PRESENCE OF CLONAL T CELL POPULATIONS
IN CHRONIC B LYMPHOCYTIC LEUKEMIA AND
SMOLDERING MYELOMA

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Chronic B lymphocytic leukemia (B-CLL)[1] and multiple myeloma (MM) represent malignancies at different stages of B cell differentiation. In B-CLL the majority of the cells have characteristics of immature B lymphocytes, whereas in MM the main part of tumor clone consists of end stage plasma cells (1–3). In both conditions, the cells of the malignant clones express surface Ig with unique antigen binding parts (idiotypes) (3–6).

Both B-CLL and MM patients can be divided into different subgroups. In B-CLL a clinical staging system is used that vary from stage 0 to 4 (7, 8) and other factors such as the isotype of the surface Ig, the expression of growth factor receptors, and number of S-phase leukemic cells are clinically important (9–11). In MM, the term smoldering myeloma is used for a special type of benign MM (12, 13).

In B-CLL there is evidence for abnormalities within the T and NK cell populations, (10, 14–17). These changes may be part of a cellular response against the tumor cells. In this paper we address this question by analyzing the occurrence of clonal T cell populations in B-CLL and MM by probing for gene rearrangements representative of the β chain of the T cell receptor loci (TCR). The presence of such clonal T cells may indicate a host response directed against leukemia-related antigens or a special T-B cell interaction. We have found evidence for predominant T cell clones in 3/13 B-CLL patients and 1/8 MM patients, all in comparatively clinical benign stages.

Materials and Methods

Diagnostic Criteria

Multiple Myeloma. The diagnosis of multiple myeloma was established if two or three of the following criteria were met: (a) >10% plasma cells in aspirate of bone marrow specimens; (b) a myeloma globulin peak on serum and/or urine electrophoresis in association with decreased concentration of at least one nonmonoclonal serum Ig; and/or (c) osteolytic and/or osteoporotic bone lesions demonstrated by x-ray examination of the skull, vertebral column,

[1] *Abbreviations used in this paper:* B-CLL, chronic B lymphocytic leukemia; MM, multiple myeloma.

and pelvic bones (18). The clinical staging system for myeloma proposed by Durie and Salmon was adopted (12).

Smoldering Multiple Myeloma.   One patient (No. 14) fulfilled the criteria for smoldering myeloma (13). At test this patient had been observed at the department for 4 yr. His condition had remained stable. However, 6 mo after test his condition deteriorated and treatment was instituted.

Chronic Lymphocytic Leukemia of B Cell Type (B-CLL).   The main criteria for B-CLL was infiltration in bone marrow of small lymphocytes and >10 × 10^9/liter of lymphocytes in peripheral blood showing restriction for κ or λ light chains (19). CLL patients were clinically staged according to the staging system of Rai et al. (7). In this study a modification of the criteria for stage 0 was used to define a truly clinically benign condition: no general symptoms (fever, night sweat, weight loss), no enlarged lymph nodes, no hepatosplenomegaly, no anemia (Hb >120 g/liter), no thrombocytopenia (platelet counts >150 × 10^9/liter), and no increase in lactodehydrogenase (LD <8.0 ukat/liter). Furthermore, during the observation period no significant increase in the blood lymphocyte count (doubling of the lymphocyte count) should have occurred. Moreover, no other symptoms or signs related to CLL should be present during the follow up (20). The observation time for stage 0 B-CLL patients ranged from 47 to 192 mo.

Isolation of Lymphocytes

Mononuclear cells were isolated on Hypaque density medium. Adherent cells were removed by passage through nylon wool. T cells were isolated by panning of $CD3^+$ cells using OKT-3 antibody. All T cell populations were >95% pure.

Probe

The cDNA probe for detecting the T cell clonality is a generous gift from Dr. Tak W. Mak (the Ontario Cancer Institute, Toronto, Ontario, Canada). It is a 770-bp fragment that contains the J and C region of the human TCR β chain gene (20), and was labeled using the commercial nick translation kit and [^{32}P]dCTP (Amersham International Ltd., Amersham, UK).

