Cancer Immunol Immunother (1996) 42: 193–199

ORIGINAL ARTICLE

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Cellular immune responses to autologous chronic myelogenous leukaemia cells in vitro

Received: 9 November 1995 / Accepted: 8 February 1996

Abstract Using a modification of the autologous mixed lymphocyte/tumour cell culture (MLTC), it is demonstrated here that lymphocytes from chronic-phase myelogenous leukaemia (CML) patients (n = 58), but not from their HLA-identical siblings, proliferated upon coculture with autologous tumour cells. However, in most cases, the level of proliferation measured was low (stimulation index <3, n = 37). This was most likely related to the amount of interleukin-10 (IL-10) released into the culture medium by the CML cells, because addition of neutralizing anti-IL-10 serum to MLTC markedly enhanced proliferative responses. In addition, supplementation of media with IL-1α further enhanced proliferative responses and a combination of anti-IL-10 serum and IL-1α was more effective than either agent alone. Only HLA-DR-matched CML cells, but not HLA-DR-mismatched CML cells or matched or mismatched PBMC restimulated proliferation of IL-2-dependent T cell lines derived from MLTC supplemented with IL-1α and anti-IL-10 serum. The responding cells under these conditions were predominantly $CD4^+$ and secreted IL-2, and interferon γ; some secreted IL-4, but none secreted IL-10. These data therefore suggest the existence of an HLA-DR-restricted DTH/Th1-type of tumour-specific immunity in CML patients, which may be down-regulated in vitro by excessive secretion of IL-10 together with depressed secretion of IL-1.

Key words Cellular immune response · Myelogenous leukemia

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Introduction

Chronic-phase myelogenous leukaemia (CML) patients commonly remain in a relatively indolent state for many years, but experience eventual progression to blast crisis characterized by the emergence of a markedly different acute-type of leukaemia. It is impossible to predict when the accelerated phase will occur; palliative chemotherapy or treatment with interferon α (IFNα) appears not to delay crisis markedly for most patients [19]. One possible mechanism among others that might contribute to the occurrence of blast crisis is the overwhelming or the suppression of immune responses against the tumour (escape from immunosurveillance). The possibility that natural immunity may be important in this context has received some attention in the past, with circumstantial evidence suggesting a relationship between natural killing (NK) and leukaemic progression, particularly under the special circumstances of relapse after allogeneic bone marrow transplantation (BMT), reviewed in [8, 9]. However, the ability of the adaptive immune system to recognize and control autologous CML tumour cells has received scant attention.

Somasundaram et al. reported that T cell clones that had the ability to lyse autologous tumour cells but not NK targets could be isolated from CML patients, but they also lysed remission bone marrow cells [16, 17]. We have presented similar data for cytotoxic effectors in CML [12]. Falkenberg et al. have demonstrated that human minor-histocompatibility-antigen-specific T cell clones may lyse CML cells in an MHC-restricted fashion, but did not investigate lysis of bone marrow cells [3, 21]. Some effort has been expended in attempting to ascertain whether peptides representing the *bcr/abl* fusion junction can be recognized by human T cells, and whether such sensitized T cells can then recognize native tumour. Barret et al. reported that the frequency of T cells stimulated to proliferate by 18-mer peptides representing b2a2 or b3a2 was higher in untreated CML patients than in post-BMT patients, suggesting an ongoing immune response to tumour-specific antigen in untreated patients [1]. We have suc-

ceeded in sensitizing normal human T cells to a 23-mer b3a2 peptide, but the frequency of responders is very low, being comparable to the frequency of autoreactive cells [13].

We have previously employed a simple modification of the mixed lymphocyte/tumour cell culture (MLTC) to investigate the question of antitumour autoimmunity in CML patients at presentation and during a period of up to 1 year's treatment with IFNα [13]. By treating CML patients' peripheral blood mononuclear cells (PBMC) in vitro with the cytostatic agent cytarabine, their proliferative response against autologous tumour cells can be visualized after a 6-day coculture. In this assay, the spontaneous proliferation of the CML cells is reduced by the cytarabine treatment, which targets the tumour cells but spares the responses of normal T cells in the population, for example, to alloantigens [10]. Evidence for presensitization of patients' cells to their own tumour was adduced from the observation that HLA-identical siblings failed to respond to the CML cells in this way [12]. We now report the results of a larger series of patients in which the weak responses observed with the majority of untreated patients in MLTC are ascribed not to a lack of potential reactivity of the patients towards their own tumour cells, but to immune suppression of the responses associated with production of large amounts of IL-10 and reduced amounts of IL-1α in vitro.

