From Mice to Men: How B Cell Immunology Helped the Understanding of Leukemia Development

Paolo Ghia*

IRCCS Ospedale San Raffaele, Università Vita-Salute San Raffaele, Milan, Italy

The work on normal B lymphocytes, immunoglobulins, and antigenic stimulation performed at the Basel institute in the early '90s, also by Anton G. Rolink, shaped and molded the way B cell lymphomas and in particular Chronic Lymphocytic Leukemia (CLL) are interpreted today.

Words like *Immunoglobulin rearrangements*, *somatic mutations,* and *B Cell Receptors*, which 25 years ago were of interest only to basic immunologists like Ton, became common and trivial (if ever possible) terms also in hematological and clinical gatherings. Nowadays immunologists are in fact regularly invited to such meetings as key-note speakers to explain the basic functionality of the immune system, which has direct implications on the daily clinical practice.

During that pioneering period we also learnt that animal models were not merely a surrogate tool for human organisms, necessary only for obvious reasons of simplicity and convenience, but on the contrary that they may provide precise insights that could be directly applied to the human system. This turned out to be true in the case of the maturation process of B lymphocyte progenitors and precursors in the bone marrow, as we demonstrated that the developmental ordering of the different stages of differentiation in human bone marrow were superimposable to those described by Ton years earlier in mice, with only few small but potentially interesting differences in phenotypic markers (1, 2). In light of such evidence, additional observations on B cell aging in mice and men have also been made, pointing to a progressive decrease of B cell production with age in both organisms (3) and to a restriction of the diversity of the repertoire, as underscored by the appearance of clonal B cell populations consisting of plasma cells or mature (mainly CD5$^+$) B cells in the spleens of aged mice (4). Monoclonal Gammopathy of Undetermined Significance (MGUS) a premalignant phase for Multiple Myeloma (5) typical and common in the elderly, can be considered the human counterpart of murine monoclonal plasma cell expansions. At that time, no expansions of CD5$^+$ mature B cells were known in aging individuals. In a subsequent publication (3), we initially suggested that CD5$^+$ B cell clonal populations present in old mice might be considered analogous to precursors of Chronic Lymphocytic Leukemia (CLL), the most frequent B cell neoplasm in the elderly (6), which is characterized by clonal expansion of CD5$^+$ B cells and for which no pre-leukemic phase was defined.

Few years later, such reasoning justified a large observational study involving hundreds of healthy elderly individuals in a quest for clonal expansions of mature B cells using high-sensitive flow cytometric techniques, not in spleens or lymphoid tissues as in mice but in the best surrogate available in humans i.e., the peripheral blood (7). This led to the discovery of either CD5$^-$ or CD5$^+$ monoclonal B lymphocytes in almost 10% of individuals above 40 years of age with an increasing frequency associated with age, peaking at an impressive 50% in healthy individuals aged more than 90 years (8). Similar observations became at the same time available from the UK (9), Spain (10), and the US (11, 12), suggesting the universality of the phenomenon that has been later defined by an international consortium as Monoclonal B cell Lymphocytosis (MBL) (13), potentially involving

either CD5$^-$ or CD5$^+$ B lymphocytes. CD5$^+$ MBL is the most frequent, accounting for two thirds of all MBL, and is characterized by the presence of monoclonal B cells with a phenotype identical to CLL (CD20^{dim}, CD5$^+$, IG^{dim}) in the peripheral blood, at concentrations lower than those required for the diagnosis of CLL (5×10^9/l) (13). Later on, we reported that MBL can be further distinguished into "low-count" MBL (LC-MBL) and "high-count" MBL (HC-MBL), based on the number of circulating CLL-like cells (more or less than 0.5×10^9/l, respectively) (14). The former does not virtually progress into a clinically relevant disease and may be considered an interesting model to study B cell aging (15), while the latter may evolve into a clinically relevant CLL at a rate of 1% per year (9, 16) and may be considered a real pre-leukemic phase of CLL (12), with all the obvious clinical and pathogenetic implications. All these findings recently provided ground for the inclusion of MBL as a new disease entity within the classification of lymphoid neoplasms by the World Health Organization (WHO), similarly to MGUS (17). Nowadays, there are patients around the world diagnosed and followed-up because of the presence of MBL.

