

Role of the B-cell receptor in chronic lymphocytic leukemia: where do we stand?

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Summary

The past 15 years have witnessed an enormous effort in studying B-cell Chronic Lymphocytic Leukemia. A great number of researches brought significant novel information and a better understanding of the natural history of this disease.

This mini review will focus on the studies related to the Immunoglobulin variable (IgV) genes rearrangements that compose the B-cell receptor (BcR) of the leukemic clones. These studies have defined a role for the antigen(s) in the paths that lead to leukemic clone generation/expansion and underscore the informative value represented by BcR analyses.

Key words

Immunoglobulin Variable Region, Chronic Lymphocytic Leukemia, Antigen stimulation

Chronic Lymphocytic Leukemia

Chronic Lymphocytic Leukemia (CLL), the most prevalent adult leukemia in the western world, is characterized by the accumulation of mature B lymphocytes in the peripheral blood and lymphoid organs. CLL cells typically express CD5, CD23 and low level of surface Immunoglobulins (sIg; reviewed by Chiorazzi et al., 2005).

Although genetic abnormalities are described in up to 80% of CLL cases by fluorescence in situ hybridization (deletion at chromosomes 13q, 11q and 17p and trisomy 12) (Dohner et al., 2000) a common genetic background needed to develop this disease has not yet been identified.

The BcR of CLL

The first studies regarding IgV gene rearrangements that compose the BcR of the leukemic clones were undertaken on few sporadic cases during the 90's. The results initially suggested that CLL cells mainly carried IgV genes with no hypersonic mutations (Kipps, 1993) thus corroborating the hypothesis that these cells were derived from virgin B-cells and possibly from CD5⁺ B-cells.

However, the first study that considered a relatively ample cohort of CLL patients (83 cases) radically changed this view. Indeed, at least 50% of CLL cases carried a significant amount of somatic mutations at IgVH gene level (Fais et al., 1998). In addition, the IgV gene usage of the CLL clones appeared to be non-random, since certain

IgVH genes as well as IgVH/D/JH gene combinations were found at higher frequency compared to the normal B-cell repertoire (i.e. VH1-69, VH3-07 and VH4-34). These observations strongly suggested that antigen selection/stimulation plays a role in the natural history of CLL clone development.

The notion that at least 50% of CLL clones has gone through a process of hyper-somatic mutation also re-opened the question regarding the cell of origin for CLL. Indeed, later studies indicated that all CLL cases have a gene expression signature similar to memory rather than virgin/unstimulated B-cells (Damle et al., 2002; Rosenwald et al., 2001).

The mutational status of CLL BcR proved to be important also to predict disease evolution (Damle et al., 1999; Hamblin et al., 1999). Indeed, CLL cases having <2% of IgVH mutation, defined as unmutated (U), have a significantly shorter survival, and inferior response to treatment compared to mutated cases (M, having $\geq 2\%$ of deviation from the germline genes). The cut-off of 2% (corresponding to 6 IgVH nucleotide mutations) to discriminate between U and M cases was a conservative choice aimed at taking into account not yet identified allelic variants. However, this cut-off proved to be the best value capable to predict disease evolution (Davis et al., 2003). The better clinical outcome of M CLL cases may be related to a diminished reactivity with certain antigens that follows the modification of BcR structure (vide infra). Currently, the IgV mutational status is considered a reliable independent prognostic factor together with CD38 and ZAP-70 expression on leukemic cells.

As mentioned, a further step in the CLL BcR characterization was given from the identification of quasi-identical BcR carried by different CLL clones. This finding was quite impressive since the mechanisms generating the diversity at the IgVH/D/JH make it highly improbable that similar IgV gene rearrangements are detected by chance among independent B-cell clones.

The first two CLL cases disclosing quasi-identical BcR were described by Chiorazzi's group (Hashimoto et al., 1995) while studying a small cohort of IgG⁺ CLL patients. Details of this BcR structure have been reported later, describing additional 3 CLL patients with this same BcR (Ghiotto et al., 2004).

