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Review

Antigens in chronic lymphocytic leukemia—Implications for cell origin and leukemogenesis

Anders Rosén[a,*], Fiona Murray[b], Chamilly Evaldsson[a], Richard Rosenquist[b]

[a] Division of Cell Biology, Department of Clinical and Experimental Medicine, Linköping University, SE-581 85 Linköping, Sweden
[b] Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden

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ABSTRACT

Several types of B cell tumors, particularly MALT lymphomas, are known to have an antigen-driven component in tumor development. Over the past two decades substantial data have accumulated regarding the restricted immunoglobulin (IG) gene repertoire in chronic lymphocytic leukemia (CLL) and its potential implications for antigenic drive in the disease development and progression. Herein we discuss how evidence first illustrated a link between certain B cell receptor (BCR) specificities and disease outcome and the subsequent large-scale IG analyses which revealed the extent of "stereotyped" BCRs in CLL. More recent studies on CLL antibody reactivity have gradually provided clues as to which antigens may be involved in the tumor development. Significantly, CLL monoclonal antibodies have been shown to resemble natural antibodies recognizing molecular motifs both on apoptotic cells (*e.g.* modified cytoskeletal proteins and oxidation-specific epitopes), as well as exogenous bacteria, indicating that CLL clones possibly arise from B cells which have dual function as scavengers of apoptotic debris, while also having the ability to bind conserved bacterial cell structures. Such revelations have led us to re-evaluate both the phenotypic and functional characteristics of the tumor B cells and the pathway by which CLL arises and develops.

1. Background

The B cell plays a central role in the immune system by means of its primary function, the recognition and elimination of foreign antigen. The B cell's ability to capture antigen depends on the immunoglobulin (IG) molecule along with several accessory molecules, which make up the B cell receptor (BCR). It is through the BCR that the B cell receives external signals which can induce it to proliferate, become anergic, edit its BCR or under certain circumstances undergo apoptosis. The final outcome of BCR stimulation depends on a number of factors; co-receptor expression at the time of antigen interaction, the maturational stage of the B cell, and the quantity and context of antigen [1]. Thus, it is the BCR specificity which dictates whether the B cell will be allowed to enter the functioning B cell repertoire.

The ability of the human immune system to create a vast array of BCR specificities against the myriad of potentially harmful exogenous pathogens is due to both the extensive diversity created by V(D)J gene recombination of the *IG* loci, and the subsequent fine-tuning of BCR specificities generated by somatic hypermutation (SHM) and class switch recombination subsequent to antigen encounter. The combinatorial effect of these mechanisms is the rapid generation of specific effector B cells which produce only the most effective antibodies (Abs) present in the adaptive immune system. Additionally, natural Abs, which appear in circulation of normal individuals independent of external antigenic stimulation [2,3], are known to target conserved pathogenic epitopes such as lipopolysaccharides and phosphorylcholine and provide a first line response against gut and blood borne antigens, thereby acting as an intermediary between the innate and adaptive immune responses [3,4].

The role of infectious disease in the development of certain entities of B cell lymphoma such as *Helicobacter pylori* in gastric MALT lymphoma, which is the example *par excellence* for antigenic-drive, and Epstein-Barr virus (EBV) in Burkitt's lymphoma, are well-established [5,6]. The chronic inflammation induced by bacteria or viruses creates the setting in which tumor cells thrive, and notably, in the case of *H. pylori*, eradication of the microbes arrests tumor growth in the majority of patients [7]. Further associations supporting the model for bacterial antigen-driven malignancy are continually emerging; *Chlamydophila psittaci* and *Borrelia burgdorferi* have more recently been implicated in ocular adnexal- and cutaneous MALT lymphomas, respectively [8,9]. These findings have opened up a line of thought that perhaps other lymphomas,

* Corresponding author.
E-mail address: Anders.Rosen@liu.se (A. Rosén).

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such as chronic lymphocytic leukemia (CLL) could also have an antigen-driven component in the disease pathogenesis. This is also indicated by recent epidemiological reports which observed an increased risk for CLL among individuals with a history of pneumococcal pneumonia [10,11].

Since the groundbreaking reports from Hamblin et al. and Damle et al. in the late 90s introducing the *IG* heavy variable (*IGHV*) gene mutational status in CLL prognostication [12,13], *IG* gene analysis has become one of the most important markers in the prediction of CLL outcome. In addition, *IG* gene rearrangements are a suitable marker for clonality assessment, while simultaneously providing insight as to the time-point of malignant transformation [14]. This extensive *IG* analysis in CLL has lead to one of the most significant hypotheses within the field in recent years; namely that selection by antigen seems to play an important part in the disease development and could even influence disease outcome. Studies on the reactivity patterns of CLL Abs are also providing clues as to which antigens are interacting with tumor B cells and provide hints as to the true cell of origin in CLL.