While MBL showed us, thanks to cross-fertilization between mouse and human immunology, how discoveries can sometimes be predictable, the progress of science remains unpredictable in other instances. This is best exemplified by Immunoglobulin genes, a unique exception to the gene-protein dogma in genetics because of the rearrangement process and the introduction of mutations, making them probably one of the most enlightening models of nature adaptation but also the least digestible concept to medical students and alike. The first study to show the developmental order of B progenitor and precursors of the B lymphocytes in the human bone marrow, performed at the Basel Institute under Ton's supervision, was entirely based on the description of the rearrangement status of the Immunoglobulin Heavy and Light chain gene loci, assessed at single cell level (2). In a pre-internet era, the gene sequences for all IG loci could only be collected one by one from printed text books and volumes and needed to be manually curated and aligned, a work that nowadays can be done online in a click. At that time, all that work appeared frustratingly far from the possibility of a concrete application in medicine. A few years later, that knowledge unexpectedly became crucial to better understand the pathogenesis of CLL, following the seminal observation that the presence of somatic mutations within the rearranged clonotypic IGHV genes associated with a better clinical outcome and prognosis in CLL patients (18, 19). This originated from the analysis of somatic hypermutation in neoplastic B cells to track back the cell of origin of mature and immature B cell neoplasms. Strangely enough, CLL, the easiest leukemia to diagnose with a typical and unique phenotype, was indeed a mixture of cases with and without somatic mutations within the IGHV genes, which puzzled all hematologists (and immunologists alike) (20). This observation fueled endless discussions and experiments aiming at clarifying the distinct origin of these 2 subsets of CLL while trying to explain the remarkable uniqueness of the surface phenotypic appearance (21). Only more recently several studies demonstrated that indeed the presence of somatic hypermutations was associated with deeper

and more prolonged responses to immunochemotherapy, in particular to the combination of fludarabine, cyclophosphamide, and rituximab (FCR), the gold standard for the treatment of young, fit CLL patients. IG-mutated patients experience long term remissions reaching a plateau on the PFS curve, with no relapses beyond 10 years, an unprecedented finding in a disease that is still considered incurable (22–24). This transformed the IGHV gene mutational status from a prognostic marker in CLL into a predictive factor, allowing for a better upfront selection of the patients who are more likely to benefit from the use of immunochemotherapy. Testing for IGHV gene mutations is now recommended in the recent update of the guidelines by the International Workshop on CLL (iwCLL) (25), where it is stated that the determination of the somatic hypermutation status of the IGHV genes should always be performed before deciding the treatment of a patient with CLL, as it has relevant implications on the choice of the most effective treatment. This is now probably one of the most prominent examples of personalized medicine approaches that is envisioned in many types of cancer but that in CLL has become reality. A validated consensus methodology for reliable clinical-grade analysis of IGHV genes has been established by the European research Initiative on CLL (ERIC) and moved out of research laboratories into diagnostic facilities (26).

From a scientific point of view, IG gene analysis also became central in the study of CLL pathogenesis and culminated with the creation, as part of an international consortium (IgCLL, www.igcll.com), of a world-wide database collecting to date over 30.000 IG sequences from CLL patients. This remarkable collection helped identify and consolidate the unexpected finding that different unrelated CLL patients share very similar if not identical sequence motifs within the—variable heavy chain complementarity determining region 3 (VH CDR3), thus defining "stereotyped" B-cell receptor (BcR) (27). This made it possible to group at least 30% of CLL patients into subsets with similar BcR that, besides similar immunogenetic features, also share similar molecular and functional features and clinical prognosis beyond the simplistic dichotomy of mutational status (28). From an immunological point of view, the presence of stereotypy suggests the recognition of common structures, thus implying that CLL ontogeny is not stochastic but rather driven by interactions between the clonogenic cells and a restricted set of antigenic elements (27).