The term "stereotyped receptor", to indicate quasi-identical BcR, has been coined by Chiorazzi's group when describing a cohort of about 300 CLL patients in which several subgroups of similar receptors were observed (Messmer et al., 2004).

Nowadays, more than 100 subgroups of stereotyped receptor have been identified by BcR sequencing of thousands of CLL clones. It is estimated that almost 30% of CLL cases use a stereotyped receptor (Darzentas et al., 2010). However, BcR stereotypy is mainly observed in U CLL cases. Indeed, in several studies more than 40% of U CLLs were found to use stereotyped BcR. There are, however, some notable exceptions, e.g. subset 2 and subset 4 carry mutated IgV3-21 and IgVH4-34 genes respectively. In addition, stereotyped receptors preferentially use IgVH1 and IgVH4 families and few IgVH genes (i.e. IgVH1-69, IgVH1-2, IgVH1-3, IgVH3-21, IgVH4-34 and IgVH4-39).

From the clinical standpoint, patients with stereotyped receptor show homogeneous behavior. In some instances the clinical course is independent from the mutational status as, for example, for subset 2 (using IgVH3-21/IgVL3-21) that shows a borderline mutational status (around 2-3%) but has an aggressive behavior.

Thus, it appears that the BcR on leukemic cells may dictate disease outcome, likely by interacting with a relatively low number of different antigens. In recent years a

great effort has been made for the identification of the antigens recognized by BcR of CLL cells.

It was already known that CLL cells produce antibodies that bind autoantigens in a polyreactivity mode, similar to “natural antibodies” (Stoeber et al., 1989). In more recent studies (Herve et al., 2005) recombinant soluble CLL U antibodies were proven to have a polyreactive pattern (80% of antibodies tested) against self and foreign antigens, whereas in CLL M antibody polyreactivity was significantly less observed (15% of antibodies tested). However, it is of note that when M antibodies were back-mutated to the germline status polyreactivity was restored in most cases.

Recently, a few antigens reacting with CLL antibodies have been identified. These are frequently represented by cytoskeleton components (for instance vimentin and non-muscle myosin IIA: Catera et al., 2008; Chu et al., 2008) which can be exposed to cell surface during apoptotic processes. In addition, other molecules generated during apoptosis by oxidative processes can be recognized by CLL antibodies. Reactivity against bacterial cell wall components is often observed as well. In most cases antibodies recognizing apoptotic cells are derived from U CLL while somatic hypermutation process appears to lead to a progressive loss of BcR ability to recognize the above mentioned structures (Chu et al., 2010). Altogether these observations suggest that M CLL cases generally have a more favorable course because the BcR has diminished its reactivity against self-antigen(s) and the B-cell clone is therefore less stimulated. This would also imply that antigen stimulation plays a role not only in the selection of the B-cell clone that will become malignant but also during overt disease. As a matter of fact, intracлонаl diversification of IgV genes was demonstrated in a significant number of CLL cases, strongly suggesting that the leukemic clone is subjected to ongoing antigenic stimulation (Bagnara et al., 2006; Sutton et al., 2009).

It has also to be reported that the signaling machinery originating from the BcR seems to be impaired mostly in M CLL cases which would again justify a more benign course for these patients (Muzio et al., 2008).

Conclusion

The reactivity of CLL BcR (in particular the U ones) appears to be similar to that of the “natural antibodies” observed in normal serum. Natural antibodies are often produced by splenic marginal zone B-cells or B-cells resident in functionally analogue structures (sub-epithelial and sub-capsular B-cells in tonsils and lymph-nodes respectively). Therefore, this B-cell subset may represent the normal counterpart of CLL cells. Figure 1 proposes the sequence of events B-cells may go through to become leukemic.

Thus, the studies on the BcR have brought an important contribution to the understanding of CLL patho-physiology and may contribute in the future to the identification of an effective therapy.

