Brief report

Splenic plasma cells can serve as a source of amyloidogenic light chains

Alan Solomon,[1] Sallie D. Macy,[1] Craig Wooliver,[1] Deborah T. Weiss,[1] and Per Westermark[1,2]

[1]Human Immunology and Cancer Program, Department of Medicine, University of Tennessee Graduate School of Medicine, Knoxville; and [2]Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden

Bone marrow-derived clonal plasma cells, as found in systemic amyloidogenic light chain–associated (AL) amyloidosis, are presumed to be the source of light chains that deposit as fibrils in tissues throughout the body. Paradoxically, people with this disorder, in contrast to multiple myeloma, often have a low percentage of such cells, and it is unknown whether this relatively sparse number can synthesize enough amyloidogenic precursor to form the extensive pathology that occurs. To investigate whether another hematopoietic organ, the spleen, also contains monoclonal light chain–producing plasma cells, we have immunostained such tissue from 26 AL patients with the use of antiplasma cell, antifree κ and λ, and anti-V_L subgroup-specific monoclonal antibodies (mAbs). In 12 cases, there was statistically significant evidence of a monoclonal population bearing the same κ or λ isotype as that within the bone marrow and identical to the amyloid. Our studies have shown that the spleen may be another source of amyloidogenic light chains. (Blood. 2009;113:1501-1503)

Introduction

Systemic amyloidogenic light chain–associated (AL) amyloidosis is characterized by the deposition in the heart, kidneys, liver, nerves, and other organs or tissues of κ or λ light chain–related fibrils.[1] These molecules are the products of plasma cells deemed monoclonal based on the finding of a predominance of κ^+ or λ^+ cytoplasmic immunoglobulin (Ig), and the presence of such cells in bone marrow is one of the diagnostic hallmarks of this disorder. In comparison with multiple myeloma, patients with AL amyloidosis typically have a relatively low number of plasmacytes in this site, that is, less than 5% to 10%[2]; thus, it is not known whether this relatively sparse population secretes sufficient amounts of amyloidogenic precursor to account for the extensive deposits that can occur throughout the body. To address this question, we have determined whether another hematopoietic organ, namely the spleen, also contains monoclonal light chain–producing plasma cells. We now report the results of immunophenotypic analyses that used monoclonal antibodies (mAbs) specific for κ and λ free light chains (FLCs),[3] as well as reagents reactive with the major V_κ and V_λ gene families.[4] Here we showed that the spleens of 4 of 8 ALκ and 8 of 18 ALλ patients had a statistically significant preponderance of plasma cells with a light chain isotype identical to that expressed by the bone marrow–derived plasma cells and/or the amyloid deposits.

Methods

Patient population

The 26 patients included in this study had a diagnosis of systemic AL amyloidosis (manifested primarily by renal, cardiac, or neurologic dysfunction) based on the presence of a serum or urinary monoclonal Ig; an abnormal serum FLC κ/λ ratio, as determined by our mAb-based enzyme-linked immunosorbent assay (ELISA)[5]; or identification of the light chain nature of amyloid extracted from autopsy-derived tissue, as documented by amino acid sequencing and/or mass spectrometry.[6,7] The study was approved by the University of Tennessee Medical Center's Institutional Review Board, and informed consent was obtained in accordance with the Declaration of Helsinki.

Immunohistochemistry

Four-micrometer-thick sections, cut from formalin-fixed, paraffin-embedded blocks of spleen, were deparaffinized and subjected to antigen retrieval by exposure in a 90°C water bath for 30 minutes to a Dako Target Retrieval Solution containing citrate buffer, pH 6.0 (Dako Cytomation, Carpenteria, CA), followed by cooling at room temperature for 20 minutes. The tissue was immunostained with a commercial antiplasma cell antibody (Dako); our murine mAbs Fκ-C3 and Fλ-G9, which react only with free κ or λ light chains, respectively[3]; and reagents specific for the major V_L subgroups (V_κ1, 2, 3, and 4; V_λ1, 2, 3, 6, and 8).[4] Immunoreactivity was visualized with the use of a streptavidin-biotin detection system (BioGenex, San Ramon, CA) and color was developed with diaminobenzidine (DAB; Vector Laboratories, Burlingame, CA). The slides were counterstained with hematoxylin (Gill #3, Sigma-Aldrich, St Louis, MO). The number of reactive plasma cells was enumerated in 15 high-power fields using a Leitz DMRB microscope (Vashaw Scientific, Norcross, GA) fitted with a 40×/0.75 dry objective and a 1.6× magnifying lens. The results were averaged and the statistical significance determined by the *t* test. Cytospin preparations of bone marrow obtained at the time of diagnosis also were evaluated with the same antibodies by methods detailed elsewhere.[8]

