Brief report

Clonal expansion of CD8$^+$ BV8 T lymphocytes in bone marrow characterizes thymoma-associated B lymphopenia

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A subgroup of thymoma patients is affected by severe immunodeficiency clinically resembling an HIV infection (Good syndrome). These individuals are characterized by B lymphopenia with B-lymphopoiesis deficiency. To investigate the pathogenesis of this unique condition, we studied the T-cell repertoire in blood and bone marrow samples by heterogeneity length analysis of CDR3 beta variable regions of the T-cell receptor (spectratyping). While no alterations were found in the peripheral blood, we detected an oligoclonal population of β variable 8 (BV8) CD8$^+$ T cells in 5 of 5 bone marrow samples. No lymphocyte expansions were found in the bone marrow of 2 thymoma patients with normal B-cell counts, 2 healthy donors, and 3 patients with diseases unrelated to thymoma. These data suggest that an immune response toward an unknown antigen is taking place in the bone marrow of B-lymphopenic thymoma patients. We propose that BV8 CD8$^+$ T cells may play a role in the pathogenesis of this immunodeficiency syndrome. (Blood. 2003;101:3106-3108)

Introduction

Thymomas are tumors of thymic epithelium characterized by severe alterations of the immune system that are not yet completely understood.[1-8] A severe immunodeficiency with B-cell defect is found in a small percentage of thymoma patients[1-6]; this condition is also known as Good syndrome.[2] The immunodeficiency may precede or follow thymectomy, and B cells are undetectable in the periphery.[4,9,10] The coexistence of autoimmunity and immunodeficiency causes life-threatening problems in the management of these patients.[7-10]

The pathogenesis of B-cell loss represents a puzzling phenomenon, the understanding of which might help us to find a better approach to treat this syndrome. It has been documented that CD8$^+$ T lymphocytes derived from blood of patients with thymoma and immunodeficiency are able to suppress proliferation of allogeneic pre-B cells. Thus, it has been suggested that an aggression toward B-cell precursors is responsible for the immunodeficiency.[4-6] The spectratyping analysis represents a novel tool to address this unresolved question.[11-15] Therefore, we examined the T-cell repertoire of peripheral and bone marrow lymphocytes in 5 thymoma patients with immunodeficiency and in 7 control individuals. The absence of B lymphocytes was always characterized by the expansion of β variable 8 (BV8)–positive T cells in the marrow. These data provide novel evidence for the involvement of CD8$^+$ T cells in the pathogenesis of Good syndrome, supporting the idea that B-cell loss is due to an aggression toward B-cell precursors.

Study design

The main characteristics of the patients included in this study are shown in Table 1. Tumor histology has been classified according to the World Health Organization (WHO) proposal.[16] The majority of the patients received thymectomy and showed an inversion of the CD4$^+$/CD8$^+$ ratio. Five patients were suffering from severe immunodeficiency syndrome characterized by recurrent infections of the lower respiratory tract. Flow cytometry analyses were performed at monthly intervals in the last 6 months, showing a stable reduction of mature CD19$^+$ peripheral cells (unique patient numbers [UPNs] 091, 157, 051, 009, 147). A marked hypogammaglobulinemia had been detected in 4 of the 5 patients, whereas 1 of them (UPN 091) had normal immunoglobulin (Ig) serum levels. On the bases of clinical signs and B lymphopenia, we classified these patients as suffering from Good syndrome. We included in the study also 2 thymoma patients with normal Ig serum levels, normal B-cell counts, and no signs of severe recurrent infections (thymoma control patients [TC] 011, TC 087). Three patients with diseases unrelated to thymoma (systemic lupus erythematosus [SLE 354], myelodysplasia [MD 27], and histiocytosis [His 104]), as well as 2 healthy controls ([HC] 1 and HC 2), were also investigated. In the year before bone marrow sampling patients 009 and 147 received prednisone and cyclosporin A. Treatments were suspended one month before bone marrow sampling. Thus, we considered all patients free of immunosuppressive treatment at the time of this study. All subjects were fully informed about the aim of the research and agreed to donate samples. Marrow samples were obtained taking advantage of material used for diagnostic purposes. Marrow samples from 2 allogenic transplantation donors were used as controls (HC 1 and 2). The immunophenotype was done on whole blood by means of standard 3-color flow cytometry. To perform the spectratyping,[11] T-cells were separated from blood and marrow by density

