Clonal non-malignant hematological disorders: unraveling molecular pathogenic mechanisms to develop novel targeted therapeutics

Antonio M. Risitano[1] and Carmine Selleri[2]
[1]Hematology, Department of Clinical Medicine and Surgery, Federico II University of Naples, Italy;
[2]Hematology and Hematopoietic Stem Cell Transplant Center, Department of Medicine and Surgery, University of Salerno, Italy.
(cselleri@unisa.it)

Abstract - **Clonal non-malignant hematological disorders are a heterogeneous group of diseases that are particularly challenging for hematologists. Indeed, most obvious and frequent hematological diseases include a broad spectrum of malignancies, such as leukemias, lymphomas, myeloma, and other myeloproliferative or lymphoproliferative disorders.**

In recent years, all these diseases have been categorized by the WHO according to a novel classification of myeloid and lymphoid malignancies, which takes in account the outstanding progress in our understanding of molecular defects underlying hematological malignancies. Regardless of a number of novel technologies, hematologists continue to deal daily with conditions where a clear diagnosis of a malignancy is missing: this is the case of several clonal hematological disorders, which are considered *bona fide* non-malignant.

Some myelodysplastic syndromes, chronic T and NK disorders of granular lymphocytes, myelofibrosis, monoclonal gammopathies, monoclonal B-cel lymphocytosis, mastocytosis and paroxysmal nocturnal hemoglobinuria are paradigmatic examples of how clonal disorders are clearly different from cancers, even if they may share with hematological malignancies similar molecular, genetic, epigenetic and immunological processes. Indeed, it is not entirely clear whether in individual conditions such pathogenic mechanisms may represent initial step(s) of malignant transformation, making a bridge between these clonal non-malignant disorders and typical hematological cancers. Some of these non-malignant disorders imply specific pathogenic mechanisms and/or clinical course, and so they have been definitely established with their own biological and clinical identity. However, the obvious question whether some of these clonal non-malignant hematological diseases form some a kind of disease-continuum with their corresponding malignant counterpart is still to be answered.

Keywords: clonal non-malignant hematological disorders, MDS, chronic T and NK disorders of granular lymphocytes, paroxysmal nocturnal hemoglobinuria.

EDITORIAL VIEW

This issue of Translational Medicine @ UniSa was conceived in the aim of providing a cutting-edge about our understanding of pathophysiology, clinical aspects, therapeutic strategies and possible malignant evolution of these clonal non-malignant hematological disorders.

Clonal non-malignant hematological disorders have continued to shake the thoughts of several researchers [1]. Whereas for most non-hematologists clonality implies cancer by definition, in hematology clonality seems to be associated with physiological of quasi-physiological processes. Indeed, recent and old studies have documented that hematopoiesis itself can be considered less polyclonal then initially thought: in fact, it has been demonstrated that even in healthy individuals the active hematopoietic stem cell pool (which account for whole blood cell production, life-long) should not exceed a few hundreds (<500, according to Buesher et al) [2-4]. This pool of active hematopoietic stem cells may be even smaller in specific circumstances not necessarily considered a disease, such as after hematopoietic stem cell transplantation [5].

One of disease discussed in this issue, named Paroxysmal Nocturnal Hemoglobinuria, exhibits a unique condition where the whole hematopoiesis can be sustained life-long by a single hematopoietic stem cells, which quite surprisingly does not show over time any exhaustion nor malignant transformation [6-7].

More commonly, oligoclonal hematopoiesis is the hallmark of myelodysplastic syndromes (MDSs) and other bone marrow failures, and is actually considered a first step toward to malignant transformation and possible leukemias. However, as extensively discussed in this issue, MDSs may sometimes imply pathogenic mechanisms which not necessarily include (at least initially) an obvious malignant transformation [8-9].

Rather, some MDSs may share immune-mediated pathogenic mechanisms which are similar to those demonstrated in typical immune-mediated bone marrow failure (i.e., acquired idiopathic aplastic anemia). In this case, oligoclonal hematopoiesis may simply reflect the contraction of a stem cell pool which is undergoing an extrinsic damage, possibly by the immune system. Even in this context, the distinction between external pressure and possible intrinsic defects (malignant, or pre-malignant) is hard: in fact, genetic lesions can be detected by either karyotype analysis or more deep molecular analysis (up to full-exome sequencing, by next-generation sequencing).

It is intriguing that even in presence of these gene mutations it is impossible to establish their actual role in the pathophysiology of the disease; in fact, with the exception of gene lesions known for their specific pathogenic role, most of these genetic defects could be detected just for the presence of a clonal hematopoiesis, becoming dominant over residual hematopoiesis for different reasons (e.g., immune selection, random damage of polyclonal hematopoietic stem cells, etc). In other words, even a mutation with no pathogenic role (i.e.,

neutral; as well as any other mutation) may become evident (i.e., fixed) just because it occurs in the context of an oligoclonal hemopoiesis (this process is often described as "neutral fixation", or "founder effect"). A clear evidence supporting this possibility is the observation of self-limiting or even transient karyotipic abnormalities demonstrated in aplastic anemia [10] or even in paroxysmal nocturnal hemoglobinuria [11].

So if (oligo-)clonality may be considered a possible finding in the hematopoietic compartment, without necessarily implying the presence of a leukemia, its meaning in the context of the immune system is even more intriguing. Indeed, acquired or adaptive immunity has evolved to improve the protection from pathogens, and it is based on the activity of highly specialized cells, mostly B and T lymphocytes, which work in a cell-based fashion as well as by fluid phase effectors (mostly antibodies, but also several cytokines).