Materials and methods

CML patients' cells

PBMC were isolated from untreated Ph^+ chronic-phase CML patients (mean leucocytes 130 000). Myeloid cells predominated in these preparations [70 ± 21% CD14, 85 ± 26% CD33, 27 ± 8% CD34 with only 11 ± 6% T cells (CD3) and 6 ± 4% B cells (CD20)]. Surface-marker phenotyping was carried out in standard fashion. Cells pre-incubated with Intraglobin were labelled with mAb. For HLA-DR staining, mAb L243 was obtained from the ATCC. The CD3 mAb OKT3 was also obtained from the ATCC. The class I mAb W6/32 was used as a positive control (W6/32.HL) and its non-binding variant as a negative control (W6/32.HK). CD18 mAb (clone 7E4) was purchased from Immunotech (Hamburg, Germany), CD54 (clone HA58) and CD58 (AICD58) mAb were purchased from Dianova (Hamburg, Germany) and CD80 (B7-24) was a kind gift of Dr. M. de Boer, Innogenetics, Belgium. CD14 (TÜK4), CD20 (B-ly1), CD33 (WM-54) and CD34 (TÜK3) were from Dako. Labelled cells were developed with fluorescein-isothiocyanate-conjugated $F(ab)_2$ rabbit anti-(mouse IgG) and analysed on a FACS IV cytometer. Dead cells were excluded by electronic gating and fluorescence histograms were area-corrected to 10 000 cells. Data are expressed as mean fluorescence channel intensity, corrected for background fluorescence with W6/32.HK. Software programs were developed by Fa. Schaudt, Gomaringen, Germany.

Cytokines and sera

Commercial enzyme-linked immunoassay (ELISA) kits were used for assessing concentrations of cytokines in culture media. Cells were cultured at 2.5×10^5/ml in RPMI-1640 medium supplemented with 10% fetal calf serum. Cell-free supernatants of CML-PBMC cultures, normal PBMC cultures or restimulated MLTC-derived cells were assayed according to the manufacturer's instructions using a Dianova, Hamburg (Immunotech) assay for IL-2 (sensitivity 10 pg/ml), an Endogen (Boston) assay for IL-4 and IFNγ (sensitivity <5 pg/ml for both), and an ICChemikalien, Munich (Perseptive) assay for IL-10 (sensitivity 10 pg/ml). Cytokines were purchased from ICChemikalien, Munich: recombinant human IL-1α, 98% purity, specific activity 10^7 U/mg; recombinant human IL-10, 98% purity, specific activity 10^6 U/mg. Neutralising antisera to IL-10 were obtained from the same source (catalogue ICC-010-3A) with an activity such that a concentration of 5 μg/ml neutralised the activity of 2 ng/ml IL-10 in a mitogenesis assay. Purified natural IL-2 (Lymphocult T-HP) was a gift of the Biotest Company, Frankfurt.

Autologous mixed lymphocyte/tumour cell cultures (MLTC)

CML patients' PBMC were irradiated and used to stimulate autologous PBMC pretreated with cytarabine, as described [12]. After cytarabine treatment, the percentage of $CD33^+$ cells in the PBMC decreased to 10 ± 14%, whereas the number of $CD3^+$ cells increased to 52 ± 25%. Cultures were performed in microtitre plates for assessment of proliferation by [^{3}H]thymidine ([^{3}H]dT) incorporation, and data analysed using in-house programs [15]. For more extended culture, 16-mm-diameter cluster plates were employed using 5×10^5 responders and 5×10^5 stimulators in medium supplemented with 10 U/ml IL-1α and 10 μg/ml anti-IL-10 sera. After 1 week in culture, they were restimulated with 1×10^6 irradiated pooled PBMC from about 20 normal donors, 1% phytohaemagglutinin and supplemented with 40 U/ml IL-2. After a further 2 weeks culture, cells were harvested, washed, counted and plated at 2×10^4/well together with 1×10^5 irradiated HLA-DR-matched and mismatched CML cells and normal PBMC, as well as autologous CML cells. Proliferation was assessed after 1, 2 and 3 days by incorporation of tritiated thymidine. Culture medium for all procedures was RPMI-1640 supplemented with 10% heat-inactived non-transfused male human serum.

