surface molecules and cytokine expression is not clear, but may indicate pre-activation of DC in vitro [68]. DC could also effectively present autologous leukaemic cells and induce a strong anti-leukaemia T cell response [48, 49]. This is in contrast to patients with progressive disease who showed a profound defect in DC derived monocytes, lacking the maturation antigen CD83 and the co-stimulatory molecule CD80. These DC were unable to induce a significant proliferative response in an MLR and had a reduced ability to release IL-12 and to direct a type 1 T cell response [62]. The reason for this difference is not clear but a possible explanation may be the production of large amounts of various immune suppressive factors by the leukaemic clone, which is higher in patients with progressive disease. One such factor contributing to the poor stimulatory activity of DC from advanced stage patients is transforming growth factor β (TGF-β). TGF-β is known to be produced by B-CLL cells [52] and has been reported to inhibit antigen presentation, T cell stimulation and migration of DC to the lymph nodes [47]

The major difference in DC functions between progressive and non-progressive disease may be of fundamental significance. A natural leukaemia specific T cell response seems to be more common at an indolent stage (see the following para) when compared to advanced disease, suggesting that a defective DC function might be part of this difference. This difference also leads to the premise that immunotherapy strategies are more likely to be efficacious in patients with indolent rather than advanced disease (see the following para).

Natural occurring leukaemia specific T cells

The presence of spontaneously occurring, leukaemia-specific T cells has been established by several studies [27, 28, 50, 70, 81]. Specific T cells were more frequently found in non-progressive when compared to progressive stages [27, 70]. The T cells recognized the leukaemic B cells through the TCR [28] and recognition induced proliferation and γ-IFN secretion [27, 28, 51]. The response could be potentially induced by unmodified CLL cells [51], but is facilitated by CD40L activation [70], which causes the upregulation of co-stimulatory and adhesion molecules. The T cell response could be inhibited by MHC class I and II antibodies. The nature of the response varied between patients since a $CD4^+$ or a $CD8^+$ response was noted in some patients while other patients demonstrated activation of both T cell types.

The specific T cells could lyse autologous leukaemic cells. CTL were shown to be MHC class II restricted [28] or to express the CD8 phenotype [33]. Lytic activity of the CTL were largely mediated through the perforin–granzyme pathway [17]. However, such autologous cytotoxic responses against native as well as CD40L activated CLL cells was infrequent compared to proliferative and cytokine responses. The fact that the CTL were functionally intact can be inferred from the observation that their allogeneic cytotoxic response was preserved [28]. The logical explanation may be that natural occurring CTLs are induced against low affinity, self epitopes presented by the CLL cells. Support for this assumption comes from experiments with heteroclitic peptides with the same specificity as the native epitope (idiotype), but with amino acid substitution aimed at increasing the MHC-binding affinity [33]. CTL generated against the heteroclitic peptide not only had enhanced reactivity against the heteroclitic peptide, but also increased killing of antigen presenting cells pulsed with the native peptide. No difference was observed in the frequency of T cells detected by MHC class I peptide tetramers after stimulation with the heteroclitic peptide compared with the native peptide. CTLs generated against heteroclitic peptides could kill the patient's tumour cells showing that the Ig-derived peptides can be presented by the tumour cells and that the failure to mount an immune response may result, among others

reasons, from the low immunogenicity of the native Ig-derived peptide. These results suggest that heteroclitic peptides may enhance the immunogenicity without changing the specificity, a finding of considerable significance for vaccine development.

Another explanation for the suppressed induction of CTL against low affinity antigens might be the presence of T_{reg} which suppress the functional activity of T cells reactive against auto-antigens [63].

T cell recognition structures on leukaemic B-CLL cells

The idiotypic immunoglobulin produced by the leukaemic B cells is a unique tumour antigen and a hallmark of B cell malignancies. Various studies have demonstrated that the idiotypic protein is naturally processed and presented by B cells and the resulting T cells are capable of recognizing the processed antigen [9, 14, 43, 44, 90]. The idiotype-specific T cells in most reports were described to recognize the antigen in conjunction with MHC class II although class I restricted T cells have also been described [14]. Additionally, a subpopulation of anti-idiotype T cells have been demonstrated to have the ability to bind Ig molecules suggesting that they may be capable of recognizing determinants on the intact immunoglobulin [1, 13]. The nature of the antigen and whether MHC restriction is required for the induction of these T cells yet remain unclear.

The most specific part of the V-regions is CDR3, the clonotypic marker. CDR1 and 2 may express conserved structures as well as the framework regions (FR1-4) with estimated shared peptide sequences of 3–4%. The VH-CDR3 region of the individual leukaemic cells has been shown to induce a VH-CDR3 specific proliferative and γ-IFN response, which was either MHC class I or II restricted in patients with indolent disease [71]. In another study idiotype reactive CD8 cells could be generated from the peripheral blood of CLL patients using VH-CDR3 peptides. CTL induced with the native peptide could lyse autologous CD40L activated tumour cells in three of five patients with non-progressive disease, but not with progressive disease. CTL generated using modified peptides where an amino acid had been replaced to improve affinity score assigned by prediction software, had increased lytic capability while the specificity was retained [93]. Similar results were also obtained by Gribben et al. [33, 51, 81]. In their studies, they also analysed FR derived peptides and found that CTL from HLA matched healthy donors could be more frequently generated against FR than against CDR. The effector population was $>90\%$ $CD8^+$ T cells and lysis could be inhibited by anti-MHC class I antibodies. In CLL patients, both FR and CDR reactive T cells could be obtained, which lysed the CLL cells. Killing was weak when native peptides were used to generate CTL, but increased significantly using heteroclitic peptides. These observations also indicate that low-affinity peptides that are ineffective in generating an immune response still can be targets of a T cell mediated immune response generated against an altered peptide. The killing of CLL cells by idiotype specific T cells could be further enhanced by CD40L activation increasing the expression of molecules involved in T–B-cell interaction.