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Table 1. Clinical and immunologic findings in patients with thymoma

Patient	Sex/age, y	Histology/ stage	Autoimmune-associated disease	Date of thymectomy	Date of BM sampling	CD3+*	CD3+ CD4+	CD3+ CD8+	CD19+	IgG†	IgA‡	IgM§
Thymoma patients with B lymphopenia												
091	M/56	B2/IVa	No	1995	1999	1915	319	1482	0	7.4	1	0.6
157	M/45	nd/IV	No	1982	1999	786	266	484	0	1.9	0.2	0.2
051	F/70	C/III	No	—‖	1999	697	252	436	25	1.2	0.3	0.1
009	F/52	AB/II	MG/A	1995	1999	2115	910	951	68	1.8	0.3	0.1
147	M/28	nd/IVa	PRCA	1998	1999	1185	402	462	58	4.2	0.8	0.3
Thymoma patients without B lymphopenia												
TC 011	F/58	B1/II	No	1995	1999	750	165	240	292	8.3	0.2	0.2
TC 087	M/48	B3/IVa	No	1998	1999	841	168	543	170	10	2.4	1.2
Control patients												
His 104	F/44	NA	NA	NA	2002	1970	1131	374	796	12	3.4	1.0
SLE354	F/38	NA	NA	NA	2002	2890	1970	750	1320	32	0.5	2.9
MD27	M/24	NA	NA	NA	2002	1350	690	570	280	19	1.5	2
HC 1	M/42	NA	NA	NA	1999	2350	1410	1090	310	12	3.2	1.8
HC 2	F/50	NA	NA	NA	2000	2420	1573	962	290	13	2.8	1.7

M indicates male; F, female; MG, myasthenia gravis, A, anemia; PRCA, pure red-cell aplasia; and NA, not applicable.
*Peripheral blood lymphocyte count (cells per cubic millimeter).
†Normal value, 7-16 g/L.
‡Normal value, 0.7-4.1 g/L.
§Normal value, 0.4-2.3 g/L.
‖No thymectomectomy performed.

gradient according to standard procedures (Lymphoprep, Oslo, Norway). When a sufficient amount of lymphocytes was available from marrow samples, T cells were separated into $CD8^+$ and $CD8^-$ cells by magnetic sorting with coated beads (Dynabeads; Dynal, Oslo, Norway). RNA was prepared with Trizol (GIBCO-BRL, Bethesda, MD) according to the manufacturer's instructions. Reverse transcription and polymerase chain reaction (PCR) amplification for the 25