Results

Autoreactivity of patients' cytarabine-treated PBMC to their own tumour cells

As part of the routine HLA typing procedure for the selection of related donors in the local BMT program, MLC typing was performed on patients and their families. In each case, patients' PBMC were treated with cytarabine for use as responders, or left untreated (irradiation only) for use as stimulators. Parents and all available siblings, as well as those established to be HLA-class I and DR-matched, were included as both responders and stimulators, as previously reported [10]. Consistent with previous data on 12 patients [12], responses of the cytarabine-treated patients' PBMC (n = 58) against their own untreated PBMC were higher than the responses of their HLA-identical siblings to tumour-cell-containing PBMC. Most of the 58 patients were tested a single time, but in the minority (n = 14) where the MLTC was repeated, similar results were obtained. When all data were summed it was found that the patients could be divided into two groups: those where the stimulation index for the response to autologous untreated PBMC was less than three (low responders, n = 37 of 58, 64%), and those where it was greater than three (high responders, n = 21 of 58, 36%), as shown in Fig. 1. For both groups, the stimulation index for the response of HLA-identical siblings to patients' untreated PBMC was less than

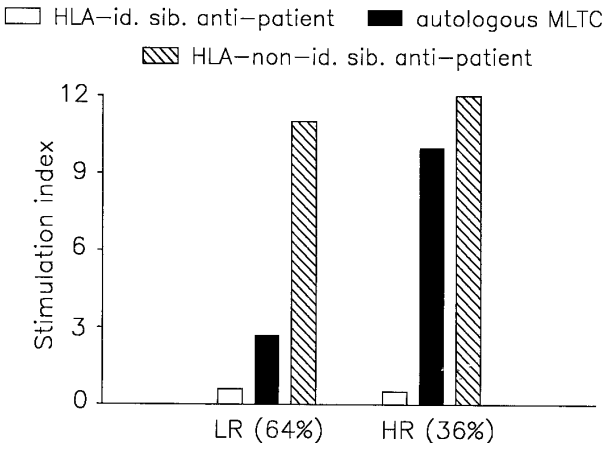

Fig. 1 Family mixed lymphocyte culture results of 58 chronic-phase chronic myelogenous leukaemia (*CML*) patients. Results are summarised as stimulation indices for proliferation in cocultures of the following combinations: HLA-identical sibling cells plus patient cells (*HLA-id. sib. anti-patient*), cytarabine-treated patient peripheral blood mononuclear cells (PBMC) and irradiated untreated autologous PBMC (autologous mixed lymphocyte/tumour cell culture, *MLTC*) and HLA-different sibling cells plus patient cells (*HLA-non-id. sib. anti-patient*). *LR* low responder, *HR* high responder

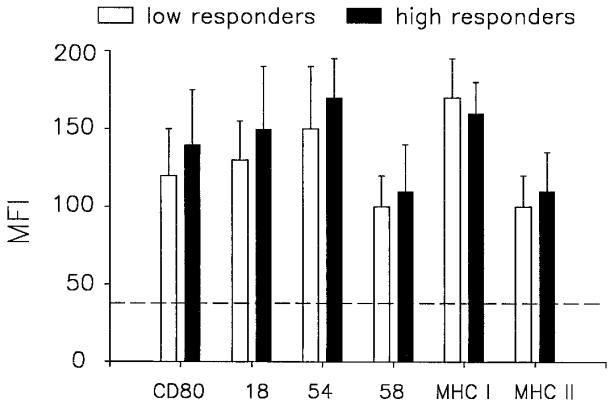

Fig. 2 Surface phenotype of CML-PBMC. CML patients were divided into groups responding weakly in autologous MLTC (stimulation index SI <3, $n = 6$) or more strongly (SI >3, $n = 8$), shown as low responders and high responders respectively. Fluorescence staining results are shown as uncorrected mean fluorescence channel intensities (*MFI*) $\pm$ SEM. --- The background staining level with a non-binding control mAb W6/32.HK