Southern Blot Analysis

DNA was extracted with phenol and chloroform, and electrophoresed through 0.7% agarose gel after complete digestion with Eco RI and Bam HI (in some cases also with Hind III). Southern transfer and hybridizations were performed according to a standard protocol (21). After hybridization, the filters were washed twice in 2 × SSC, 0.2% SDS at room temperature for 30 min and twice in 0.2 × SSC, 0.2% SDS at 65°C for 20 min. The autoradiograms were developed after the exposure at −70°C for 2-4 d.

Results

13 patients with B-CLL and 8 patients with MM were studied. Eight of the B-CLL patients were classified as stage 0 patients, a clinically benign condition with a good prognosis (7, 8, 10). One of the MM cases was classified as a smoldering myeloma (13) at time of testing, but later required treatment (case 14).

T cells were isolated from patients by using anti-CD3 antibodies on solid phase. CD3 is a five-chain complex associated with TCR and a marker for mature T cells. DNA extracted from patient T cells was analyzed on Southern blots and compared with human germline, placental DNA. In four cases, evidence for T cell clonality was found (cases 1-3 and 14) (Table I). In all these cases rearranged bands could be seen in the Bam I digestions. In DNA from patients 1, 2, and 4, rearranged bands can also be seen with Eco RI or Hind III digestions. This suggests rearrangements in either the Cβ1 or the Cβ2 gene, as both these can be detected in Bam HI diges-

TABLE I

Clinical Status and Presence of Clonal T Cell Populations in Patients
with B-CLL and Myeloma

Pat no.	Diagnoses	Sex	Age	Clinical stage	Total ly count	Clonal rearrangements
					× 10⁹/liter	
1	CLL	F	72	0	14.9	+
2	CLL	F	63	0	38.4	+
3	CLL	F	75	0	14.8	+
4	CLL	M	70	0	12.6	−
5	CLL	M	69	0	18.0	−
6	CLL	F	72	0	81.7	−
7	CLL	F	71	0	24.4	−
8	CLL	M	72	0	31.5	−
9	CLL	M	79	III	104	−
10	CLL	M	68	III	25.2	−
11	CLL	F	72	IV	40.0	−
12	CLL	F	71	IV	86.0	−
13	CLl	M	72	IV	43.5	−
14	Smoldering myeloma	M	57	—	1.3	+
15	Myeloma	M	61	IIIB	0.8	−
16	Myeloma	M	72	IIIA	1.6	−
17	Myeloma	F	78	IIIB	0.7	−
18	Myeloma	M	54	IIA	0.9	−
19	Myeloma	F	77	III	1.2	−
20	Myeloma	F	60	IA	1.6	−
21	Myeloma	M	78	IA	1.6	−

tions while Eco RI and Hind III only detect single rearrangements of the C1 and C2 loci, respectively.

Case 1 showed two clones, both using Cβ2 genes (Fig. 1). Case 2 showed one rearranged band in the Bam HI digestion, indicating one dominant T cell clone using the Cβ2 gene (Fig. 2). Case 3 and 14 both had two rearranged bands in the Bam HI digest, but only one band in the Eco RI digestion, indicating rearrangements in the C1 locus (Figs. 3 and 4).

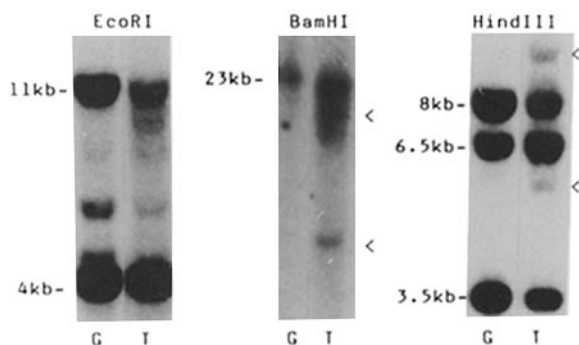Cases 1-3 were B-CLL patients in stage 0 and case 14 was a smoldering MM.

