Contrasting antigenic maturation patterns in M0–M2 versus M3 acute myeloid leukemias

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Abstract

Acute myelogenous leukemia (AML) is divided into 8 FAB subgroups based on differentiation and maturation properties of the neoplastic cells. Acute promyelocytic leukemia (APL), or M3 AML, is associated with disseminated intravascular coagulation (DIC). Flow cytometric immunophenotyping differentiates among the AML subtypes. Key markers in this classification include the myeloid antigens CD13 and CD33 and the hematopoietic precursor markers CD34 and HLA-DR. The present study analyzes and compares differences in the expression of these markers in 27 M0–M2 cases and 8 M3 cases. The M0–M2 cases generally expressed all four antigens. CD13 and CD33 were positively expressed in 23 (85.2%) and 21 (77.8%) of the 27 cases, respectively. CD34 and HLA-DR were present in 25 (92.6%) and 26 (96.3%) of the 27 cases, respectively. Analysis of the M3 cases revealed a different immunophenotype as CD13 and CD33 were each positive in all 8 (100%) M3 AML cases while CD34 and HLA-DR were negative in 6 (75%) and 8 (100%) of the 8 M3 cases, respectively. In contrast to expression of the early markers CD34 and HLA-DR in the M0–M2 group, these were negative in the M3 cases which were characterized by heterogeneous CD13 and generally homogeneous and bright CD33 expression.

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Introduction

As a broad category of hematological malignancy, acute myeloid leukemia (AML) is defined as a condition in which myeloid blasts have clonally expanded in the blood, bone marrow, or other tissue (Jaffe et al., 2001). However, due to the nature of hematopoiesis there are numerous points along the differentiation process of myeloid cells where the expansion of leukemic cells of various maturation stages is possible. In 1976, the French–American–British cooperative group (FAB) published guidelines for the classification of AML into six subclasses, M1–M6, which were based upon both the differentiation and maturation patterns of the leukemic cells (Bennett et al., 1976). With increased scientific knowledge and the advancement of technological methods, this classification has been revised over the years to incorporate further distinguishing marks of subclasses of AML, including proposals of the M0 and M7 groups (Bennett et al., 1985, 1991). Though separate entities themselves, the M0–M3 subclasses all demonstrate some degree of differentiation which primarily affects granulocytes and thus differ mainly in their respective maturation stages along the granulocytic pathway (Bennett et al., 1976).

Minimally differentiated acute myeloid leukemia (M0) is the least differentiated form of AML and bears resemblance morphologically to the L2 and L1 subclasses of acute lymphoblastic leukemia (ALL). The diagnosis of M0 requires more than morphological evaluation alone where myeloid antigen expression demonstrated by reactivity with monoclonal antibodies specific for myeloid associated antigens, such as CD13 and CD33, along with the lack of reactivity towards the majority of antigens of lymphoid origin is considered to be strong evidence for minimal myeloid differentiation and thus M0 AML (Bennett et al., 1991). Myeloblastic leukemia without maturation (M1) shows evidence of differentiation along the granulocytic pathway without demonstrating any further maturation whereas myeloblastic leukemia with maturation (M2) shows both
granulocytic differentiation and maturation of cells to promyelocytes and beyond. Acute promyelocytic leukemia (M3) is characterized by a large population of abnormal promyelocytes where the majority of cases are hypergranular with a minority of cases presenting as a microgranular variant form of M3 AML. The prompt and correct diagnosis of APL is especially important due to the dangerous clinical association of APL with disseminated intravascular coagulation (DIC).

Upon presentation, flow cytometric analysis of the blast population provides an efficient means of analyzing the antigenic repertoire of the leukemic cells. In AML some of the antigens which are most consistently expressed by the leukemic cells are CD13, CD33, CD34, and HLA-DR (Terstappen et al., 1992; Reading et al., 1993). However, these are also the antigens regularly expressed by normal precursor cells of the hematopoietic lineage, thus complicating immunophenotypic analysis in states of remission (Reading et al., 1993). When describing antigen expression by leukemic cells in flow cytometry, there are two principal patterns of expression distribution which are generally related to the differentiation or maturation within the population. Homogeneous expression is used to describe the pattern of distribution in which there is a discrete population of cells demonstrating expression, and in theory is usually related to a population of cells which is relatively uniform in distribution as would be expected at the maturational end stage of that population. Heterogenous expression describes the pattern of distribution expression where the population is not dense and compact, but instead more irregular and spread out, spanning over a more extensive area and indicating a cell population which is both maturing and differentiating (Terstappen et al., 1992). In addition to describing the antigenic expression patterns as homogeneous or heterogeneous, flow cytometric analysis allows the intensity, i.e., brightness, of expression to be evaluated. If the population of cells is found to be expressed in the first decade only, antigen expression is considered to be negative. If the population of cells extends past the first decade and antigen expression is thus positive, it is classified as being dimly, moderately, or brightly expressed based upon the furthest decade into which the population of cells extends.