Results and discussion

The predominant site of deposition and the V_L nature of the amyloid in all 26 patients are given in Table 1. Immunocytochemical analyses of bone marrow–derived specimens obtained at the time of diagnosis were performed in 19 cases in which clonal

Submitted April 29, 2008; accepted November 18, 2008. Prepublished online as *Blood* First Edition paper, December 2, 2008; DOI 10.1182/blood-2008-04-154484.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 1. Number of plasma cells per high-power field immunoreactive with free κ and λ immunoglobulin light chains in sections of spleen from patients with AL amyloidosis

		Immunophenotype			Splenic plasma cells			Serum*		
Case	Site†	AL‡	Marrow plasma cells	Spleen plasma cells	Free κ	Free λ	*P*	Free κ, μg/mL§	Free λ, μg/mL§	Free κ/λ ratio
1	H	λ6	λ6	λ6	0.6 ± 0.2	87.1 ± 7.2	<.001	1.57	396.2	0.004
2	H	λ1	λ1	λ1	7.4 ± 1.1	15.2 ± 1.1	<.001	8.96	525.8	0.017
3	L	κ1	κ1	κ1	102.1 ± 3.7	9.0 ± 1.7	<.001	n.d.	n.d.	n.d.
4	N, LU	λ6	λ6	λ6	12.8 ± 1.6	28.3 ± 1.5	<.001	12.6	277.8	0.045
5	K	λ2	λ2	λ2	7.7 ± 0.7	15.3 ± 1.0	<.001	n.d.	n.d.	n.d.
6	I	κ1	n.d.	κ1	4.9 ± 0.8	0.5 ± 0.3	<.001	634.4	14.5	43.8
7	K, TH	λ1	λ1	λ1	1.1 ± 0.3	16.1 ± 1.6	<.001	22.6	71.7	0.32
8	L	κ1	κ1	κ1	15.4 ± 1.4	1.7 ± 0.4	<.001	332.8	43.7	7.62
9	H, T	λ1	λ1	λ1	6.3 ± 0.8	11.1 ± 1.0	<.001	46.1	258.3	0.18
10	L, N	κ1	κ1	κ1	5.7 ± 1.7	0.3 ± 0.2	<.01	738.3	50.1	14.7
11	H	λ8	λ8	λ8	5.5 ± 0.8	9.5 ± 0.9	<.01	17.5	471.7	0.04
12	L, S, A	λ2	λ2	λ2	0.7 ± 0.3	2.2 ± 0.3	<.01	n.d.	n.d.	n.d.
13	K, H, S	λ3	λ3	λ3	1.9 ± 0.4	2.4 ± 0.5	n.s.	11.6	120.2	0.10
14	H, K	λ2	λ2	—¶	0.3 ± 0.2	0.1 ± 0.1	n.s.	36.4	329.0	0.11
15	L	κ4	κ4	—	0.2 ± 0.1	0.3 ± 0.2	n.s.	103.4	123.9	0.83
16	S, L	λ1	n.d.	—	0.3 ± 0.2	1.1 ± 0.6	n.s.	n.d.	n.d.	n.d.
17	K	λ1	λ1	λ1	18.1 ± 1.6	14.5 ± 1.2	n.s.	15.0	844.3	0.02
18	K	λ2	λ2	—	0.1 ± 0.1	0.3 ± 0.1	n.s.	21.9	320.8	0.07
19	K	λ3	λ3	λ3	2.1 ± 0.4	1.6 ± 0.3	n.s.	36.2	221.8	0.16
20	K, H	λ3	λ3	—	0.2 ± 0.1	0.0	n.s.	8.9	106.9	0.08
21	L	λ2	λ2	λ2	1.7 ± 0.7	1.6 ± 0.5	n.s.	48.8	279.4	0.17
22	L, S	κ3	n.d.	κ3	3.6 ± 0.5	4.5 ± 0.9	n.s.	n.d.	n.d.	n.d.
23	S	λ3	n.d.	—	0.1 ± 0.1	1.3 ± 0.3	n.s.	n.d.	n.d.	n.d.
24	K, S, LN	κ1	n.d.	κ1	2.9 ± 0.7	2.5 ± 0.6	n.s.	n.d.	n.d.	n.d.
25	K, L, S	λ2	n.d.	—	0.3 ± 0.2	0.6 ± 0.2	n.s.	n.d.	n.d.	n.d.
26	I	κ3	n.d.	κ3	8.7 ± 0.8	9.9 ± 1.4	n.s.	n.d.	n.d.	n.d.
Normal					5.0 ± 0.6	3.5 ± 0.9	n.s.	4.2-13.0	16.4-127.3	0.07-0.28