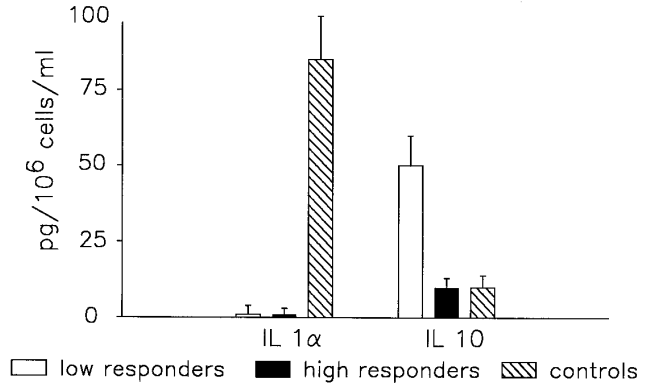

Fig. 3 Secretion of interleukin-1α (IL-1α) and IL-10 by CML-PBMC. Patients' and controls' PBMC were cultured at 2.5×10^5/ml at a density of 5×10^5/16-mm-diameter culture well for 48 h in medium with 10% fetal calf serum (low responders, $n = 5$; high responders, $n = 6$; controls, $n = 11$). Cell-free supernatants were assayed by enzyme-linked immunosorbent assay for content of IL-10 and IL-1α and results were expressed as pg produced $(10^6 \text{ cells})^{-1}$ ml^{-1} in 48 h

1, whereas the response of one-haplotype-different siblings and parents to patients' PBMC was commonly above 10 (Fig. 1). Thus, at least two-thirds of this larger group of patients examined manifested only equivocal responses in MLTC against their own tumour cells. The low-responder group did not differ obviously from the high-responder group in terms of time elapsed since diagnosis or peripheral tumour load. Because the responding cells after cytarabine treatment were greatly depleted of tumour cells it is unlikely that the reason for poor responses was a dilution effect and that low responsiveness was directly related to numbers of leukaemia cells. Similarly, the different proportions of $CD3^+$ cells in the responder populations did not correlate with levels of responsiveness, because the samples from many non-responders contained higher numbers of T cells than did responders and because they retained a capacity to respond to allogeneic PBMC stimulators (data not shown). The reasons for low responsiveness were therefore sought elsewhere.

Expression of MHC class II and costimulatory molecules by CML cells

Of the many possible reasons for weak responses observed with 37 of the 58 (64%) patients tested, it seems unlikely that the lack of or low expression of stimulatory class II molecules and/or costimulatory and accessory/adhesion molecules is a major contributor. A side-by side comparison of weak and strong stimulators illustrates this, at least for HLA-DR, CD18, CD54, CD58 or CD80, as shown in Fig. 2. This shows the median fluorescence intensity reflecting the density of expression of these molecules at the cell surface, and is clearly very similar for both groups of patients. In addition, there was no noticeable difference in the percentage of cells in either population staining for any of the markers (data not shown).

Cytokine production by PBMC from CML patients

Another possible reason for low responsiveness in MLTC may be either the production of immunosuppressive substances by the tumour cells or the failure to produce sufficient immunostimulatory substances to achieve adequate costimulation. To investigate some aspects of these possibilities, untreated PBMC from CML patients were cultured in vitro for 48 h and cell-free supernatants ana-

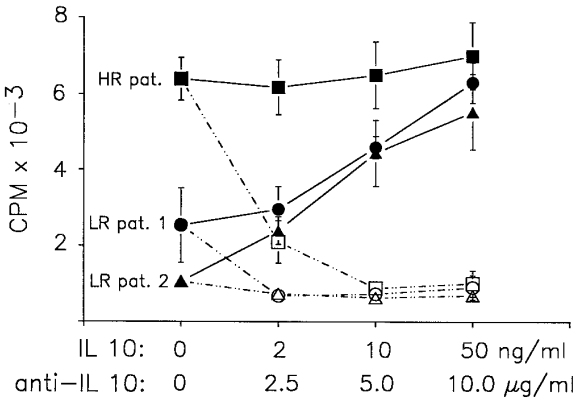

Fig. 4 Influence of IL-10 on proliferation in CML-MLTC. Stimulator cells from high- (*HR*) and low-responder (*LR*) patients were 30-Gy-irradiated PBMC; responder cells were CML-PBMC pretreated for 1 h with 100 μg/ml cytarabine. MLTC were set up in microtitre plates with titrated amounts of IL-10 or anti-IL-10 sera as shown. –··– Addition of IL-10; —— addition of anti-IL-10 sera. Data are given as mean radioactivities (cpm) of triplicate cultures ± SEM after 6 days of culture