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Figures

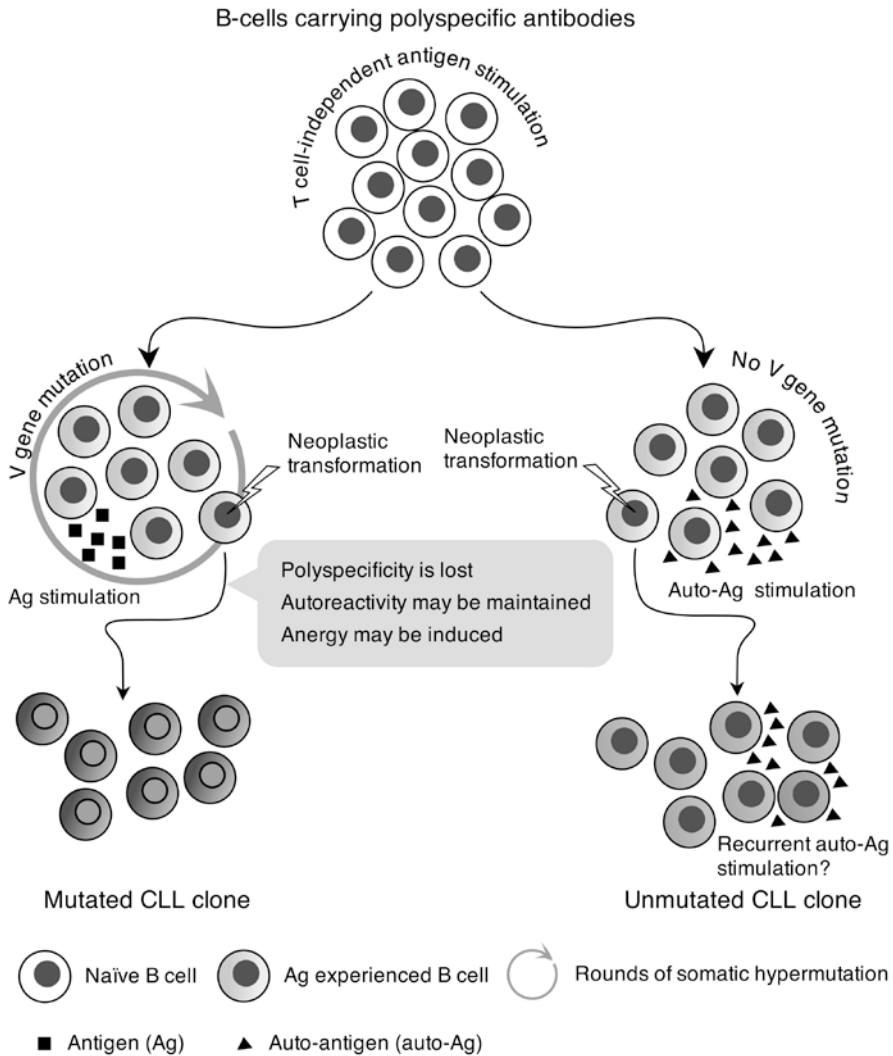


Figure 1 – A model describing the role of the B-cell receptor in the generation of the CLL clone.

B-cells carrying polyspecific antibodies are stimulated in a T-cell independent fashion. Some of these cells may bear (or acquire) a genetic lesion that allows prolonged survival.

These cells can later be recruited in immunological responses that require (or do not require) IgV hypersonic mutations, likely depending on the type of the (auto)antigens.

In the first case (on the left) the mutation process causes loss of polyreactivity. In addition, a more specific autoreactivity may arise possibly leading to an anergic state. This would justify a less aggressive course by cells that eventually become leukemic.

In the second case (on the right), B-cells are recruited in an immune response that does not require BcR mutation. After leukemic transformation these cells are still subjected to antigen stimulation (mostly autoantigens) and display, therefore, a more activated status and higher aggressiveness.