2. IG analysis reveals a potential antigenic involvement in CLL

2.1. Early hints of antigenic involvement in CLL

During the mid 90s the first reports arose describing a non-stochastic Ab repertoire in CLL, which appeared to be distinct from the normal B cell repertoire [15–18]. An over-usage of certain *IG* genes such as *IGHV1-69*, *IGHV3-21*, *IGHV4-34*, and *IGHV3-7* was clearly evident, and while the exact gene frequencies varied slightly between cohorts, the overall pattern was the same. Later in the same decade, reports emerged from two independent groups describing a striking observation; a number of CLL patients in their respective cohorts carried *IGHV1-69* rearrangements with highly similar heavy complementarity determining region 3:s (HCDR3s), which were typically long and carried specific amino acid motifs [15,19]. As investigators began to perform larger scale *IG* gene sequencing, it became clear that these findings were not incidental. An abundance of publications reported similar observations; unrelated CLL patients, often from distinct geographical locations, expressed almost identical *IG* gene rearrangements, even within the most variable region of the molecule, the HCDR3 (Fig. 1) [15,20–24]. Analysis of light chain *IG* genes within these patient groups confirmed that the *IG* usage on the light chain was also frequently restricted and displayed homogeneity within the kappa or lambda CDR3 [21–23,25].

Considering the random manner in which the CDR3 is generated, this was extremely surprising. Going back to the logistics of *IG* gene rearrangement, recombination at the *IGH* and light chain loci permits the creation of over 1.2×10^9 permutations, taking into account the activity of terminal deoxynucleotidyl transferase (TdT) and exonuclease at the V-D and D-J joints and the alterations of the *IG* genes during affinity maturation [26]. Thus, the likelihood of randomly selecting two B cells with almost identical *IG* rearrangements from the same individual is remote. It therefore seemed credible that the restriction seen in CLL in terms of the *IG* repertoire and CDR3 composition, which essentially confers the antigen-specific binding properties of the B cell, could be due to a selective pressure by antigen(s), potentially conferring a growth advantage to tumors.

2.2. IGHV3-21-utilizing CLL

Although the *IGHV* gene mutational status is considered to be one of the best predictors of disease outcome in CLL, there

SUBSET #2 (IGHV3-21/IGLV3-21)

	HCDR3	LCDR3
CON	CARDANGMDVW	CQVWDSGSDHPWVF
S1	....	....
S3	....	.........Q....
G3	....	....
IT3	......A....	....
F3	......A....	....
F1	....R......	....
F2	....L......	.......T......
IT1	....PT.....	.....D.....Y..
IT4	....G......	....
S4	....G......	.........Q....
S5	....Q......	....
S6	....G......	....
S2	....Q.V....	.....G........
IT2	....M.A....	....
G1	....L.A....	......T.......
G2	....Q.A....	....

SUBSET #4 (IGHV4-34/IGKV2-30)

	HCDR3	KCDR3
CON	CARGYGDTAVTRRYYYYGMDVW	CMQGTHWP-YTF
G14	.V...A....V..........	........P...
G12	.....A..PTF..........	........-...
G13	.V...P....VK.......E..	......G.P...
G11	.....P..P.V..........	........P...
S10	.....PL...M..........	........-...
IT10	......T..T..........	........-...
S11	......S..T..........	........-...
G10	.....PLDT............	........-...
G15	.......SPD.K....F.L...	........-...
G16	......TSDD.....F......	....S...-...
F10	.......SSDV.......L...	........-...
G18	.....A.SD.I..........	........P...
G17	....WPED...........EI.	........-...
G19	......TS.T.K.........	.....Y..-...

Fig. 1. Highly similar CDR3s evident among unrelated CLL patients. Alignment of the heavy and light chain CDR3s of a selection of subset #2 and subset #4 cases from different European centers. CON, consensus sequence; K, kappa; L, lambda. A dot denotes the same amino acid as in the uppermost sequence [33,34].

appeared to be one exception to this rule. In 2002, our group first reported a proportion of CLL patients, making up 11% of the cohort, all displaying rearrangements of the *IGHV3-21* gene [27]. Moreover, alignment of the *IGH* sequences of these Scandinavian patients revealed an almost identical HCDR3 and the majority of patients carried a distinctively short nine amino-acid long HCDR3 matching, or differing only slightly from, the consensus motif DANGMDV [25,27]. Subsequent analysis of the light chain locus in these patients revealed a restricted usage of the *IGLV3-21* light chain gene which also displayed highly homologous lambda CDR3s (Fig. 1) [25]. These findings provided strong evidence that a common antigen epitope was being recognized by non-related *IGHV3-21* tumors. Another striking finding of this analysis was that patients carrying an *IGHV3-21* rearrangement had a poor clinical outcome regardless of their *IGHV* mutational status, and appeared to define a distinct clinical subgroup. Subsequent analysis of larger cohorts reconfirmed these findings, although it also became apparent that the frequency of *IGHV3-21* usage differed significantly between cohorts: analyses in Scandinavia and the UK reported frequencies of