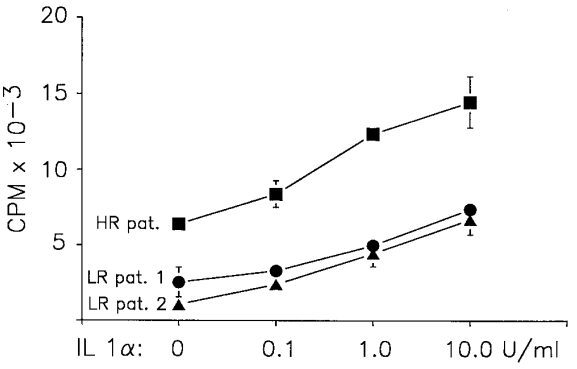

Fig. 5 Influence of IL-1α on proliferation in CML-MLTC. Stimulator cells from HR and LR patients were 30-Gy-irradiated PBMC; responder cells were CML-PBMC pretreated for 1 h with 100 μg/ml cytarabine. MLTC were set up in microtitre plates with titrated amounts of IL-1α as shown. Data are given as mean radioactivities (cpm) of triplicate cultures ± SEM after 6 days of culture

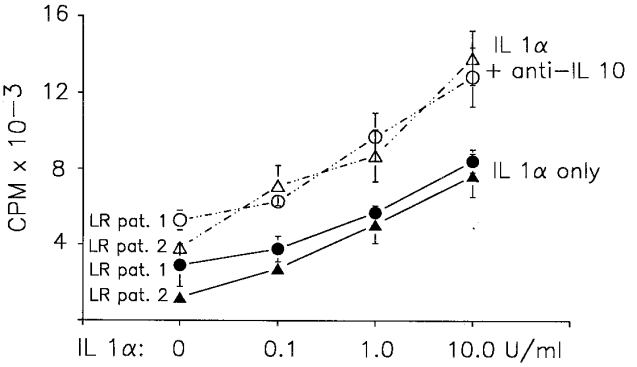

Fig. 6 Influence of IL-1α and IL-10 on proliferation in CML-MLTC. Stimulator cells from HR and LR patients were 30-Gy-irradiated PBMC; responder cells were CML-PBMC pretreated for 1 h with 100 μg/ml cytarabine. MLTC were set up in microtitre plates with titrated amounts of IL-1α as shown (IL-1α alone: ● —— ●, ▲ —— ▲ or with titrated amounts of IL-1α and a fixed amount (10 μg/ml) of anti-IL-10 sera –··○··–, –··△··–. Data are given as mean radioactivities (cpm) triplicate cultures ± SEM after 6 days of culture

lysed for production of a number of cytokines by ELISA. Figure 3 shows the spontaneous production of IL-10 and IL-1α (Fig. 3). PBMC from CML patients in the high-responder group secreted little IL-10 under these culture conditions, whereas cells from patients in the low-responder group secreted greater amounts of this potentially immunosuppressive cytokine. There were no kinetic differences between CML groups and controls, because the same patterns were also observed after 24 h or after 72 h (data not shown). IL-1α was completely lacking in supernatants of CML patients' PBMC, regardless of whether they belonged to the high- or low-responder group, whereas normal controls' PBMC secreted modest amounts of IL-1α and very little IL-10. The same patterns were observed when the culture medium was supplemented with 20 U/ml IL-2, 100 U/ml IFNα or a combination of both (data not shown).

The presence of tumour necrosis factor α (TNFα), granulocyte/macrophage-colony-stimulating factor (GM-CSF), and IFNγ was also assessed in the same supernatants, but no clear differences could be found between the high- and low-responder patient groups for these cytokines (data not shown).