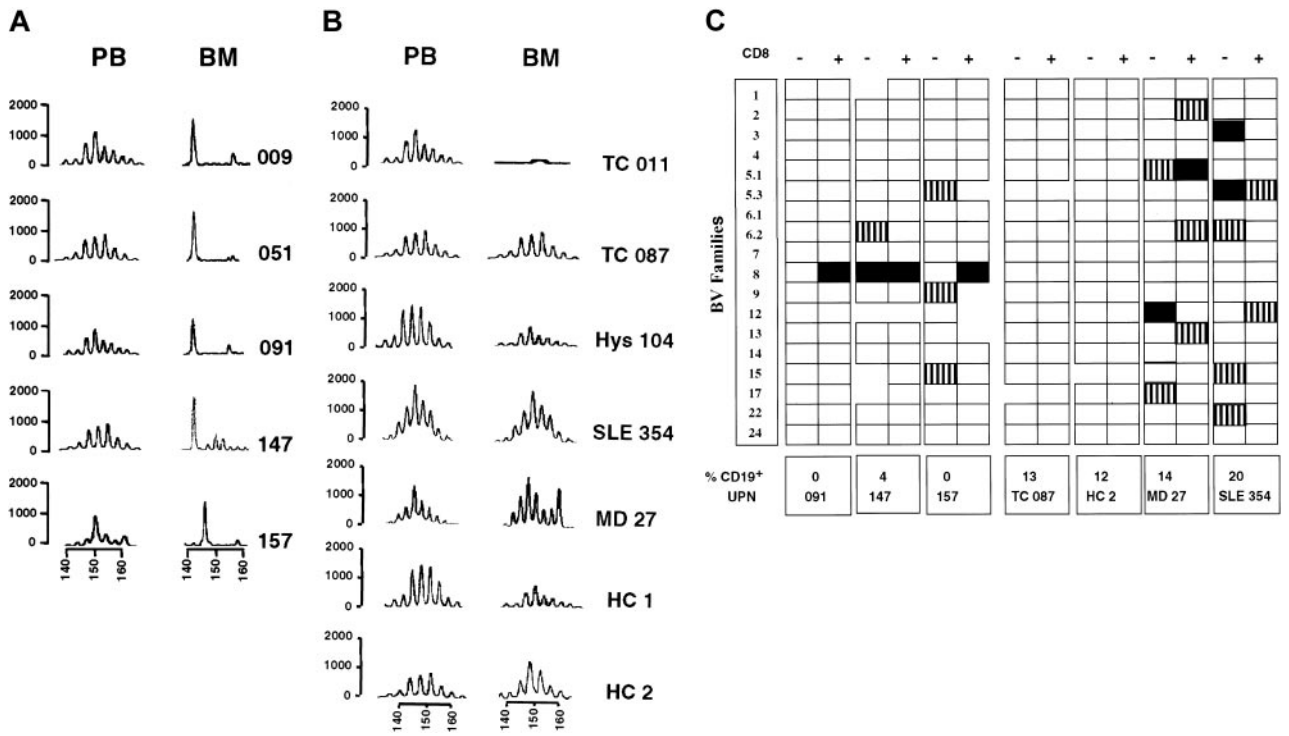

Figure 1. T-cell–receptor diversity of bone marrow–derived lymphocytes in thymoma patients. (A) CDR3 heterogeneity length analysis of β variable 8 (BV8) families of T cells isolated from peripheral blood and bone marrow (PB and BM, respectively) of 5 patients with thymoma and B lymphopenia. In the cases of patients 091, 147, and 157, we refer to $CD8^+$ T cells. Profiles for patients 051 and 009 have been generated from unfractionated T cells. (B) BV8 profiles of 2 patients with thymoma and normal B-cell counts (TC 011 and TC 087, respectively), 3 patients with thymoma-unrelated diseases (His 104, SLE 354, and MD27), and 2 healthy donors (HC 1 and HC 2). All profiles in panel C refer to $CD8^+$ T cells, except for TC 011, His 104, and HC 1, in which data were generated on unsorted T cells. PCR products were separated on DNA sequencing polyacrylamide gel by using an automated ABI PRISM 377 apparatus (Perkin Elmer, Applied Biosystems, Warrington, United Kingdom). Band intensity and size were evaluated with the Gene Scan software (Perkin Elmer) and expressed as relative fluorescence units (rfu) and base pairs (bp). (C) Synopsis of TCR repertoire expressed in bone marrow–derived $CD8^+$ and $CD8^-$ T-cell subsets. Open, gridded, and filled boxes, respectively, represent BV families showing 3 standard profiles: normal (Gaussian distribution), altered (< 5 peaks with a non-Gaussian distribution), and mono-oligoclonal profile (< 3 major peaks). The absence of boxes represents undetectable BV families.

different β variable (BV) T-cell receptor (TCR) families was performed by the one-step PCR protocol (GIBCO-BRL). To resolve the composition of each BV TCR family, the PCR products were run on a sequencing gel in a fluorescence-based DNA sequencer.[11-13] Normally, each TCR family is resolved by this technique as a series of bands having a Gaussian distribution. Each alteration, in either the distribution or the intensity of single bands, represents a perturbation in the given BV TCR family reflecting the involvement of that family in an immune response toward one or more antigens.[11-15] PCR products corresponding to BV8 regions showing a single peak were separated on polyacrylamide gel. The major band was then cut from the gel, eluted, and sequenced by direct PCR method (Big Dye Sequencing kit, V3.0; Applied Biosystem, Warrington, United Kingdom).