Survivin, a member of the inhibition of apoptosis protein family, is over-expressed in most malignancies including CLL. Over-expression of survivin is largely restricted to the neoplastic cells. MHC class I restricted natural peptides have been used to generate CTL from CLL patients. These peptides had a low to intermediate affinity. In the majority of patients, survivin specific T cells could be expanded, which cells could kill the autologous leukaemic cells in an MHC class I restricted manner [5, 76]. Modifying the peptide to increased binding affinity augmented the cytolytic response [6].

The catalytic subunit of telomerase can also be regarded as a universal tumour antigen expressed in most human malignancies including CLL, but not in normal differentiated tissues [92]. Shortening of the telomere length and increased telomerase activity is seen in CLL patients to varying degrees and seem to be more frequently found in progressive disease [19]. A proliferative and γ-IFN response could be detected against a 17-aa long peptide (p611–626) of the catalytic subunit of human telomerase (hTERT). CTL against autologous leukaemic cells could be generated by three rounds of stimulation with the peptide (unpublished data from our laboratory).

CD8 T cells in CLL patients reactive to peptide sequences of CD19 and CD20 with an intermediate affinity score (21–27) have also been detected in CLL, but not in other B cell malignancies. T cells with the same specificity could be found in healthy donors but at a much lower frequency. The specific T cells of CLL patients exhibited a proliferative response but could not lyse autologous CLL cells [30]. There might be several reasons for the specific T cells not being able to lyse the tumour cells. Intermediate affinity peptides may have too low binding. However, this is probably not the case as survivin peptides [76, 93] were of intermediate to low affinity, but still be able to induce CTL. The same was also true for low to intermediate affinity idiotypic peptides [33, 51, 81]. The peptide concentration on B-CLL cells may be too low, or peptides may not be adequately presented on the leukaemic cells. Alternatively, the lack of accessory molecules or the presence of T_{reg} cells may be factors responsible for suppressing the functional activity of T cells reactive against these auto-antigens [63].

A summary of reactive and functional capability of leukaemia specific T cells in B-CLL is presented in Table 1.

Vaccination of B-CLL patients

In most CLL patients, the disease presents as a slow smouldering malignancy that progress over years. Taken

Table 1 Summary of recognition structures and functional capability of spontaneously occurring leukaemia specific T cells in B-CLL

Antigens	Proliferative response	γ-IFN response	CTL activity against CLL cells
Leukaemic cells	+	+	+
Defined antigens leukaemia cell derived			
Idiotypic Ig	+	+	+
Survivin	ND	ND	+
Telomerase	+	+	+
CD19	+	ND	–
CD20	+	ND	–

ND not done

together with the advanced age of the patients, low toxicity immunotherapy approaches seem ideally suited for the treatment of this disease. Ideally, a vaccine candidate should be able to induce both CD4 and CD8 T cell responses. In addition to providing help to CD8 cells, CD4 cells are themselves known to have lytic ability. Spontaneously occurring cytotoxic CD4 T cells have been shown in CLL [28]. Additionally, CD4 T cells are also of importance for mounting a humoral response and tumour specific antibodies has been shown to be of clinical benefit in solid tumours [82].

Previous studies have reported several clinical vaccination trials where whole tumour cell antigens were used with varying degrees of success [11, 42, 88]. The use of whole tumour preparations for vaccination uses both defined and occult tumour antigens for generating immune response. However, these genre of vaccines are typically labor intensive to formulate and patient-specific.

CD40 ligand stimulation of B-CLL cells results in upregulation of co-stimulatory (CD80/86) and adhesion molecules (CD54/58) thereby improving their T cell stimulatory ability. CD40L activated CLL cells induced CD4 and CD8 proliferative response of autologous T cells and γ-interferon production of CD4 T cells but not a CD8 CTL response. CD4 specific T cells appeared to lyse the leukaemic B cells in a Fas-mediated manner [10]. The concept of using CD40L activated CLL cells as a vaccine was improved by transduction of CLL cells with a replication-defective adenovirus vector (Ad-CD154). In vitro studies demonstrated that CLL cells became highly proficient at antigen presentation and could induce autologous T cell responses leading to the generation of CLL specific CTL [45]. A phase I trial testing this concept was performed. Autologous CLL cells were transduced ex vivo with Ad-CD154 and transfected cells were given back as an intravenous infusion [89]. Several interesting observations were made. The absolute T cell count increased. CLL specific T cells capable of proliferating and producing γ-IFN upon challenge were induced. The CLL specific T cell response was noted within a few days of vaccination indicating that a pre-existing immune response was probably boosted by the vaccine rather than a de novo initiation of an anti-leukaemic response. Decrease of circulating leukaemic cells as well as shrinking of lymph node size was noted. High concentrations of IL-12 and γ-IFN were found in the serum of the patients. γ-IFN can be released from NK cells, activated T cells and monocytes. The source of IL-12 is not clear, but probably not from the leukaemic cells or T cells. IL-12 is produced among others by monocytes and dendritic cells. It cannot be excluded that the immune response was partly induced by activated DC, which had taken up and processed Ad-CD154 transduced CLL cells and that IL-12 was produced by activated DC. As a bystander effect, CD95 was upregulated on non-transfected CLL cells. The expression of CD95 coincided with the reduction of leukaemic cell counts and lymph node size, which may have mediated by lytic CD4 T cells expressing Fas ligand [15].