Enhancement of MLTC with IL-1α and neutralising IL-10 antisera

To test whether the low level of IL-1α secretion in both high- and low-responder groups, and whether the high IL-10 production of the low-responder group was functionally relevant, IL-1α supplementation and IL-10 neutralization experiments were performed. Addition of titrated amounts of anti-IL-10 sera enhanced MLTC proliferation of two low-responder patients strikingly in a dose-dependent manner (low-responder patients 1 and 2 in Fig. 4), resulting in an increase in thymidine incorporation by up to a factor of five. In contrast, proliferative responses of a high-responder patient were enhanced little if at all (Fig. 4). The addition of anti-IL-10 serum did not affect the proliferation of cells from either high or low responders against allogeneic PBMC in regular MLC (data not shown). Reciprocally, addition of exogenous IL-10 to cultures resulted in markedly reduced responses of both high and low responders, resulting in essentially complete inhibition of MLTC (Fig. 4) and standard mixed lymphocyte culture (data not shown). Addition of IL-1α, on the other hand, enhanced the MLTC of both the same high- and low-responder patients approximately equally (Fig. 5) and also enhanced standard mixed lymphocyte culture (data not shown). That IL-1α and anti-IL-10 have different mechanisms of action was suggested by the finding of additive effects of the two on MLTC of low-responder patients (Fig. 6). Similar results have been obtained in 2 of 2 other high responders tested and in 2 of 3 low responders (data not shown).

Table 1 Specificity of proliferative responses. The irradiated peripheral blood mononuclear cells (*PBMC*) were from the same chronic myelogenous leukaemia (CML) patient as the responding cells (autologous), or from HLA-DR matched or mismatched donors, or were normal PBMC from DR-matched and mismatched donors. The responding cells were from mixed lymphocyte/tumour cells cultured for 2 weeks with interleukin-2 (4 of 8 patients giving similar results are shown). Results are mean radioactivities (cpm) ± SEM of triplicate cultures

Responders (patient no.)	Stimulators				
	CML PBMC			Normal PBMC	
	Autologous	DR-matched	DR-mismatched	DR-matched	DR-mismatched
1	19836 ± 2122	20672 ± 2961	1782 ± 623	256 ± 34	195 ± 50
2	13279 ± 1566	11285 ± 931	902 ± 319	136 ± 21	391 ± 18
3	18934 ± 4511	25629 ± 3006	3400 ± 388	2194 ± 230	726 ± 127
4	15560 ± 1425	16348 ± 562	1284 ± 309	371 ± 33	276 ± 62

Table 2 Cytokine secretion by T cells derived from mixed lymphocyte/tumour cell cultures (MTLC). Cytokines were detected in cell-free supernatants, collected 48 h after phytohaemagglutinin stimulation and measured in enzyme-linked immunosorbent assay; data given in pg ml^{-1} $(2.5\times10^5$ cells$)^{-1}$ $(48\ h)^{-1}$. Cell lines 1–8 derived from MLTC performed in the presence of 10 μg/ml anti-IL-10 sera and 10 U/ml IL-1α, and supplemented with IL-2 after 1 week; total of 3 weeks old. *C* cells derived from mixed lymphocyte culture and cultured with IL-2 for 1 month thereafter. *IL* interleukin, *IFN* interferon

	Secreted cytokine			
Line	IL-2	IL-4	IFNγ	IL-10
1	280	33	860	12
2	170	190	910	4
3	140	30	898	3
4	170	267	788	0
5	430	13	824	2
6	190	17	812	0
7	540	17	488	9
8	370	163	666	6
C	250	144	745	138

Properties of patients' T cells responding to autologous CML cells

T cell lines were developed from MLTC from 8 different patients by short-term cultivation in medium containing IL-1α and anti-IL-10 at concentrations chosen as a result of the experiments shown in Figs. 4–6 (10 U/ml IL-1α and 10 μg/ml anti-IL-10 sera), followed by subculturing on pooled allogeneic PBMC with non-specific stimulation by PHA and IL-2 supplementation. The majority of the cells in these populations 3 weeks after initiation of culture were $CD4^+$ T cells carrying α/β T cell receptors and capable of autocrine proliferation when restimulated in the absence of IL-2. The specificity of the proliferative responses of these lines for CML cells was examined by stimulating them with autologous untreated PBMC and HLA-DR-matched or mismatched PBMC either from normal donors or from CML patients. Proliferative responses were recorded only when autologous CML cells or HLA-DR-matched CML cells were used as stimulators, but not when normal PBMC were used as stimulators, whether or not they were HLA-DR-matched (Table 1).

