Recurrent cytogenetic findings in subsets of patients with chronic lymphocytic leukemia expressing IgG-switched stereotyped immunoglobulins

We report a remarkable association of recurrent (*stereotyped*) cytogenetic aberrations with two subsets of chronic lymphocytic leukemia (CLL) cases expressing IgG-switched, stereotyped B-cell receptors (BCRs). Comparison with cases with heterogeneous BCRs showed that these recurrent cytogenetic aberrations were *subset-biased*. These findings further support a role for antigen in CLL development.

Athanasiadou A, Stamatopoulos K, Gaitatzi M, Stavroyianni N, Fassas A, and Anagnostopoulos A. Recurrent cytogenetic findings in subsets of patients with chronic lymphocytic leukemia expressing IgG-switched stereotyped immunoglobulins. Haematologica 2008 Mar; 93(3):473-474. doi: 10.3324/haematol.11872

CLL is uniquely characterized by the existence of subsets of cases with closely homologous (*stereotyped*) BCRs.[1-6] As we and others have recently reported, CLL cases expressing stereotyped BCRs may also share unique molecular, immunophenotypical and clinical features,[2,6] suggesting that a particular antigen-binding site can have a differential impact on clinical presentation and perhaps also outcome, regardless of IGHV mutation status. The few relevant studies have not provided a cytogenetic profiling of patients in subsets. Given the established prognostic and pathogenetic implications of specific cytogenetic aberrations in CLL, in this study we explored whether patients belonging to subsets with stereotyped BCR sequences also shared recurrent (*stereotyped*) cytogenetic findings.

We evaluated 179 typical, unselected CLL cases. Detailed descriptions of the study design and methods have been previously reported.[7] Combining results from classic cytogenetic and FISH analysis, the overall incidence of trisomy 12 and abnormalities of 13q, 11q23, and 17p13 was 23.3%, 31.3%, 3.7%, and 2.5% respectively. Clonal IGHV-D-J sequences were available for all cases of the present study. Using the 98% cut-off for homology to germline, 101/179 IGHV-D-J rearrangements (56.4%) were considered *mutated*, while the remainder (78/179 cases; 43.6%) were considered *unmutated*.

Cluster analysis of HCDR3 sequences following previously described criteria[3,6] showed 30/179 cases (16.8%) to belong to 8 different subsets with stereotyped HCDR3, all of which have been previously reported.[1-6] Five out of the 8 subsets included only pairs of sequences and will not be considered further, since the possibility that shared cytogenetic findings may be due to chance alone cannot be *a priori* excluded. Therefore, we focus on 3 subsets of cases with stereotyped BCRs which included 3-8 cases each. The heavy chain isotype was the same among members of the 3 subsets. The first subset included 8 IgM/IgD cases with stereotyped BCRs expressing unmutated IGHV genes of the 1/5/7 clan, who carried heterogeneous cytogenetic aberrations. In contrast, the remaining 2 subsets of IgG-switched cases with stereotyped BCRs (Figure 1) showed recurrent, *subset-biased* genomic aberrations.

In particular: (i) 3 out of 4 cases with stereotyped, unmutated IGHV4-39/IGKV1-39(1D-39) BCRs carried trisomy 12; the remaining case had no cytogenetic abnormalities. In contrast, IGHV4-39-expressing cases with heterogeneous HCDR3s (*non-stereotyped*) carried heterogeneous aberrations. Comparison with all other IgG-switched cases as well as non-switched CLL cases revealed that the association of trisomy 12 with this stereotyped subset was statistically significant ($p<0.01$ for either comparison); (ii) 5 out of 8 cases with stereotyped, mutated IGHV4-34/IGKV2-30 BCRs carried del(13)(q14), detected either by classic cytogenetic and FISH analysis (1 case) or by FISH analysis alone 4 cases); the remaining 3 cases were negative for cytogenetic aberrations. In contrast, IGHV4-34 cases with heterogeneous HCDR3s (non-stereotyped) also

Subset #1

CASE	IGHV	%	HCDR3	IGKV	%	KCDR3
P1114	4-39*01	100	ARRTGYSSSWYSTYNWFDP	1-39*01/1D-39*01	100	QQSYSTPLT
P1615	4-39*01	100	-S-R------FNVVA----	1-39*01/1D-39*01	100	-------P-
P242	4-39*01	100	--QE------- R--L	1-39*01/1D-39*01	99,7	-------R-
P2446	4-39*01	99,7	-SL--------TPA-----	1-39*01/1D-39*01	100	-------R-

Subset #2

CASE	IGHV	%	HCDR3	IGKV	%	KCDR3
P103	4-34*01	95,9	ARGYPDTPVVRRYYYYGMDV	2-30*01	97,3	MQGTHWPPYT
P1422	4-34*01	91,9	----AD--TF----------	2-30*01	95,7	------- --
P2451	4-34*01	91,7	V---A--A----------	2-30*01	98,4	----------
P1939	4-34*01	94,5	V------A--K-------E-	2-30*01	96	----------
P2920	4-34*01	93,5	----G-S-DTK----F-L--	2-30*01	97,3	------- --
P3020	4-34*01	90	----G-SDDT----F-----	2-30*01	91,1	---S--- --
P907	4-34*01	93,2	----G-SATTK---------	2-30*01	97	----Y-- --
P1626	4-34*01	94,8	-S- S--TT----------	2-30*01	96,2	------- --