Ex vivo generated DC are probably the most potent cellular adjuvants that can be used in conjunction with whole tumour antigens for the induction of an anti-tumour response. There are various approaches for antigen loading of DC such as tumour lysate, total RNA, or apoptotic tumour cells. Hybrids can be produced between tumour cells and DC using chemical or electrochemical methods. In a comparative study, DC were loaded with apoptotic tumour cells, tumour cell lysate, or total RNA or hybridized with autologous CLL cells and examined for their ability to stimulate T cell responses. Apoptotic tumour cells were found to induce the strongest T cell response evaluated as a proliferative response and γIFN production. Both MHC class I and II restricted T cells were detected. Interestingly, apoptotic tumour cells mainly evoked a Th_1 cytokine response pattern, while lysate, RNA and hybrids induced a mixed Th_1/Th_2 response. Perforin was also induced indicating cytotoxic potential [48, 49].

Another in vitro study used only RNA-transfected dendritic cells for induction of a T cell response in vitro [60]. A proliferative MHC class II restricted T cell response was induced. The specific T cells could lyse native autologous leukaemic cells. Cytotoxicity could be blocked by MHC class I antibodies. However, MHC class II antibodies were not used in this test. One of the antigens recognized by the T cells was survivin, indicating that survivin might be an immunodominant antigen in CLL. The cellular response was also induced against shared antigens on the leukaemic cells. Importantly, leukaemia specific T cells did not lyse autologous non-malignant B cells. Induction of CTL recognizing shared antigens was also found using lysate to stimulate T cells [28].

An advantage offered by apoptotic bodies is that presentation of intracellular proteins released into tumour lysate or necrotic cells that are not relevant to induction of anti-tumour responses may be avoided [35]. DC that have endocytosed apoptotic tumour cells is also

an attractive alternative since phagocytotized cellular fragments are 300 times more efficient in forming MHC-peptide complexes than processed peptides[41]. Higher IL-12 levels were produced by DC loaded with apoptotic tumour cells in an animal model compared to tumour lysate or necrotic cells [50]. High IL-12 production can polarize T cells toward a Th_1 response [77]. Apoptotic tumour cells and DC can activate CTL as well as effector cells of the innate immune system, which are of importance for initating an adaptive immune response [77]. DC pulsed with apoptotic bodies from myeloma cells were more effective than lysate in inducing CTL against the autologous myeloma cells [34]. The reasons for the differences in efficacy between different tumour cell preparations are not clear. Further studies are needed to elucidate the best tumour cell preparation for vaccination. The ultimate testimony, however, will be their efficacy in human clinical trials.

In a vaccination attempt using apoptotic tumour cells, CLL patients in clinical stages 0 and I were vaccinated s.c. with irradiated leukaemia cells (to induce apoptosis). Ten patients have so far been included in the study. CD8 T cells increased during vaccination with a concomitant decrease in the CD4/CD8 ratio. In one patient the leucocyte count decreased. In all other patients the disease has been stabilized for varying periods up to 18 months [40].

Vaccination strategies in B-CLL

A few considerations for the development of vaccination strategies using whole tumour antigens in B-CLL is presented in Table 2.

Table 2 Considerations for designing vaccination strategies against B-CLL

Vaccine preparation
–Whole tumour cell preparations at present, but defined tumour antigens in the future.
Adjuvants
–Dendritic cells loaded with whole tumour cell antigen prepared as apoptotic bodies or RNA.
–Appropriate cytokines, e.g., GM-CSF, IL-12 and IL-2.
Route and schedule
–Subcutaneous or intradermal. Repeated immunizations over a long boosting period.
Clinical setting
–Low clinical stage, preferably before chemo/radiotherapy when the disease starts to slowly accelerate. Alternatively stable phase after cytoreductive treatment provided immune functions are preserved.

This table summarizes the various considerations for vaccination therapy against B-CLL. The recommendations suggested are based on the cumulative clinical observations and experimental observations in generating T cell responses with anti-leukaemic reactivity in B-CLL. Factors such as nature of the vaccine preparation, route and frequency of vaccine administration, use of immune adjuvants and the disease status in patients at the time of vaccination may be critical for generating a therapeutic immune response

In B-CLL natural cellular immunity is evoked against several B-CLL associated antigens, most of them yet undefined. However, three antigens have so far been characterized, the idiotype, survivin and telomerase. Modified leukaemic cells for vaccination are easy and inexpensive to produce in B-CLL. Unmodified B-CLL cells alone cannot be used as the vaccine. Autologous ex vivo generated DC pulsed with autologous leukaemic cells may be an attractive approach. Tumour cells should probably be presented to DC as apoptotic cells or total tumour cell RNA. An alternative might be to culture leukaemic cells together with CD40L and IL-4, which upregulate immunostimulatory molecules and abrogate production of inhibitory factors by the leukaemic cells. Such modified leukaemic cells can drive T cells towards a Th_1 response [59] and might be used as a vaccine. Discovery of defined B-CLL antigens with the ability to generate therapeutic responses would also offer new vaccine candidates. With regard to the route of vaccine administration, a large body of evidence now establishes that intradermal and subcutaneous administration allows greater availability of the vaccine within the lymph nodes, which facilitates an immune response of higher magnitude. In contrast, intravenous administration of vaccine preparations diminish their ability to elicit immune responses.