H indicates heart; L, liver; LN, lymph node; LU, lung; K, kidney; I, intestine; TH, thyroid; T, tongue; A, adrenal; n.d., not determined; and n.s., not significant.
*Specimens obtained less than 1 month before death, except in cases 9 and 21 (taken 3 and 4 months before death, respectively).
†Principal site of amyloid deposition based on postmortem examination.
‡Determined by chemical analyses of amyloid extracts.
§Values derived from a mAb-based enzyme-linked immunosorbent assay (ELISA).
¶No clonal plasma cells detected.

populations of plasma cells were found, as evidenced by their reactivity with either the antifree κ or λ reagent, as well as a particular anti-V_L subgroup mAb. In each instance, there was complete concordance between the chemical nature of the AL amyloid and the predominant plasma cell immunophenotype. Further, a striking and statistically significantly similar population was found in the spleens from 12 patients (κ1, 4; λ1, 3; λ2, 2; λ6, 2; and λ8, 1), as illustrated for cases 8 (ALκ) and 7 (ALλ; Figure 1). Even in 7 patients in whom there was no κ or λ predominance and who had more than 2 plasma cells per high power field, the presence of such cells having the same cytoplasmic V_L phenotype as that of the amyloid was evidenced by their staining with specific anti-V_L mAbs (Table 1). Bone marrow specimens taken within 2 months of death from 6 patients also were evaluated; in the 5 who had statistically significant clonal splenic plasma cells, comparable results were found in the marrow. In the 6th case, as in the spleen, there was no clonal predominance.

Serum samples obtained less than 1 month before death from 15 subjects (and, in 2 cases, 3 and 4 monhs before death) were available for FLC assay. In 7 of 11 cases with a statistically significant population of splenic plasma cells, the FLC κ/λ ratios were concordantly abnormal. Among the remaining 8, where a clonogenic population was not detected, these values were abnormal in 2.

On the basis of the results of these studies, it was shown that in AL amyloidosis, the spleen (in addition to the bone marrow) can contain a population of clonal plasmacytes that produce amyloidogenic light chains. Experimental data suggest that these cells presumably arise from bone marrow–derived transitional B lymphocytes that home to the spleen and there mature into low-level native follicular (or marginal zone) B cells.[9-11] Their further development within germinal centers and evolution into plasma cells results

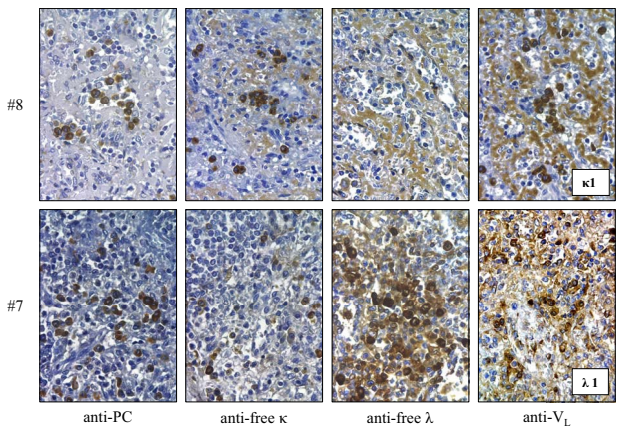

Figure 1. Photomicrographs of clonal plasma cells present in the spleens of patients with systemic ALκ (case 8) and ALλ (case 7) amyloidosis. The primary antibodies are as indicated (avidin biotin complex [ABC] technique; secondary antibody, biotinylated mouse IgG). Original magnification ×640. Photographs were taken with a SPOT RT color camera using SPOT RT software, Version 3.0 (Diagnostic Instruments, Sterling Heights, MI).

from exposure to an antigenic stimulus that leads to transcriptionally regulated clonal expansion and production of somatically mutated antibody molecules. This terminally differentiated population, which contains long-lived plasma cells, resides in splenic and (predominantly) bone-marrow survival niches.[12,13]

At present, therapeutic options for patients with AL amyloidosis are limited to antiplasma cell chemotherapy given in conventional amounts or high doses combined with autologous stem cell transplantation (or other "antimyeloma" regimens).[14-22] The rationale for this approach is to reduce the synthesis of amyloidogenic precursor light chains,[23] although the sensitivity of clonogenic bone marrow versus splenic plasma cells to such agents remains to be determined.

Acknowledgments

This work was supported in part by US Public Health Service Research Grant CA10056 from the National Cancer Institute (Bethesda, MD), the Aslan Foundation (Knoxville, TN), the Swedish Research Council (Stockholm, Sweden), and the European Union Sixth Framework Programme (Brussels, Belgium). P.W. was a Harry and Elsa Jiler American Cancer Society International Visiting Scientist. A.S. is an American Cancer Society Clinical Research Professor.