Figure 1. HCDR3 and KCDR3 amino acid sequences of IgG-switched cases belonging to 2 subsets with different stereotyped BCRs (dashes indicate amino acid identity). Amino acid differences at the same HCDR3 or KCDR3 position in cases belonging to a subset were evaluated according to amino acid physicochemical properties (hydropathy, volume and chemical characteristics). Functionally similar amino acids are highlighted in grey. %: homology to germline.

carried heterogeneous cytogenetic aberrations. Comparison of stereotyped IGHV4-34/IGKV2-30 cases to: (i) *non-stereotyped* IGHV4-34 cases, (ii) all other IgG-switched cases and, (iii) non-switched CLL cases revealed that the association with del(13)(q12q14) is *subset-specific* (p=0.05, p<0.001 and p<0.01 respectively).

IgG-positive CLL is a relatively rare variant of CLL, whose origin and ontogenetic relationship to the common IgM/IgD variant remains unknown.[8] Ghiotto *et al*. first reported a subset of IgG-switched cases expressing IGHV4-39/IGKV1-39(1D-39) BCRs;[2] similar cases were subsequently reported by other groups, including ours.[5,6] Here, we document a remarkable association of trisomy 12 with cases belonging to this subset. Although trisomy 12 is among the more common recurring chromosomal defects in CLL,[7,9,10] it is generally detected at a frequency (~20%) which is significantly lower than its frequency in this subset (3/4 cases; 75%).

The IGHV4-34 gene is over-represented in the repertoire of IgG-switched CLL cases.[6,8] Several groups, including ours, have reported a subset of IgG-switched cases expressing restricted IGHV4-34/IGKV2-30 BCRs.[3,5-6,8] In the present study, we document a remarkable, statistically significant association of this subset with del(13)(q12q14). One might argue that the frequent occurrence of del13q in patients of this subset is merely a reflection of the overall high frequency of this genomic aberration in CLL (9-10). However, del13q was detected at a significantly higher frequency in stereotyped compared to *non-stereotyped* IGHV4-34-expressing cases, all other IgG-switched cases or non-switched CLL cases from our series.

In conclusion, recurrent (*stereotyped*) cytogenetic findings in IgG-positive CLL patients with stereotyped HCDR3 sequences indicate that selective antigenic pressures play an important role in CLL leukemogenesis, for at least some CLL patients. Because the number of cases studied is admittedly small, it will be necessary to confirm our results on a larger cohort of these rare CLL IgG-positive patients with stereotyped BCRs.

Anastasia Athanasiadou, Kostas Stamatopoulos, Maria Gaitatzi, Niki Stavroyianni, Athanasios Fassas, and Achilles Anagnostopoulos

Hematology Department and HCT Unit, G. Papanicolaou Hospital, Thessaloniki, Greece

Key words: chronic lymphocytic leukemia, cytogenetic diagnosis, FISH, immunoglobulin genes, hematopoietic recovery.

Correspondence: Kostas Stamatopoulos, M.D., Hematology Department and HCT Unit, G. Papanicolaou Hospital, 57010 Thessaloniki, Greece. Phone: international +30.2310350538. Fax: international +30.2310350579. E-mail: kostas.stamatopoulos@gmail.com

References

1. Tobin G, Thunberg U, Johnson A, Eriksson I, Soderberg O, Karlsson K, et al. Chronic lymphocytic leukemias utilizing the VH3-21 gene display highly restricted Vλ2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. Blood 2003;101:4952-7.
2. Ghiotto F, Fais F, Valetto A, Albesiano E, Hashimoto S, Dono M, et al. Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. J Clin Invest 2004;113:1008-16.
3. Messmer BT, Albesiano E, Efremov DG, Ghiotto F, Allen SL, Kolitz J, et al. Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. J Exp Med 2004;200:519-25.
4. Widhopf GF 2nd, Rassenti LZ, Toy TL, Gribben JG, Wierda WG, Kipps TJ. Chronic lymphocytic leukemia B cells of more than 1% of patients express virtually identical immunoglobulins. Blood 2004;104:2499-504.
5. Tobin G, Thunberg U, Karlsson K, Laurell A, Willander K, Enblad G, et al. Subsets with restricted immunoglobulin gene rearrangement features indicate a role for antigen selection in the development of chronic lymphocytic leukemia. Blood 2004;104:2879-85.
6. Stamatopoulos K, Belessi C, Moreno C, Boudjograh M, Guida G, Smilevska T, et al. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood 2007;109:259-70.
7. Athanasiadou A, Stamatopoulos K, Tsompanakou A, Gaitatzi M, Kalogiannidis P, Anagnostopoulos A, et al. Clinical, immunophenotypic, and molecular profiling of trisomy 12 in chronic lymphocytic leukemia and comparison with other karyotypic subgroups defined by cytogenetic analysis. Cancer Genet Cytogenet 2006;168:109-19.
8. Potter KN, Mockridge CI, Neville L, Wheatley I, Schenk M, Orchard J, et al. Structural and functional features of the B-cell receptor in IgG-positive chronic lymphocytic leukemia. Clin Cancer Res 2006;12:1672-9.
9. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-6.
10. Stilgenbauer S, Bullinger L, Lichter P, Dohner H. Genetics of chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. Leukemia 2002;16:993-1007.