Thanks to all these molecular findings, it became evident that immunoglobulins and in particular the entire B cell receptor are central players in CLL pathogenesis, even overshadowing in some occasions the role of genetic aberrations. At variance with many other B cell neoplasias characterized by a single distinct genetic abnormality, a number of gross genomic abnormalities including deletion 11q, 13q, 17p, and trisomy 12, are found in different proportions of CLL cases (29). Moreover, high-throughput studies have revealed a further remarkable genetic heterogeneity, with certain genes (*NOTCH1*, *SF3B1, TP53*) mutated in only 10–15% of patients (30–32) and others mutated at even lower frequencies (e.g., *NFKBIE, RSP15, EGR2*) (33). No unifying genetic mechanisms or lesions have thus been identified insofar.

On the contrary it has been demonstrated that the signaling pathway downstream of the BcR is active in all cases of CLL, regardless of the mutational status and immunogenetic features of the patient (34). This is particularly true in the leukemic cells obtained from lymph nodes, where CLL cells are believed to encounter the relevant antigenic elements in the context of the so-called *proliferation centers,* considered the reservoir of the disease: there, leukemic cells get to proliferate and expand also thanks to additional signals originating from T cells, stromal cells and monocytic-derived cells, or through other immune receptors such as the TLRs.

In a proportion of cases, a number of foreign or auto-antigens (35, 36) have been identified as able to interact and stimulate the leukemic BcR, in particular neoantigens, newly exposed in the context of apoptotic bodies produced during physiological cell turnover. However, the notion that classic auto-antigen recognition and binding is important for CLL ontogeny has been challenged by the recent demonstration of cell-autonomous signaling, a novel type of signal generation, occurring specifically in CLL amongst B cell lymphomas, through self recognition as a result of the interaction of the leukemic BcR IG with a conserved epitope of the same or adjacent BcR IGs (37). Autonomous signaling has first been demonstrated in B cell precursors where the pre-BCR has evolved to ensure self-recognition, allowing for positive selection at the pre-B cell stage (38). Interestingly, different portions of the Immunoglobulin are recognized by different stereotyped receptors, each with affinities that appear to associate with distinct clinical outcomes and/or biological responses (39). This heterogeneity resembles the long-known evidence that antigen-dependent stimulation in CLL may also be pleiotropic and may associate with clinical outcome, ranging from full activation and proliferation to anergy and survival (40). The former appears to be typical of more aggressive CLL, particularly with unmutated IGs, while the latter associates with more indolent cases, usually with mutated IGs, thus providing a functional basis for the clinical heterogeneity of the disease. It still remains to be elucidated how antigen-dependent and autonomous signaling cooperate in the onset and maintenance of CLL.

Despite the molecular evidence of the centrality of the BcR in CLL, the final proof that the BCR stimulation is crucial in all cases of CLL, regardless of the differences in mutational status, antigenic affinities or strength of autonomous signaling, has come from the impressive and virtually universal efficacy of the therapeutic inhibition of the BcR signaling pathway in CLL patients.

The accumulating knowledge of the biology and immunology behind CLL has been applied to the research for innovative therapies, leading to the design and approval of novel mechanism-based drugs such as ibrutinib (41) and Idelalisib (in association with the anti-CD20 antibody Rituximab) (42), targeting molecules downstream the BcR, namely BTK and PI3Kδ. Both drugs have shown greater efficacy and better tolerability than chemoimmunotherapy and have even dramatically changed the prognosis of high-risk CLL patients, including those with *TP53* aberrations.

Thanks to the immunological knowledge (and human wisdom) that has developed at the Basel Institute also thanks to Ton Rolink, all physicians may find themselves less at loss in a changing world of hematology where immunology has become central for understanding neoplastic diseases to the benefit of the patients. As unexpected as this was, it has become reality and it should encourage us all to be in part immunologists if we want to better understand the pathogenesis of leukemias and lymphomas. And we should all teach future generations to do so with fun every day!

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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