Authorship

Contribution: A.S. and D.T.W. wrote the paper; S.D.M. and C.W. performed the studies; and P.W. wrote the paper and evaluated the data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Alan Solomon, MD, University of Tennessee Graduate School of Medicine, 1924 Alcoa Highway, Knoxville, TN 37920; e-mail: asolomon@utmck.edu.

References

1. Solomon A, Weiss DT. Protein and host factors implicated in the pathogenesis of light chain amyloidosis. Amyloid. 1995;2:269-279.
2. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin Hematol. 1995;32:45-59.
3. Abe M, Goto T, Kosaka M, Wolfenbarger D, Weiss DT, Solomon A. Differences in kappa to lambda (κ:λ) ratios of serum and urinary free light chains. Clin Exp Immunol. 1998;111:457-462.
4. Abe M, Goto T, Kennel SJ, et al. Production and immunodiagnostic applications of antihuman light chain monoclonal antibodies. Am J Clin Pathol. 1993;100:67-74.
5. Davern S, Tang LX, Williams TK, et al. Immunodiagnostic capabilities of anti-free immunoglobulin light chain monoclonal antibodies. Am J Clin Pathol. 2008;130:702-711.
6. Murphy CL, Eulitz M, Hrncic R, et al. Chemical typing of amyloid protein contained in formalin-fixed paraffin-embedded biopsy specimens. Am J Clin Pathol. 2001;116:135-142.
7. Murphy CL, Wang S, Williams T, Weiss DT, Solomon A. Characterization of systemic amyloid deposits by mass spectrometry. Methods Enzymol. 2006;412:48-62.
8. Solomon A, Weiss DT, Macy SD, Antonucci RA. Immunocytochemical detection of kappa and lambda light chain V region subgroups in human B-cell malignancies. Am J Pathol. 1990;137:855-862.
9. Sze DM, Toellner KM, García de Vinuesa C, Taylor DR, MacLennan CM. Intrinsic constraint of plasmablast growth and extrinsic limits of plasma cell survival. J Exp Med. 2000;192:813-821.
10. Shapiro-Shelef M, Calame K. Plasma cell differentiation and multiple myeloma. Curr Opin Immunol. 2004;16:226-234.
11. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol. 2005;5:230-242.
12. Manz RA, Arce S, Cassese G, Hauser AE, Hiepe F, Radbruch A. Humoral immunity and long-lived plasma cells. Curr Opin Immunol. 2002;14:517-521.
13. Ellyard JI, Avery DT, Phan TG, Hare NJ, Hodgkin PD, Tangye SG. Antigen-selected, immunoglobulin-secreting cells persist in human spleen and bone marrow. Blood. 2004;103:3805-3812.
14. Comenzo RL, Getz MA. Autologous stem cell transplantation for primary systemic amyloidosis. Blood. 2002;99:4276-4282.
15. Sanchorawala V, Seldin DC. An overview of high-dose melphalan and stem cell transplantation in the treatment of AL amyloidosis. Amyloid. 2007;14:261-269.
16. Jaccard A, Moreau P, Leblond V, et al. High-dose melphalan versus melphalan plus dexamethasone for AL amyloidosis. N Engl J Med. 2007;357:1083-1093.
17. Cohen AD, Zhou P, Chou J, et al. Risk-adapted autologous stem cell transplantation with adjuvant dexamethasone +/− thalidomide for systemic light-chain amyloidosis: results of a phase II trial. Br J Haematol. 2007;139:224-233.
18. Kastritis E, Anagnostopoulos A, Roussou M, et al. Treatment of light chain (AL) amyloidosis with the combination of bortezomib and dexamethasone. Haematologica. 2007;92:1351-1358.
19. Palladini G, Russo P, Nuvolone M, et al. Treatment with oral melphalan plus dexamethasone produces long-term remissions in AL amyloidosis. Blood. 2007;110:787-788.
20. Sanchorawala V, Wright DG, Rosenzweig M, et al. Lenalidomide and dexamethasone in the treatment of AL amyloidosis; results of a phase 2 trial. Blood. 2007;109:492-496.
21. Dispenzieri A, Lacy MQ, Zeldenrust SR, et al. The activity of lenalidomide with or without dexamethasone in patients with primary systemic amyloidosis. Blood. 2007;109:465-470.
22. Wechalekar AD, Lachmann HJ, Offer M, Hawkins PN, Gillmore JD. Efficacy of bortezomib in systemic AL amyloidosis with relapsed/refractory clonal disease. Haematologica. 2008;93:295-298.
23. Dispenzieri A, Zhang L, Katzmann JA, et al. Appraisal of immunoglobulin free light chain as a marker of response. Blood. 2008;111:4908-4915.