Materials and methods

Thirty-five cases of M0–M3 acute myelogenous leukemia (AML) were diagnosed over the course of 3 years in the Department of Pathology at the University of Mississippi Medical Center where twenty-seven of the cases were classified as M0–M2 AML and the remaining eight were classified as M3 AML. These cases were diagnosed based on morphology, immunophenotype, and clinical evaluation. Immunophenotyping was performed by analysis of peripheral blood samples collected in EDTA, analysis of bone marrow aspirates, and lymph node cell preparations by flow cytometry (Epics XL and FC500, Fig. 1. Flow cytometry histograms representing cases of acute myelogenous leukemia used in this study: (A) positive expression of both CD13 and HLA-DR in M0–M2 AML; (B) expression of both CD33 and CD34 in M0–M2 AML; (C) heterogeneous expression of CD13 and absence of HLA-DR in M3 AML; (D) homogeneous bright expression of CD33 and absence of CD34 in M3 AML.

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Beckman and Coulter, Miami, FL). Standard techniques were used in the processing of samples. The relationships between antigen expression and FAB classification were analyzed by Fisher’s exact test where all calculations were executed using the SigmaStat statistical program.

Results

Among the 35 cases of AML in this study, 27 were of the M0–M2 classification and the remaining 8 were of the M3 classification. Of the 27 M0–M2 AML cases, CD13 demonstrated expression in 23 (85.2%) cases. Expression of CD33 was observed in 21 (77.8%) of the 27 cases, respectively. Among the M0–M2 AML cases in this study, CD34 and HLA-DR were expressed in 25 (92.6%) and 26 (96.3%) of the 27 cases, respectively. In the 8 cases of M3 AML, CD13 was expressed in all 8 (100%) cases where it was generally observed to be expressed heterogeneously. CD33 was also expressed in all 8 (100%) cases of M3 AML, and the expression of CD33 was noted to usually be both bright and homogeneous. CD34 was not present in 6 (75%) of the 8 M3 AML cases, and HLA-DR was negative in all 8 (100%) cases of M3 AML (Fig. 1).

The expression of CD13 and CD33 in M0–M2 cases was found to have no statistically significant difference when compared to expression level in M3 cases, where \( P = 0.553 \) and \( P = 0.29 \) respectively. The expression of CD34 in M0–M2 cases versus expression of CD34 in M3 cases did show a statistically significant difference \( P < 0.001 \). HLA-DR expression observed in M0–M2 cases differed significantly statistically when compared to the expression of HLA-DR in M3 cases \( P < 0.001 \).

Discussion

Due to the great degree of heterogeneity which is displayed by AML, the practice of immunophenotyping in AML is somewhat more difficult and interpretation less clear than in other hematological malignancies such as ALL. However, immunophenotyping of AML by flow cytometry is of value in the utilization of monoclonal and polyclonal antibodies which are specific for both different paths of differentiation and for different stages of maturation and thus the differentiation and maturation of the subclass of AML. CD13, CD33, CD65, CD117, and anti-myeloperoxidase are all of pan myeloid specificity. For cases of AML which show differentiation that is not granulocytic, there are antibodies with specificities to detect differentiation along monocytic, erythroid, and megakaryocytic pathways. Regarding maturation of the blast cells, reactivity with antibodies for CD34, HLA-DR, and anti-TDT shows specificity for immaturity whereas reactivity with antibodies to CD15 and CD11b shows specificity for maturation of cells (Bain et al., 2001).

While the diagnosis of AML and its further classification is generally dependent on several methods including morphology and cytochemistry, immunophenotyping is also significant. Immunophenotyping is especially useful in the distinction of M0 AML, which is minimally differentiated, from acute lymphoblastic leukemias, as well as in the identification of M7 AML. Additionally, immunophenotyping can provide information on both the maturation and differentiation of malignant cells and is thus used to reveal characteristic antigenic expression in many of the remaining subclasses. Since subclasses M0–M3 are of the myeloid differentiation, they all generally express the myeloid antigens CD13 and CD33. The distinguishing differences of antigenic expression and FAB classification within the M0–M3 subclasses of AML have been noted to be the lack of precursor antigens CD34 and HLA-DR in M3 cases compared to their presence in the M0–M2 cases of AML (Bennett et al., 1988; Campos et al., 1989; Terstappen et al., 1992; Jennings and Foon, 1997; Venditti et al., 1997; Manaloor et al., 2000).

CD13 is a myeloid-associated antigen which is found to be expressed by macrophages, monocytes, granulocytes, endothelial, epithelial, stem cells and precursor cells. CD33 is a pan myeloid marker which is found to be expressed by macrophages, monocytes, granulocytes, and stem cells and precursor cells (Cruse et al., 2004). In the assignment of leukemias to different differentiation pathways, CD13 and CD33 expression is reliable evidence of myeloid differentiation. Thus, monoclonal antibodies to these myeloid antigens have been established to be of significant worth in the diagnosis of AML, whether they are utilized in the initial determination or subsequent confirmation of the diagnosis (Bennett et al., 1988). In a study by Manaloor et al. (2000), CD33 was found, by flow cytometric analysis, to be more intensely positive than CD13.