The use of adjuvant cytokines should be considered to further enhance the therapeutic immune responses. Dranoff et al. [20, 21] have demonstrated that GM-CSF used as an adjuvant in conjunction with whole tumour cell vaccination can induce a potent and long lasting anti-tumour immunity in animal models and cancer patients. GM-CSF may initiate the maturation of DC and promote migration to local lymph nodes [58]. IL-12 might amplify the immune response and direct towards a type 1 T cell response [66]. Idiotype immunization together with IL-12 induced a Th_1 biased cellular response in patients with low stage myeloma, which was associated with clinical responses (own unpublished data). IL-2 may amplify and maintain an memory T cell response [53].

It is evident that a natural immune response in B-CLL is more frequently noted in a non-progressive stage as compared to progressive disease. Furthermore, T cell function including the expression of T cell signalling pathway molecules are also better preserved in indolent as compared to advanced disease (unpublished data). So far, the most encouraging clinical effects of therapeutic cancer vaccines in various malignancies have been seen in the adjuvant setting or in low stage disease [8, 11, 32, 42, 57, 88]. Collectively, these data, extrapolated to B-CLL patients, suggest that the preferred clinical settings for immunotherapy are previously untreated patients where a slow progression of the disease is seen or in an indolent stage after cytoreductive therapy, provided DC and T cell functions are preserved in the patients. Moreover, a prolonged series of booster vaccinations would serve to protract the therapeutic effects of immunotherapy [56].

The use of vaccine therapy for clinical management of B-CLL patients

As described in previous sections, vaccine therapy in B-CLL patients has not been extensively tested in clinical settings although the disease is ideally suited for such a therapeutic approach. In addition to considerations of vaccine design and adjuvants, the disease status of the patients receiving vaccine therapy may be a crucial factor in determining the clinical outcome post-vaccination. Previous studies have demonstrated that there is an inverse correlation between tumour burden and therapeutic benefit of vaccine therapy [37, 88]. Moreover, it is well established that B-CLL patients with advanced disease have a greater degree of T cell dysfunction than indolent or early stage patients [74, 75]. Agents like fludarabine and Alemtuzumab, used in the treatment of B-CLL also have a profound effect on T cell function. Taken together, indolent, treatment naïve patients with low disease burden represent the ideal population, who would potentially benefit the most from vaccine therapy. It is conceivable that application of vaccine therapy in adjuvant settings may result in eradication of residual disease and long term remissions.

Concluding remarks

The relationship between T cells and leukaemic cells in B-CLL almost assumes the character of a Faustian bargain. This malignancy represents a unique situation where the T cells that normally serve as sentinels against cancer, actually abet the growth and sustenance of the malignant clone. In exchange, the B-CLL cells facilitate the polyclonal expansion of T cells and prevent their apoptotic death from normal immunoregulatory mechanisms. We speculate that the key to therapy of B-CLL lies in a two-pronged approach. In addition to strategies designed at debulking the leukaemia, one aspect of therapy lies in ''resetting'' the regulatory switch on T cells, breaking their nexus with the leukaemic clone. The second aspect involves stimulating and specifically expanding the pre-existing, albeit minor, pool of T cells with therapeutic potential through vaccination strategies. Appropriately designed clinical studies should be able to answer the questions whether these concepts may be successful.

