**Recent Advances in the Cytobiology of Leukemias**

**CD10 IN ACUTE LEUKEMIAS**

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**ABSTRACT**

**Background and Objective.** CD10, initially known as cALLA, was identified as one of the earliest markers expressed by leukemic cells of the lymphoblastic lineage. This review summarizes what has been discovered about this ubiquitous molecule since anti-cALLA antibodies allowed its detection on leukemic cells. It also attempts to specify the selectivity of CD10 in acute leukemias as a subclassification or prognostic tool.

**Evidence and Information Sources.** The material used for this review includes articles identified as relevant through a meta-analysis in the Medline database, as well as personal publications or data issued within the French Study Group on the Immunology of Leukemias (GEIL).

**State of Art.** CD10 now stands as much more than a mere leukemic marker. It belongs to a rather large family of exopeptidases expressed in a variety of tissues and mostly involved in the activation or deactivation of peptides through the removal of terminal amino acids. CD10 expression on leukemic cells remains a useful subclassification tool for B-lineage leukemias, but it can also be found on other types of leukemic cells.

**Perspectives.** Much remains to be discovered regarding the physiological role of CD10 during the maturation of normal B-lineage lymphocytes and about its functions - or lack of activity - on leukemic cells. It could also provide a valuable tool for further dissecting B-lineage leukemias when the quantitative aspect of its expression on blast cells is taken into account.

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**CD10: the molecule and its functions**

The neutral endopeptidase NEP, or KII-NA, is coded by a gene located on chromosome 3. Cloning of this gene revealed it was identical to CD10. It also allowed the production of transfected COS cells that were able to display surface enzymatic activity. CD10 is also known as enkephalinase and, more recently, acquired the official name of Neprilysin or EC 3.4.24.11. It is a 90-100 Kd transmembrane type II molecule (with an intracellular N-terminus and an extracellular C-terminus) expressed as a single chain of 795 amino acids (Figure 1). The intracytoplasmic tail consists of only 24 amino acids. Among the 700 amino acids of the extracellular portion, there are 12 cystein residues, which suggests that numerous intrachain disulphide bonds may exist. There also are numerous potential glycosylation sites, and the position of at least 3 zinc-binding sequences has been identified. CD10 is indeed a metallo-peptidase, requiring Zn cations to be efficient. The enzymatic activity is located in a 5-amino-acid portion (His-Glu-[Ile,Leu,Met]-Xaa-His) similar to that found on other exopeptidases. Indeed CD10 belongs to a family of at least 3 other exoenzymes: aminopeptidases A and N, and dipeptidylpeptidase IV. Amazingly, aminopeptidase N is also known as CD13 and dipeptidylpeptidase IV as CD26. It might not be fortuitous that CD10, CD13 and CD26 are present on activated mature cells of the three major lineages: respectively, B cells, myeloid cells and T cells. Carboxypeptidase M and angiotensin conversion enzyme also belong to this large family of exopeptidases.

CD10 is specialized in the cleavage of 1-3 N-terminal amino acids from peptides, preferably cleaving neutral amino acids such as valine, iso-leucine,...
phenylalanine, leucine or alanine. Many physiological activities of CD10 can be deduced from the list of its known substrates reported in Table 1. Since the major expression sites of this molecule are the brush border of enterocytes and renal tubules and glomeruli (Figure 2), it is likely that CD10 is actively involved, respectively, in the degradation of small food-derived peptides and of renal mediators. CD10 also plays an important part in the maturation of the lung through the cleavage of bombesin-like peptides. In the brain, it was initially described as enkephalinase since it is able to inactivate the opioid peptides met- and leu-enkephalins. On circulating cells, CD10 is actively involved in the regulation of chemotactic and inflammatory processes involving neutrophils. The association of CD10 and CD13 on the surface of polymorphonuclears allows them to hydrolyze and inactivate such chemotactic peptides as fMet-Leu-Phe. Soluble CD10 could be involved in the regulation of the kininogen-kinin system, since KII-NPE is also a kininase that is able to inactivate both kallidin and bradikinin within 30 seconds of their release from kininogen.

In the hematopoietic system, CD10 regulates stromal cell-dependent B lymphopoiesis, either by inactivating B-stimulating peptides or by activating inhibitory propeptides (Figure 3), although these specific substrates have not yet been identified. Furthermore, this activity seems to be specific for the human hematopoietic system because similar observations could not be made in some mouse models, although other authors have reported CD10/NAP expression on mouse Thy-1+ progenitors. Indeed CD10 expression, which could be involved in early B-cell proliferation, also seems to be increased on thymocytes, perhaps through contact with thymic epithelial cells, and might be involved in T-cell differentiation and maturation as well. It has clearly been demonstrated to be able to increase T cell IL-2 production. As for CD13 and CD26, which are found on functionally mature cells, CD10 expression is not limited to early B (or T) cells. Indeed CD10 re-expression has been reported on physiologically mature B cells in germinal centers as well as on myeloma and lymphoma cells. Therefore CD10, like other exopeptidases, seems to be involved in late stage regulation of hematopoietic cells.

CD10 and acute leukemias

B-lineage ALL

As mentioned above, CD10, which was originally known as cALLA, was initially investigated using the polyclonal antiserum produced by Melvin Greaves in rabbits. Monoclonal antibodies were soon developed and cALLA acquired a CD number at the first workshop on leukocyte differentiation antigens. Not all CD10 antibodies are similar; about 18 different clones are currently commercially available, and they may yield different results both on leukemic and non leukemic cells. That CD10 was not the ALL antigen was gradually discovered, even before the identicalness between CD10 and the neutral endopeptidase was evidenced. CD10 was discovered to be physiologically present on a small subset of maturing B cells in human bone marrow, and is in fact transiently expressed during B-cell differentiation. This is consistent with the possible roles of CD10 in the regulation of differentiation and maturation of peptides or propeptides. This is not true however in murine models.