FIGURE 1. Case 1. Two rearranged bands were shown on both blots with either Bam HI or Hind III digestion. G, Germline DNA (from human placenta); T, DNA from T cells.
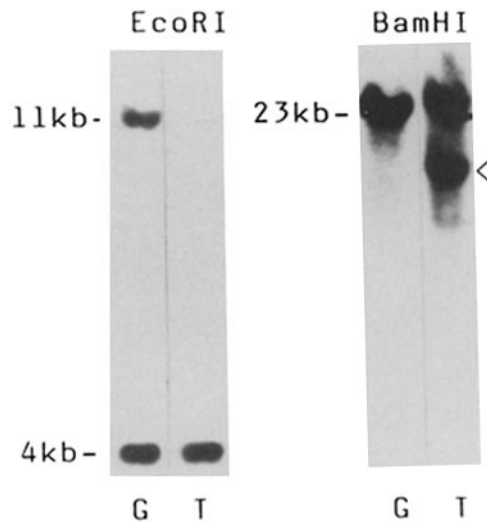
FIGURE 2.   Case 2. One rearranged band can be seen on the Bam HI blot. G, Germline DNA (from human placenta); T, DNA from T cells.
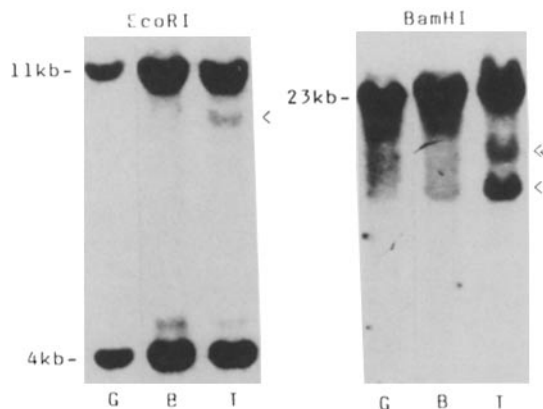

FIGURE 3.   Case 3. Two rearranged bands are clearly shown on the Bam HI blot, one of them can also be seen on the Eco RI blot. DNA from adherent B cells isolated by nylon wool binding (lane B) show no rearranged band. G, Germline DNA (from human placenta); T, DNA from T cells; B, DNA from B cells.

No patients with a more advanced disease had signs of clonal T cell rearrangements, suggesting that the level of clonal T cells may be <5%.

Discussion

In 3 of 13 B-CLL patients and 1 of 8 myeloma patients evidence was found for a clonal predominance in the mature, peripheral T cell population. The method used was based on the detection of specific rearrangements within in the gene for the TCR β chain and has a sensitivity of 1-5% of the whole T cell population (22). In other systems we have found that the development of detectable clones only occurs with defined antigens presented by autologous cells. Thus, a substantial part of the T cell population in the B-CLL and myeloma patients seems to be involved in recognition of certain restricted antigens, presumably expressed by the leukemic cells. In patients with rheumatoid arthritis, T cells derived from synovial membranes,
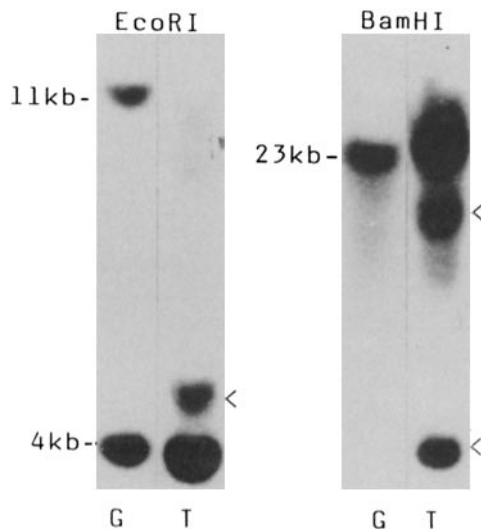
EcoRI BamHI

11kb-

23kb-

4kb-

G T G T

FIGURE 4. Case 14. Two rearranged bands were shown on the Bam HI blot with one of them on the Eco RI blot. G, Germline DNA (from human placenta); T, DNA from T cells.

and short-term tissue cultured in IL-2, have been found to contain a predominance of a limited number of clones by using the same technique as used in this report (23). These cells may be reactive against autoantigens.