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References

1. Abbas AK, Perry LL, Bach BA, Greene MI (1980) Idiotype-specific T cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma idiotypic determinants. J Immunol 124:1160–1166
2. Aguilar-Santelises M, Loftenius A, Ljungh C, Svenson SB, Andersson B, Mellstedt H, Jondal M (1992) Serum levels of helper factors (IL-1 alpha, IL-1 beta and IL-6), T-cell products (sCD4 and sCD8), sIL-2R and beta 2-microglobulin in patients with B-CLL and benign B lymphocytosis. Leuk Res 16:607–613
3. Akbar AN, Borthwick NJ, Wickremasinghe RG, Panayoitidis P, Pilling D, Bofill M, Krajewski S, Reed JC, Salmon M (1996) Interleukin-2 receptor common gamma-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (bcl-2, bcl-xL) but not pro-apoptotic (bax, bcl-xS) gene expression. Eur J Immunol 26:294–299
4. Amos CL, Woetmann A, Nielsen M, Geisler C, Odum N, Brown BL, Dobson PR (1998) The role of caspase 3 and BclxL in the action of interleukin 7 (IL-7): a survival factor in activated human T cells. Cytokine 10:662–668
5. Andersen MH, Pedersen LO, Becker JC, Straten PT (2001) Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res 61:869–872
6. Andersen MH, Pedersen LO, Capeller B, Brocker EB, Becker JC, thor Straten P (2001) Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes in situ as well as ex vivo in cancer patients. Cancer Res 61:5964–5968
7. Bartik MM, Welker D, Kay NE (1998) Impairments in immune cell function in B cell chronic lymphocytic leukemia. Semin Oncol 25:27–33
8. Bendandi M, Gocke CD, Kobrin CB, Benko FA, Sternas LA, Pennington R, Watson TM, Reynolds CW, Gause BL, Duffey PL, Jaffe ES, Creekmore SP, Longo DL, Kwak LW (1999) Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. Nat Med 5:1171–1177
9. Bogen B, Malissen B, Haas W (1986) Idiotope-specific T cell clones that recognize syngeneic immunoglobulin fragments in the context of class II molecules. Eur J Immunol 16:1373–1378
10. Buhmann R, Nolte A, Westhaus D, Emmerich B, Hallek M (1999) CD40-activated B-cell chronic lymphocytic leukemia cells for tumor immunotherapy: stimulation of allogeneic versus autologous T cells generates different types of effector cells. Blood 93:1992–2002
11. Bystryn JC, Zeleniuch-Jacquotte A, Oratz R, Shapiro RL, Harris MN, Roses DF (2001) Double-blind trial of a polyvalent, shed-antigen, melanoma vaccine. Clin Cancer Res 7:1882–1887
12. Caligaris-Cappio F (2003) Role of the microenvironment in chronic lymphocytic leukaemia. Br J Haematol 123:380–388
13. Cerny J, Smith JS, Webb C, Tucker PW (1988) Properties of anti-idiotypic T cell lines propagated with syngeneic B lymphocytes. I. T cells bind intact idiotypes and discriminate between the somatic idiotypic variants in a manner similar to the anti-idiotopic antibodies. J Immunol 141:3718–3725
14. Chakrabarti D, Ghosh SK (1992) Induction of syngeneic cytotoxic T lymphocytes against a B cell tumor. III. MHC class I-restricted CTL recognizes the processed form(s) of idiotype. Cell Immunol 144:455–464
15. Chu P, Deforce D, Pedersen IM, Kim Y, Kitada S, Reed JC, Kipps TJ (2002) Latent sensitivity to Fas-mediated apoptosis after CD40 ligation may explain activity of CD154 gene therapy in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 99:3854–3859
16. Chu P, Deforce D, Rassenti L, Kipps T (2000) Characterization of CD4 cytotoxic T cells specific for autologous chronic lymphocytic leukemia B cells. Blood (Suppl):157a
17. Chu P, Wierda WG, Kipps TJ (2000) CD40 activation does not protect chronic lymphocytic leukemia B cells from apoptosis induced by cytotoxic T lymphocytes. Blood 95:3853–3858
18. Cordingley FT, Bianchi A, Hoffbrand AV, Reittie JE, Heslop HE, Vyakarnam A, Turner M, Meager A, Brenner MK (1988) Tumour necrosis factor as an autocrine tumour growth factor for chronic B-cell malignancies. Lancet 1:969–971