It has to be reminded that the efficiency of acquired immunity lies on the possibility of selecting, via somatic mutation of specific genes, immune cells with a particular specificity for foreign antigens/pathogens. Thus, clonal expansion of immune cells (either B or T lymphocytes) is a common para-physiological phenomenon which occurs daily for an effective protection from pathogens.

There is a plethora of data demonstrating that both humoral or cellular responses against pathogens are clonal (for instance after vaccinations), and the ability of mounting such clonal responses correlates with the efficiency of protection. Thus, it is not surprising if this highly regulated mechanism may quite easily run into mistakes, leading to the selection of clonal cells which can be useless, or even detrimental for the whole organism. Given with this background, it is intelligible that clonality within the immune system may represent a uncontrolled, deranged, immune response, as well, as obvious, a cancer of immune cells. Indeed, oligoclonality, seen both as the presence of some (auto-)antibodies and/or of clonal B or more commonly T cells is considered as a surrogate marker of autoimmune disorders. In this issue some experts review clonal expansions of myeloid cells, plasmacells, B, T and NK cells, discussing the problems of differential diagnosis with malignancies of these cells [7-9,12-16].

Once again, they deal with pathogenic mechanisms which may share common pathways, finally concluding that some clonal conditions should not be considered cancers, even if a possible future malignant transformation cannot be ruled out.

Thus, even for the immune system, clonality *per se* is not cancer, but may represent an initial step possibly leading to malignant transformation: in other words, clonality (seen as the uncontrolled extreme of a physiological oligoclonality) may be considered a bridge between normal immune response and cancer.

The observation that lymphoproliferative disorders are possibly associated with some common pathogens (mostly viruses such as EBV, HBV, HCV, HTLV, HHV8, but also Helycobacter Pilorii) is a clear confirmation of this possible process, which on the other end remains largely unpredictable, given that most antigen-driven clonal expansion may persist as non-malignant, possibly leading to clinical manifestations which are independent from a malignant transformation (e.g., cytopenia of large granular lymphocyte expansions, paraprotein-associated symptoms, etc.).

In conclusion most (if not any) cancers are clonal, but not all clones are malignant: the reviews included in this issue describe different scenarios, and represent a useful guide to face the specific topics discussed, but even more to a thoughtful and critical view of possible cases that a hematologist may observe during his professional life.

REFERENCES

[1] WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Fourth Edition. Edired by the World Health Organization, 2008.

[2] Buescher ES, Alling DW, Gallin JI. Use of an X-linked human neutrophil marker to estimate timing of lyonization and size of the dividing stem cell pool. J Clin Invest 1985;76:1581–1584.

[3] Shepherd BE, Guttorp P, Lansdorp PM, Abkowitz JL. Estimating human hematopoietic stem cell kinetics using granulocyte telomere lengths. Exp Hematol 2004;32:1040–1050.

[4] Dingli D, Pacheco JM. Ontogenic growth of the haemopoietic stem cell pool in humans. Proc Biol Sci 2007;274:2497–2501.

[5] Nash R, Storb R, Neiman P. Polyclonal reconstitution of human marrow after allogeneic bone marrow transplantation. Blood 1988;72:2031–2037.

[6] Nishimura Ji J, Hirota T, Kanakura Y, Machii T, Kageyama T, Doi S, Wada H, Masaoka T, Kanayama Y, Fujii H, Inoue N, Kuwayama M, Inoue N, Ohishi K, Kinoshita T.Long-term support of hematopoiesis by a single stem cell clone in patients with paroxysmal nocturnal hemoglobinuria. Blood 2002;99(8):2748-2751.

[7] Risitano AM. Anti-complement treatment in paroxysmal nocturnal hemoglobinuria: where we stand and where we are going. Transl Med UniSa 2013;8: 43-52

[8] Visconte V, Selleri C, Maciejewski JP, Tiu RV. Molecular Pathogenesis of Myelodysplastic Syndromes. Transl Med UniSa 2013:8. [Epub ahead of print].

[9] Serio B, Risitano AM, Giudice V, Montuori N, Selleri C. Immunological derangement in Hypocellular Myelodysplastic Syndromes. Transl Med UniSa 2013;8: 31-42

[10] Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. Blood 2002;99(9):3129-3135.

[11] Araten DJ, Swirsky D, Karadimitris A, Notaro R, Nafa K, Bessler M, Thaler HT, Castro-Malaspina H, Childs BH, Boulad F, Weiss M, Anagnostopoulos N, Kutlar A, Savage DG, Maziarz RT, Jhanwar S, Luzzatto

L. Cytogenetic and morphological abnormalities in paroxysmal nocturnal haemoglobinuria. Br J Haematol 2001;115(2):360-368.

[12] Tabarroki A, Tiu RV. Molecular Genetics of Myelofibrosis and its associated Disease Phenotypes. Transl Med UniSa 2013;8: 53-64

[13] Magliacane D, Parente R, Triggiani M. Current Concepts on Diagnosis and Treatment of Mastocytosis. Transl Med UniSa 2013;8: 65-74

[14] Palladino C, Bruno B, Boccadoro M. Discovering the meaning of monoclonal gammopathy of undetermined significance: current knowledge, future challenges. Transl Med Unisa 2013;8: 12-18

[15] D'Arena G, Musto P. Monoclonal B-cell lymphocytosis. Transl Med Unisa 2013;8

[16] Zambello R, Teramo A, Gattazzo C, Semenzato G. Are T-LGL leukemia and NK-chronic Lymphoproliferative disorders really two distinct diseases? Transl Med UniSa 2013:8: 4-11