CD34 is an antigen expressed mainly by hematopoietic precursor cells. Of clinical significance in numerous hematological malignancies, it has been observed that CD34 expression is correlated significantly with the expression of the multidrug resistance gene (MDR-1) product P-170 and thus that expression of CD34 by malignant cells carries a poor prognosis (Campos et al., 1992; Jennings and Foon, 1997). Additionally, in one study it was found that in the cases studied, the expression of P-170 held no significant relationship with FAB classification except for the M3 subclass where negative P-170 expression levels were more common (Sato et al., 1990; Campos et al., 1992). Since the M3 subclass is also associated with the general absence of CD34, this is further evidence of the correlation between CD34 and drug resistance. HLA-DR is a class II histocompatibility antigen where major histocompatibility complex (MHC) class II molecules are expressed on B cells, macrophages, and a few other cell types (Cruse et al., 2004). The negativity of HLA-DR has been reported to be significant as a marker in the identification of AML as FAB M3 (Jennings and Foon, 1997; Kaleem et al., 2003).

Minimally differentiated AML, M0, shows expression of at least one pan myeloid antigen by the blast population, with more than one myeloid antigen often being expressed. CD13 and CD33 are two of the myeloid antigens which are usually present in M0. As M0 is the least differentiated form of AML and thus the most immature, most cases demonstrate expression of hematopoietic precursor markers such as HLA-DR and CD34 which are early maturation antigens (Jaffe et al., 2001). Acute myeloblastic leukemia without maturation, M1, possesses an

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immunophenotype which is characterized by expression of at least two myeloid antigens, including CD13 and CD33. As there is no maturation of the blast cells, CD34 is also usually found to be expressed (Jaffe et al., 2001). Acute myeloblastic leukemia with maturation, M2, has an immunophenotype consisting of at least one, if not more, antigens associated with myeloid lineage, including CD13 and CD33. Even though there is further maturation with M2, the blast cells still express antigens associated with immaturity, CD34 and HLA-DR (Jaffe et al., 2001). CD13 and CD33 were demonstrated to be positive in 23 (85.2%) and 21 (77.8%) of the 27 M0–M2 cases, respectively. In this study, CD34 was observed in 25 (92.6%) of the 27 M0–M2 cases analyzed. HLA-DR was positively expressed in 26 (96.3%) of the 27 M0–M2 cases analyzed in this study.

Acute promyelocytic leukemia, M3, cannot be diagnosed by immunophenotyping but does possess immunologic features which are characteristically specific for M3 versus the other forms of AML with granulocytic differentiation. As there is myeloid differentiation, CD33 and CD13 are generally present. In particular, CD33 is bright and homogeneous while CD13 demonstrates heterogeneous expression when evaluated by flow cytometry. HLA-DR and CD34 are not present in most cases of M3 AML (Jaffe et al., 2001). In the cases of M3 AML, CD13 and CD33 were expressed in all 8 (100%) cases. Thus, these myeloid antigens demonstrate similar expression in both M0–M2 and M3 AML cases. Additionally, CD13 was observed to be generally heterogeneous while CD33 usually displayed expression that was both bright and homogeneous. In contrast to the expression of CD34 in M0–M2 cases, in 6 (75%) of the 8 M3 cases CD34 was negative. Upon statistical analysis, expression of CD34 in M0–M2 cases compared with expression in M3 cases proved to be statistically significantly different ($P<0.001$). Unlike the M0–M2 cases which revealed usual expression of HLA-DR, it was absent in all 8 (100%) cases of M3 included in this study. Expression of HLA-DR was therefore shown to be of a statistically significant difference among FAB subclasses ($P<0.001$).

Conclusions

The antigens CD13 and CD33 are myeloid-associated antigens that are generally expressed by subclasses which display granulocytic differentiation. Since M0–M3 show granulocytic differentiation to some degree, they characteristically express the myeloid antigens CD13 and CD33. Both CD34 and HLA-DR are hematopoietic precursor antigens as they appear early in development, and they are, therefore, usually expressed by those subclasses that are less mature. Thus, the less mature classes M0–M2 usually express CD34 and HLA-DR, while these markers are not expressed by M3. This lack of CD34 and HLA-DR in M3 cases is due to the advanced degree of maturation of this subclass, whereas the M0–M2 subclasses which are less mature do express these precursor hematopoietic antigens.

No statistically significant difference among FAB subclasses regarding the expression of CD13 and CD33 was detected in this analysis. However, in this study we found the difference in expression of both CD34 and HLA-DR to be significantly different statistically at ($P<0.001$) such that the probability of observing these specific differences in proportions of expression between the two groups by chance is very unlikely. We also observed trends in the antigenic expression patterns in the M3 cases studied as CD34 was observed to be usually heterogeneous, whereas CD33 was generally expressed homogeneously and brightly.

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