19. Damle RN, Batliwalla FM, Ghiotto F, Valetto A, Albesiano E, Sison C, Allen SL, Kolitz J, Vinciguerra VP, Kudalkar P, Wasil T, Rai KR, Ferrarini M, Gregersen PK, Chiorazzi N (2004) Telomere length and telomerase activity delineate distinctive replicative features of the B-CLL subgroups defined by immunoglobulin V gene mutations. Blood 103:375–382
20. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 90:3539–3543
21. Dranoff G, Soiffer R, Lynch T, Mihm M, Jung K, Kolesar K, Liebster L, Lam P, Duda R, Mentzer S, Singer S, Tanabe K, Johnson R, Sober A, Bhan A, Clift S, Cohen L, Parry G, Rokovich J, Richards L, Drayer J, Berns A, Mulligan RC (1997) A phase I study of vaccination with autologous, irradiated melanoma cells engineered to secrete human granulocyte-macrophage colony stimulating factor. Hum Gene Ther 8: 111–123
22. Farace F, Orlanducci F, Dietrich PY, Gaudin C, Angevin E, Courtier MH, Bayle C, Hercend T, Triebel F (1994) T cell repertoire in patients with B chronic lymphocytic leukemia. Evidence for multiple in vivo T cell clonal expansions. J Immunol 153:4281–4290
23. Furman RR, Asgary Z, Mascarenhas JO, Liou HC, Schattner EJ (2000) Modulation of NF-kappa B activity and apoptosis in chronic lymphocytic leukemia B cells. J Immunol 164:2200–2206
24. Garcia C, Rosen A, Kimby E, Aguilar-Santelises M, Jondal M, Bjorkhilm M, Holm G, Mellstedt H (1989) Higher T-cell imbalance and growth factor receptor expression in B-cell chronic lymphocytic leukemia (B-CLL) as compared to monoclonal B-cell lymphocytosis of undetermined significance (B-MLUS). Leuk Res 13:31–37
25. Ghia P, Caligaris-Cappio F (2000) The indispensable role of microenvironment in the natural history of low-grade B-cell neoplasms. Adv Cancer Res 79:157–173
26. Ghia P, Strola G, Granziero L, Geuna M, Guida G, Sallusto F, Ruffing N, Montagna L, Piccoli P, Chilosi M, Caligaris-Cappio F (2002) Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4+, CD40L+ T cells by producing CCL22. Eur J Immunol 32:1403–1413
27. Gitelson E, Hammond C, Mena J, Lorenzo M, Buckstein R, Berinstein NL, Imrie K, Spaner DE (2003) Chronic lymphocytic leukemia-reactive T cells during disease progression and after autologous tumor cell vaccines. Clin Cancer Res 9:1656–1665
28. Goddard RV, Prentice AG, Copplestone JA, Kaminski ER (2001) Generation in vitro of B-cell chronic lymphocytic leukaemia-proliferative and specific HLA class-II-restricted cytotoxic T-cell responses using autologous dendritic cells pulsed with tumour cell lysate. Clin Exp Immunol 126:16–28
29. Granziero L, Ghia P, Circosta P, Gottardi D, Strola G, Geuna M, Montagna L, Piccoli P, Chilosi M, Caligaris-Cappio F (2001) Survivin is expressed on CD40 stimulation and interfaces proliferation and apoptosis in B-cell chronic lymphocytic leukemia. Blood 97:2777–2783
30. Grube M, Rezvani K, Wiestner A, Fujiwara H, Sconocchia G, Melenhorst JJ, Hensel N, Marti GE, Kwak LW, Wilson W, Barrett JA (2004) Autoreactive, cytotoxic T lymphocytes specific for peptides derived from normal B-cell differentiation antigens in healthy individuals and patients with B-cell malignancies. Clin Cancer Res 10:1047–1056
31. Hahn S, Erb P (1999) The immunomodulatory role of CD4-positive cytotoxic T-lymphocytes in health and disease. Int Rev Immunol 18:449–464
32. Hansson L (2004) Natural and induced idiotype immunity in patients with multiple myeloma. Karolinska Institute, Stockholm, ISBN91-7349-820-3
33. Harig S, Witzens M, Krackhardt AM, Trojan A, Barrett P, Broderick R, Zauls AJ, Gribben JG (2001) Induction of cytotoxic T-cell responses against immunoglobulin V region-derived peptides modified at human leukocyte antigen-A2 binding residues. Blood 98:2999–3005
34. Hayashi T, Hideshima T, Akiyama M, Raje N, Richardson P, Chauhan D, Anderson KC (2003) Ex vivo induction of multiple myeloma-specific cytotoxic T lymphocytes. Blood 102:1435–1442
35. Henry F, Boisteau O, Bretaudeau L, Lieubeau B, Meflah K, Gregoire M (1999) Antigen-presenting cells that phagocytose apoptotic tumor-derived cells are potent tumor vaccines. Cancer Res 59:3329–3332
36. Hideshima T, Anderson KC (2002) Molecular mechanisms of novel therapeutic approaches for multiple myeloma. Nat Rev Cancer 2:927–937
37. Hsu FJ, Caspar CB, Czerwinski D, Kwak LW, Liles TM, Syrengelas A, Taidi-Laskowski B, Levy R (1997) Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma–long-term results of a clinical trial. Blood 89:3129–3135
38. Huard B, Schneider P, Mauri D, Tschopp J, French LE (2001) T cell costimulation by the TNF ligand BAFF. J Immunol 167:6225–6231
39. Hulkkonen J, Vilpo J, Vilpo L, Hurme M (1998) Diminished production of interleukin-6 in chronic lymphocytic leukaemia (B-CLL) cells from patients at advanced stages of disease. Tampere CLL Group. Br J Haematol 100:478–483
40. Hus I, Kawiak J, Rolinski J, Tabarkiewics J, Wojas K, Wasik-Szczepanek E, Kosek A, Dmoszynska A (2004) Vaccination of B-cell chronic lymphocytic leucaemia patients with peripheral blood autologous apoptotic leucaemic cells. European Society of Oncology Congress, Wien 2004 Abstract 578PD
41. Inaba K, Turley S, Yamaide F, Iyoda T, Mahnke K, Inaba M, Pack M, Subklewe M, Sauter B, Sheff D, Albert M, Bhardwaj N, Mellman I, Steinman RM (1998) Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. J Exp Med 188:2163–2173
42. Jocham D, Richter A, Hoffmann L, Iwig K, Fahlenkamp D, Zakrzewski G, Schmitt E, Dannenberg T, Lehmacher W, von Wietersheim J, Doehn C (2004) Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. Lancet 363:594–599
43. Jorgensen T, Hannestad K (1980) H–2-linked genes control immune response to V-domains of myeloma protein 315. Nature 288:396–397
44. Jorgensen T, Hannestad K (1982) Helper T cell recognition of the variable domains of a mouse myeloma protein (315). Effect of the major histocompatibility complex and domain conformation. J Exp Med 155:1587–1596
45. Kato K, Cantwell MJ, Sharma S, Kipps TJ (1998) Gene transfer of CD40-ligand induces autologous immune recognition of chronic lymphocytic leukemia B cells. J Clin Invest 101:1133–1141
46. Kimby E, Mellstedt H, Nilsson B, Bjorkholm M, Holm G (1987) T lymphocyte subpopulations in chronic lymphocytic leukemia of B cell type in relation to immunoglobulin isotype(s) on the leukemic clone and to clinical features. Eur J Haematol 38:261–267
47. Kobie JJ, Wu RS, Kurt RA, Lou S, Adelman MK, Whitesell LJ, Ramanathapuram LV, Arteaga CL, Akporiaye ET (2003) Transforming growth factor beta inhibits the antigen-presenting functions and antitumor activity of dendritic cell vaccines. Cancer Res 63:1860–1864
48. Kokhaei P, Choudhury A, Mahdian R, Lundin J, Moshfegh A, Osterborg A, Mellstedt H (2004) Apoptotic tumor cells are superior to tumor cell lysate, and tumor cell RNA in induction of autologous T cell response in B-CLL. Leukemia 18:1810–1815
49. Kokhaei P, Rezvany MR, Virving L, Choudhury A, Rabbani H, Osterborg A, Mellstedt H (2003) Dendritic cells loaded with apoptotic tumour cells induce a stronger T-cell response than dendritic cell-tumour hybrids in B-CLL. Leukemia 17:894–899