Table 2. Direct sequencing data of the major band found in BV8 families of patients 009, 051, 091, 147, 157

UPN	BV8	CDR3	BJ	
009	CAS	SFGDGVN QPQH	FGDGTRLSIL	1.5*
051	CAS	SLGTGNN SP LH	FGNGTRLTVT	1.6
091	CAS	SLGRGAN ETQY	FGPGTRLTVT	2.7
147	CAS	SLGVGAN QPQH	FGDGTRLSIL	1.5
157	CAS	SFGSGEN ETQY	FGPGTRLTVT	2.7

See Figure 1.
*BJ gene used.

Results and discussion

The immunophenotype analysis of peripheral lymphocytes derived from thymoma patients with immunodeficiency showed a stable decrease in the level of mature $CD19^+$ B cells (Table 1) and $CD20^+$ B cells (data not shown). Unexpectedly, the BV8 family showed a single-peak profile in bone marrow, but not in peripheral blood lymphocytes, in 5 of 5 B-lymphopenic patients (Figure 1A). We reasoned that these BV8 families could share similarity in the CDR3 sequences, too. The direct sequencing analysis confirmed this hypothesis, revealing a conserved CDR3 motif (SF/LGXGXNXXQ/LH/Y) in all BV8 products found to be expanded (Table 2). T cells isolated from marrow and blood samples of patient TC 087 and from the other control individuals expressed detectable amounts of BV8 mRNA with a Gaussian CDR3 profile (Figure 1B). In patient TC 011 we found only a barely detectable level of BV8 mRNA in marrow-derived lymphocytes (Figure 1A). In Figure 1C, we present a synopsis of the whole repertoire analysis expressed in $CD8^+$ and $CD8^-$ T cells. A very limited number of altered families can be found in the marrow of B-lymphopenic patients (Figure 1C). Of note, only BV8 families display a single-peak profile. In 2 of 3 patients (UPN 091 and 157) BV8 mRNA have been found expanded only in the $CD8^+$ subset. In patient 147, BV8 mRNA was expanded in $CD8^+$ as well as in $CD8^-$ T cells. However, in this case the expression level was much higher in $CD8^+$ than in $CD8^-$ lymphocytes (data not shown).

In conclusion, the whole body of data confirmed that bone marrow of B-lymphopenic thymoma patients was infiltrated by the oligoclonal expansion of $CD8^+$ $BV8^+$ T lymphocytes. Since in the periphery $BV8^+$ lymphocytes were normal, it was likely that the selective expansion detected in the marrow resulted from an immune response toward an unknown pathogen (eg, B lymphotropic virus) or from a direct autoimmune aggression against B-cell precursors. Autoimmune reactions against hematopoietic precursors seem to be responsible for disorders that are associated with thymoma and disorders that are not.[17-20] Clonal lymphocyte expansion has been found in the peripheral blood of patients with pure red-cell aplasia.[17-19] Furthermore, the analysis of the CDR3 size distribution in the bone marrow of patients with aplastic anemia indicates that an antigen-driven expansion of T cells is involved in the pathogenesis of this disease.[20] Our findings provide novel evidence supporting the hypothesis that a T-cell–mediated reaction against hematopoietic precursors is responsible for the selective B-cell loss in a subgroup of thymoma patients. In light of this, the immunodeficiency with severe B lymphopenia syndrome associated with thymoma has to be included in the autoimmune phenomena occurring in the patients affected by this form of tumor. Further experiments are warranted to characterize the (auto)antigen(s) possibly involved in triggering this phenomenon.

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