The pattern of cytokine secretion by these sensitised cells was assessed by ELISA 48 h after mitogenic stimulation. As shown in Table 2, all the lines secreted IL-2 and IFNγ, some secreted low levels of IL-4, but none secreted detectable IL-10. In contrast, a non-clonal alloreactive T cell line derived from a normal donor secreted IL-2, IL-4, IFNγ and IL-10. Therefore, T cells originally reacting against autologous CML cells are unlikely to contribute to the increased IL-10 levels suppressing antitumour immunity in these patients. Rather, they seem to represent a population of DTH-like or Th1-like cells, which may be involved in immunosurveillance of the tumour and which might be supressed by IL-10.

Discussion

The present report documents in vitro evidence for T-cell-mediated antitumour immunity in CML patients as demonstrated by the proliferative responses of patients' tumour-depleted PBMC on challenge with irradiated tumour-containing PBMC. These data on 58 patients are consistent with previous preliminary data concerning 12 patients [12] and go further to divide such patients into two groups, based on their level of antitumour autoimmunity. The majority of patients (64%) responded only weakly to their autologous tumour cells, with a stimulation index of less than 3 (generally taken as the cut-off point below which responses are considered negative in the majority of published studies). However, among the remaining patients some responded very strongly, as strongly as against an alloantigen, demonstrating the potential for CML patients to mount vigorous responses when their cells are co-cultured with their own tumour cells. Because the majority of patients demonstrated only weak responses to their tumour cells, some so weak as to be equivocal, it was deemed important to pursue the possible reasons for low antitumour reactivity.

In the absence of expression of defined tumour antigen(s) on the CML cells, it was not possible to investigate whether certain tumour cells failed to stimulate simply because they did not express tumour antigen. The CML cells clearly expressed both MHC class I and class II antigens, as noted by others previously [20], and thus should have been able to present putative tumour antigen to CD4 and CD8 cells. Therefore, the first possibility

considered was that the CML cells may have lacked second-signal molecules for T cell activation, particularly costimulatory molecules CD80 and CD86 and adhesion/accessory molecules known to be important for T cell activation. The results obtained in this study did not provide evidence for the lack of accessory or costimulatory molecules on CML cells, which could be responsible for the lack of stimulation. Thus, CML cells from both high- and low-responder groups expressed similar densities of CD18, CD54, CD58 and CD80 (B7). The expression of CD86 on CML cells has not yet been evaluated but, in another tumour immunology model, CD80 and CD86 both appeared equally effective as costimuli [4]. Both in terms of the percentage of cells staining with the various antibodies and in terms of the density of expression of the relevant molecules at the cell surface, no difference between low- and high-responder patients could be determined (Fig. 2). In addition, earlier functional studies have shown that CML PBMC were able to restimulate T cell lines and clones previously sensitized to alloantigens [11], further making it unlikely that the CML cells suffered a general inability to stimulate adequately. At the level of the responder cells, a general inability of the cells to respond proliferatively to stimuli was excluded by their reactivity to control stimulators (data not shown).

The next step was to explore whether the tumour cells might be secreting substances capable of inhibiting T cell activation, and whether neutralization of any such factors would convert low responders to high responders. It was observed that the accumulated levels of IL-10 but not IL-1α, TNFα, GM-CSF, and IFNγ in supernatants of untreated CML PBMC (mostly tumour cells) were higher for MLTC non-responders than for responders. IL-10 was considered a good candidate for suppression of MLTC in low responders because it is known to block lymphocyte stimulation caused by alloantigens, antigens and superantigens by direct and indirect pathways [5, 18] and can inhibit antitumour reactivity [6]. Moreover, addition of neutralizing anti-IL-10 sera to MLTC enhanced proliferation of low-responder cells but not of high-responder cells (Fig. 4), confirming that autocrine suppression in MLTC was at least in part mediated by IL-10.

Both low-responder and high-responder patients essentially failed to secrete IL-1α and, accordingly, supplementation of MLTC with IL-1α enhanced proliferative responses against autologous CML cells in both the high- and low-responder groups (Fig. 5). Moreover, for the latter, a combination of both IL-1α and anti-IL-10 sera had an additive effect (Fig. 6). It may therefore be concluded that a prime cause of low responses to autologous CML cells in vitro in one group of patients is their hyperproduction of IL-10, whereas both high- and low-responder groups appear to share the apparent defect of production of low levels of IL-1α. Whether these observations are of relevance in vivo is difficult to say. We are not aware of any trials in which CML patients have been treated with IL-1α or neutralising antibodies to IL-10. The questionable benefits of treating patients in vivo with IFNα are probably not reflected in decreased IL-10 production or enhanced IL-1α production in this system, because supplementation of CML cultures with IFNα in vitro did not affect these parameters (data not shown).