50. Kotera Y, Shimizu K, Mule JJ (2001) Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immunization. Cancer Res 61:8105–8109
51. Krackhardt AM, Harig S, Witzens M, Broderick R, Barrett P, Gribben JG (2002) T-cell responses against chronic lymphocytic leukemia cells: implications for immunotherapy. Blood 100:167–173
52. Kremer JP, Reisbach G, Nerl C, Dormer P (1992) B-cell chronic lymphocytic leukaemia cells express and release transforming growth factor-beta. Br J Haematol 80:480–487
53. Lotem M, Shiloni E, Pappo I, Drize O, Hamburger T, Weitzen R, Isacson R, Kaduri L, Merims S, Frankenburg S, Peretz T (2004) Interleukin-2 improves tumour response to DNP-modified autologous vaccine for the treatment of metastatic malignant melanoma. Br J Cancer 90:773–780
54. Luo HY, Rubio M, Biron G, Delespesse G, Sarfati M (1991) Antiproliferative effect of interleukin-4 in B chronic lymphocytic leukemia. J Immunother 10:418–425
55. Mainou-Fowler T, Miller S, Proctor SJ, Dickinson AM (2001) The levels of TNF alpha, IL4 and IL10 production by T-cells in B-cell chronic lymphocytic leukaemia (B-CLL). Leuk Res 25:157–163
56. Matzinger P (1998) An innate sense of danger. Semin Immunol 10:399–415
57. Mellstedt H (2004) In: Gahrton DBG, Samson DM (eds) Immunolocial approaches multiple myeloma and related disorders. Arnold, London, pp 305–316
58. Mellstedt H, Fagerberg J, Frodin JE, Henriksson L, Hjelm-Skoog AL, Liljefors M, Ragnhammar P, Shetye J, Osterborg A (1999) Augmentation of the immune response with granulocyte-macrophage colony-stimulating factor and other hematopoietic growth factors. Curr Opin Hematol 6:169–175
59. Mohty M, Isnardon D, Charbonnier A, Lafage-Pochitaloff M, Merlin M, Sainty D, Olive D, Gaugler B (2002) Generation of potent T(h)1 responses from patients with lymphoid malignancies after differentiation of B lymphocytes into dendritic-like cells. Int Immunol 14:741–750
60. Muller MR, Tsakou G, Grunebach F, Schmidt SM, Brossart P (2004) Induction of chronic lymphocytic leukemia (CLL)-specific CD4- and CD8-mediated T-cell responses using RNA-transfected dendritic cells. Blood 103:1763–1769
61. Novak AJ, Bram RJ, Kay NE, Jelinek DF (2002) Aberrant expression of B-lymphocyte stimulator by B chronic lymphocytic leukemia cells: a mechanism for survival. Blood 100:2973–2979
62. Orsini E, Guarini A, Chiaretti S, Mauro FR, Foa R (2003) The circulating dendritic cell compartment in patients with chronic lymphocytic leukemia is severely defective and unable to stimulate an effective T-cell response. Cancer Res 63:4497–4506
63. Piccirillo CA, Thornton AM (2004) Cornerstone of peripheral tolerance: naturally occurring CD4+CD25+ regulatory T cells. Trends Immunol 25:374–380
64. Podhorecka M, Dmoszynska A, Rolinski J, Wasik E (2002) T type 1/type 2 subsets balance in B-cell chronic lymphocytic leukemia–the three-color flow cytometry analysis. Leuk Res 26:657–660
65. Porakishvili N, Roschupkina T, Kalber T, Jewell AP, Patterson K, Yong K, Lydyard PM (2001) Expansion of CD4+ T cells with a cytotoxic phenotype in patients with B-chronic lymphocytic leukaemia (B-CLL). Clin Exp Immunol 126:29–36
66. Portielje JE, Gratama JW, van Ojik HH, Stoter G, Kruit WH (2003) IL-12: a promising adjuvant for cancer vaccination. Cancer Immunol Immunother 52:133–144
67. Pu QQ, Bezwoda WR (1997) Interleukin-4 prevents spontaneous in-vitro apoptosis in chronic lymphatic leukaemia but sensitizes B-CLL cells to melphalan cytotoxicity. Br J Haematol 98:413–417
68. Rezvany MR, Jeddi-Tehrani M, Biberfeld P, Soderlund J, Mellstedt H, Osterborg A, Rabbani H (2001) Dendritic cells in patients with non-progressive B-chronic lymphocytic leukaemia have a normal functional capability but abnormal cytokine pattern. Br J Haematol 115:263–271
69. Rezvany MR, Jeddi-Tehrani M, Osterborg A, Kimby E, Wigzell H, Mellstedt H (1999) Oligoclonal TCRBV gene usage in B-cell chronic lymphocytic leukemia: major perturbations are preferentially seen within the CD4 T-cell subset. Blood 94:1063–1069
70. Rezvany MR, Jeddi-Tehrani M, Rabbani H, Lewin N, Avila-Carino J, Osterborg A, Wigzell H, Mellstedt H (2000) Autologous T lymphocytes may specifically recognize leukaemic B cells in patients with chronic lymphocytic leukaemia. Br J Haematol 111:608–617
71. Rezvany MR, Jeddi-Tehrani M, Rabbani H, Ruden U, Hammarstrom L, Osterborg A, Wigzell H, Mellstedt H (2000) Autologous T lymphocytes recognize the tumour-derived immunoglobulin VH-CDR3 region in patients with B-cell chronic lymphocytic leukaemia. Br J Haematol 111:230–238
72. Rezvany MR, Jeddi-Tehrani M, Wigzell H, Osterborg A, Mellstedt H (2003) Leukemia-associated monoclonal and oligoclonal TCR-BV use in patients with B-cell chronic lymphocytic leukemia. Blood 101:1063–1070
73. Rossi E, Matutes E, Morilla R, Owusu-Ankomah K, Heffernan AM, Catovsky D (1996) Zeta chain and CD28 are poorly expressed on T lymphocytes from chronic lymphocytic leukemia. Leukemia 10:494–497
74. Rossmann ED, Jeddi-Tehrani M, Osterborg A, Mellstedt H (2003) T-cell signaling and costimulatory molecules in B-chronic lymphocytic leukemia (B-CLL): an increased abnormal expression by advancing stage. Leukemia 17:2252–2254
75. Rossmann ED, Lewin N, Jeddi-Tehrani M, Osterborg A, Mellstedt H (2002) Intracellular T cell cytokines in patients with B cell chronic lymphocytic leukaemia (B-CLL). Eur J Haematol 68:299–306
76. Schmidt SM, Schag K, Muller MR, Weck MM, Appel S, Kanz L, Grunebach F, Brossart P (2003) Survivin is a shared tumor-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. Blood 102:571–576
77. Schnurr M, Scholz C, Rothenfusser S, Galambos P, Dauer M, Robe J, Endres S, Eigler A (2002) Apoptotic pancreatic tumor cells are superior to cell lysates in promoting cross-priming of cytotoxic T cells and activate NK and gammadelta T cells. Cancer Res 62:2347–2352
78. Scrivener S, Goddard RV, Kaminski ER, Prentice AG (2003) Abnormal T-cell function in B-cell chronic lymphocytic leukaemia. Leuk Lymphoma 44:383–389
79. Soderberg O, Christiansen I, Carlsson M, Nilsson K (1997) CD40 ligation inhibits IL-2 and SAC+IL-2 induced proliferation in chronic lymphocytic leukaemia cells. Scand J Immunol 45:706–714
80. Trentin L, Zambello R, Agostini C, Enthammer C, Cerutti A, Adami F, Zamboni S, Semenzato G (1994) Expression and regulation of tumor necrosis factor, interleukin-2, and hematopoietic growth factor receptors in B-cell chronic lymphocytic leukemia. Blood 84:4249–4256
81. Trojan A, Schultze JL, Witzens M, Vonderheide RH, Ladetto M, Donovan JW, Gribben JG (2000) Immunoglobulin framework-derived peptides function as cytotoxic T-cell epitopes commonly expressed in B-cell malignancies. Nat Med 6:667–672
82. Ullenhag GJ, Frodin JE, Jeddi-Tehrani M, Strigard K, Eriksson E, Samanci A, Choudhury A, Nilsson B, Rossmann ED, Mosolits S, Mellstedt H (2004) Durable carcinoembryonic antigen (CEA)-specific humoral and cellular immune responses in colorectal carcinoma patients vaccinated with recombinant CEA and granulocyte/macrophage colony-stimulating factor. Clin Cancer Res 10:3273–3281
83. Wallgren A, Festin R, Gidlof C, Dohlsten M, Kalland T, Totterman TH (1993) Efficient killing of chronic B-lymphocytic leukemia cells by superantigen-directed T cells. Blood 82:1230–1238
84. van Kooten C, Banchereau J (1997) Functions of CD40 on B cells, dendritic cells and other cells. Curr Opin Immunol 9:330–337