One potential class of antigens, which may be involved in B lymphoproliferative diseases, are idiotypes expressed by the tumor cell related surface Ig (3-6). However, at present, we have no information regarding the specificity of the clonal T cell populations, although in one case (No. 14), a short-term established T cell line could respond by DNA synthesis when challenged with purified idiotype protein and autologous antigen-presenting cells (data to be published). However, it can not be excluded that the clones were reactive against nonleukemic antigens although no patients had any apparent infection at time of testing.

Clonal crosslineage rearrangements of the TCR β chain can occur in B-type leukemias, more commonly in B-CLL, than MM (24-30). However, we have several reasons to believe that the clonality we have detected was present in the T population rather than in monoclonal leukemic cells. First, mature T cells were isolated by solid phase separation with anti-CD3 antibodies. CD3 is a marker for mature T cells and only expressed by cells that express TCR at the cell surface. Second, in at least one case (No. 14), the patient had no malignant B cells in the peripheral circulation. Third, in three of the four cases with predominant clones, there was evidence for two clones. This strongly suggests that these are T cell derived as leukemic cells are clonal in nature (3). Fourth, in at least one patient with predominant clones, adherent leukemic cells that bound to nylon wool columns, were clearly negative for rearrangements of the TCR β chain gene (Fig. 3).

All patients with clonal predominance in the T cell population were clinically benign cases, stage 0 B-CLL, or smoldering MM. This may indicate either that the reactive clones are involved in the control of the leukemic cells and/or that the T cell system is deregulated in more advanced cases. Some tumors are clearly immunogenic and an active human response may limit their growth (31-33). In experimental

B cell leukemias, isolated Ig idiotypes have been used to induce a humoral and cellular immune response against the corresponding leukemic cells (34, 35). Campbell et al. (36) have synthesized peptides, corresponding to defined determinants binding to the TCR, and found that such peptides also could induce a similar effect.

The reasons why a clonal T cell predominance was only detected in 4 of 21 cases may be related either to a generalized T cell dysfunction or to properties of the leukemic cells. We and others have found a progressive T cell imbalance in later stages of B-CLL and myeloma, reflected by an inverted CD4/CD8 ratio and the appearance of suppressor T cells (3, 10, 12–14). These suppressor cells may disturb a normal cellular immune response. In later stages the properties of the leukemic cells also change. One characteristic that may be important, and that we recently have studied, is the decreased capacity of more progressively growing B-CLL cells to secrete IL-1β (37). Stage 0 B-CLL cells secrete normal levels of IL-1β in vitro and they also express cell surface markers typical for myelomonocytic cells (38). Thus stage 0 B-CLL cells may be more active in antigen presentation, including processing of their own antigens than leukemias in more advanced stages.

In summary, in some cases of benign B-CLL and MM, there are predominant T cell clones present in the peripheral blood. Whether these clones are biologically and clinically important is presently unclear. To study this question, the clones need to be expanded in vitro, and characterized with regard to functional activity against tumor cells.

Summary

Clonality in the non-neoplastic T cell population was investigated in 21 patients with B cell chronic leukemic (B-CLL) or multiple myeloma (MM) by probing for TCR β chain gene rearrangements using Southern blot analysis. In three patients with a benign form of B-CLL (stage 0), and in one patient with smoldering MM, evidence was found for predominant T cell clones. As cellular immunity against the malignant cells may be important in leukemia, the results are discussed in view of the potential role of T cell immunity in B-CLL and MM.

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