The responding cells obtained shortly after MLTC of both low and high responders were shown to be predominantly $CD4^+$ T cells with classical TCR2 antigen receptors, rather than $CD8^+$ T cells, TCR1-T cells or natural killer cells, all of which might have been able to proliferate under the culture conditions employed. They are thus likely to recognize antigen in an HLA-class-II-dependent fashion, and are more likely to represent helper cells than cytotoxic cells. That some of these expectations were met by the cell lines obtained was demonstrated by examining their patterns of cytokine secretion and their proliferative specificity. Thus, the lines recognized autologous or HLA-DR-matched CML cells, but not DR-mismatched CML and neither DR-matched nor mismatched normal PBMC. These data show that the MLTC-derived cells are restricted by HLA-DR, one of three class II isotypes that is commonly observed to be immunodominant over the other two, HLA-DQ and HLA-DP, in most systems. The data further show that the lines were not autoreactive, because normal PBMC were not recognized. However, they do not completely exclude the possibility that the responding cells recognized a normal myeloid antigen, not necessarily tumour-specific, because the relevant normal myeloid lineage cells may have been missing, or present in too low numbers, in PBMC. However, even substituting normal bone marrow for PBMC controls would still not be completely satisfactory to resolve this question, because it would not be possible to be certain that the tumour cell differentiation stage was really present in sufficient numbers even in bone marrow. Therefore, we can only conclude that the responding cells are likely to be tumour-specific, but that this is not formally proven. A class-II-restricted response in CML may be particularly important because normal $CD34^+$ progenitor cells in CML patients express little or no HLA-DR, whereas the neoplastic cells are strongly DR^+ [22].

The $CD4^+$ responding cells from MLTC secreted large amounts of IL-2 and IFNγ, and 3 of the 8 tested also secreted lesser amounts of IL-4. Therefore, the main phenotype was that of a DTH/Th1 cell, but as the lines were polyclonal it is not clear whether the IL-4 is derived from some Th2 cells present within 3 of the lines. Significantly, however, none of the cells produced large amounts of IL-10, and even those that did secrete some were very close to the limit of detection of the ELISA. It has been reported that, unlike in the mouse, IL-10 is produced by both Th1 and Th2 clones in humans [2]; the failure of MLTC-derived cell lines in this study to produce IL-10 may, therefore, be related to the presence of anti-IL-10 neutralizing sera during primary culture, and possibly also to supplementation with IL-1α. It is well-known that the presence of cytokines during the initiation of priming (and therefore also that anti-cytokines have the opposite influence) can “divert” immune responses towards one or another cytokine secretion pattern in vitro, although whether this also occurs in vivo is controversial [14]. Anti-IL-10 in this context might therefore be expected to

further Th1 development, because IL-10 was originally described as a factor produced by Th2 cells to down-regulate Th1 cells in the mouse [7].

The results presented here suggest that $CD4^+$ $TCR2^+$ T cells present in the peripheral blood of chronic-phase CML patients possess the potential to recognize autologous tumour cells in an HLA-DR-restricted fashion and to develop into DTH/Th1-like cells secreting IL-2 and IFNγ, but little IL-4 and no IL-10. However, in the majority of patients, increased secretion of IL-10 from other cells, possibly coupled with decreased availability of IL-1α, contributes to a blockade of antitumour responses. If these inhibitory factors could be manipulated in vivo, possible benefits to CML patients might accrue. At the very least, this auto-MLTC protocol may allow the generation of CML-specific T cell lines for use in adoptive immunotherapy.

Acknowledgements We thank Prof. C. A. Müller for critical comments and Dr. M. de Boer, Innogenetics, Belgium, for the CD80 mAb B7-24. This work was supported by the Mildred Scheel Foundation (Deutsche Krebshilfe) grants numbers W2/91/Pa1 and W14/94/Pa2 and by the Deutsche Forschungsgemeinschaft (SFB 120).

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