85. van Kooten C, Rensink I, Aarden L, van Oers R (1992) Interleukin-4 inhibits both paracrine and autocrine tumor necrosis factor-alpha-induced proliferation of B chronic lymphocytic leukemia cells. Blood 80:1299–1306
86. Wang H, Marsters SA, Baker T, Chan B, Lee WP, Fu L, Tumas D, Yan M, Dixit VM, Ashkenazi A, Grewal IS (2001) TACI-ligand interactions are required for T cell activation and collagen-induced arthritis in mice. Nat Immunol 2:632–637
87. Wen T, Mellstedt H, Jondal M (1990) Presence of clonal T cell populations in chronic B lymphocytic leukemia and smoldering myeloma. J Exp Med 171:659–666
88. Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, Scheper RJ, Meijer CJ, Bloemena E, Ransom JH, Hanna MG Jr, Pinedo HM (1999) Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet 353:345–350
89. Wierda WG, Cantwell MJ, Woods SJ, Rassenti LZ, Prussak CE, Kipps TJ (2000) CD40-ligand (CD154) gene therapy for chronic lymphocytic leukemia. Blood 96:2917–2924
90. Wilson A, George AJ, King CA, Stevenson FK (1990) Recognition of a B cell lymphoma by anti-idiotypic T cells. J Immunol 145:3937–3943
91. von Bulow GU, Bram RJ (1997) NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. Science 278:138–141
92. Vonderheide RH, Hahn WC, Schultze JL, Nadler LM (1999) The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity 10:673–679
93. Vuillier F, Maloum K, Thomas EK, Magnac C, Dumas G, Payelle-Brogard B, Oppezzo P, Dighiero G, Scott-Algara D (2003) Idiotype-pulsed dendritic cells are able to induce antitumoral cytotoxic CD8 cells in chronic lymphocytic leukaemia. Br J Haematol 120:243–250
94. Zaknoen SL, Kay NE (1990) Immunoregulatory cell dysfunction in chronic B-cell leukemias. Blood Rev 4:165–174