In B-lineage leukemias, CD10 expression is involved in the definition of EGIL B-I versus B-II stages: it must be absent from B-I blasts and present on B-II blasts. Analysis of large series of leukemias, as in the GEL, shows that the percentage of CD10-positive cases decreases in more mature forms of B-lineage ALL (Figure 4). This was also reported on normal lymphocytes during their maturation. CD10 is supposed to be expressed during the first stages of heavy chain gene rearrangement and can be co-expressed with surface µ-VpreB or µ-λ 5 pre-BCR.

A quantitative analysis of CD10 expression, this
time in terms of CD10 molecules on blasts, was performed within the GEIL using the QIFI-kit, and this also showed decreasing CD10 cell density on more mature blasts. Similar data have also been reported by others.

**CD10-negative B-lineage ALL**

CD10 expression on B-lineage leukemias defines the largest group of ALL, whether they be classified as B-II or a later differentiation stage. The absence of CD10 therefore defines a peculiar subtype of ALL that deserves special attention. The incidence of B-I ALL is difficult to determine in the literature, owing both to discrepancies in clinical studies and to frequent reports of mixed series of patients described without sufficient immunophenotyping criteria to define sIg-/cµ- B-I cases among CD10- patients. Moreover, such patients usually represent small series of individuals.

Figure 5 reports data from 4 studies and demonstrates that the incidence of B-I ALL cases varies from 5 to 55% among series. Pui’s pediatric report emphasizes a higher incidence in infants, although a lower rate was observed in Garand’s GEIL study. All studies agree on a rate of approximately 5% in children between the ages of 1 and 16 years. B-I ALL, however, also occur in adults, with a similar incidence in French and German studies (around 15%). Specific characteristics of B-I ALL have seldom been analyzed in detail. A GEIL study on 64 consecutive patients reported clinical features similar to those of patients diagnosed as suffering from B-II ALL and significantly different from T-ALL patients, but with an outcome more comparable to that of T-ALL in both children and adults. This con-
firms previous reports about the poor outcome of these patients\(^{33,43}\) and reinforces the importance of properly diagnosing this subtype of ALL, which involves not only showing the absence of CD10 but also demonstrating the intracytoplasmic absence of immunoglobulins.

Other immunophenotypic peculiarities of B-I ALL observed in the GEIL series are summarized in Figure 6. These include a lesser expression of DR, CD24 and CD34, and a higher incidence of variants of biphenotypic ALL, mostly because of the aberrant expression of myeloid lineage markers. Gene analyses show relatively frequent IgH and sometimes \(\text{V}_{\beta}\) rearrangements, suggesting that anomalies in the rearrangement machinery may have stopped cell differentiation.\(^{34}\)

Great emphasis has been given in several reports to the relationship between a B-I ALL immunophenotype and chromosome 11 abnormalities, especially \(t(4;11)\). Indeed many \(t(4;11)\) or \(11q23\) anomalies are associated with this ALL subtype;\(^{43,44}\) however, a reverse analysis, i.e. the incidence of chromosome 11 alterations within the B-I ALL subtype, shows a weaker association. The percentages of B-I ALL with \(t(4;11)\) range between 30% and 50% in literature series. In the GEIL series, 25% of B-I ALL patients available for karyotypic analysis presented a \(t(4;11)\), and 31% of the patients had anomalies involving chromosome 11. However, no difference was noted in the clinical, immunophenotypic or evolutionary characteristics of these patients as compared to ones with a normal karyotype or other anomalies. This suggests that it might be more important to diagnose B-I ALL on immunophenotypic criteria than on karyotypic features, and that all B-I ALL patients could benefit from better adapted therapeutic protocols.
CD10 in acute leukemias

The expression of CD10 in other types of AL has been described in several series.\textsuperscript{11-14} It does not seem to be a rare feature in T-ALL, with similar incidences of about 30% being reported in the literature (Figure 7). This could be related to the possible physiologic roles of CD10 in normal thymic maturation and on activated T cells. Nevertheless, although CD10 is definitely an important and well-expressed surface molecule on polymorphonuclears, AML cells very seldom appear to be CD10\textsuperscript{+}, with between 0 and 10% incidence given in literature series.

Conclusions

Today CD10 definitely has a different image from that of the ALL common antigen known to hematologists and immunologists. It appears as a ubiquitous and functionally important exoenzyme, the expression of which is certainly not restricted to maturing B cells. Much however remains to be investigated regarding the functions of CD10 during physiologic B-lineage differentiation. This involves the identification of its peptide substrates, as well as further investigation of its possible relationship with other enzymes physiologically associated with CD10 or its fellow exopeptidases, such as the carboxypeptidases,\textsuperscript{47} CD38,\textsuperscript{47} CD73\textsuperscript{48} or CD26-associated adenosine deaminase.\textsuperscript{49} All these other compounds could be involved either in activating or deactivating growth factors, or in providing material and/or energy for dividing cells.

Better knowledge of these interactions could perhaps shed some light on the still obscure mechanisms of leukemogenesis. Indeed CD10 peptidase activity might be relevant in the leukemogenic process of B-II ALL, which remain the most frequent type of ALL.

It also appears to be important to evaluate properly the absence of CD10 on B-lineage ALL, since B-I ALL clearly represent a subgroup with poor prognosis. Finally, further developments may be expected from a quantitative approach to assessing CD10 expression on leukemic cells.

References

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