Loss of red cell A, B, and H antigens is frequent in myeloid malignancies

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Introduction

The A, B, and H antigens (hereafter referred to as ABH antigens) are complex carbohydrate structures found on glycoproteins and glycolipids present on the surface of erythrocytes, endothelial cells, and on most epithelial cells. Alleles of the ABO gene code for glycosyltransferases that act on the precursor H antigen. In red blood cells, the H antigen is determined by a fucosyltransferase coded for by the FUT1 gene. If the H antigen is absent, there is no substrate for the ABO glycosyltransferases to modify. Homozygous deficiency of FUT1 gives rise to the Bombay phenotype where there are no A or B antigens on the red cell due to lack of the H precursor. The major alleles of the ABO gene are A, which adds N-acetylgalactosamine to give the A antigen; B, which adds galactose to give the B antigen; and O, which is a null allele coding for an inactive transferase incapable of modifying the H antigen. The most frequent of the minor A and B alleles is the A2 allele, which gives rise to a lower density of A antigen. Weaker alleles such as A3 and A4 show progressively less A antigen.

Alteration of ABH antigens in hematologic malignancy was first reported by van Loghem et al., who described very weak A antigen expression on the red cells of a patient with acute myeloid leukemia (AML), who had previously shown normal A antigen expression. Loss of A, B, or H antigens from the surface of red blood cells is now recognized as a recurrent observation in hematologic malignancy. In a healthy individual of type A, B, or AB, complete agglutination of the red blood cells is observed after incubation with antibodies reactive against their blood group. In patients with loss of ABH antigens, a varying proportion of red blood cells do not agglutinate, giving a characteristic mixed-field reaction. Mixed-field reactions can also occur in healthy individuals where the reactions are associated with rare alleles of the ABO gene such as A3 and B3.

Loss of ABH antigens is also seen in the tumor cells of many types of carcinoma, including bladder, lung, head and neck, cervical, and thyroid, where it has been associated with tumor grade, metastatic potential, and poor prognosis. Furthermore, laboratory studies have shown that tumorigenicity is reduced when A or B antigens are expressed. Whether the mechanisms of loss of ABH antigens in epithelial tumors and hematologic malignancies are truly analogous to each other remains to be determined.

Unlike epithelial malignancies where the loss of ABH antigens is seen in dedifferentiated tumor cells, loss of ABH antigens in myeloid malignancies is only seen on red cells as most hematopoietic cells and precursors do not express ABH antigens. However, malignant stem cells often retain the potential to differentiate along the erythroid lineage. Thus, loss of ABO expression in the population of red cells derived from a malignant stem cell is an indicator of genetic changes that have occurred in the malignant stem cell. Consistent with this, Salmon et al. showed that the red blood cells deficient in A or B antigen had strongly decreased A or B transferase activity consistent with a genetic change at the ABO gene rather than an alteration at the cell surface or of membrane precursors.

The infrequent reporting of the loss of ABH antigens belies the likely importance of this loss as a signpost to recurrent mechanisms in the development of hematologic malignancy. We have found that blood with as many as 50% of cells showing loss of A or B antigens can be scored as normal in transfusion laboratories. We therefore developed a sensitive flow cytometric method to identify alterations in ABH antigens. This allowed us to determine how frequently alterations occurred in patients with myeloid malignancies.
Patients, materials, and methods

Patients

The patients analyzed in this study presented to the Haematology-Oncology Department at The Queen Elizabeth Hospital during the period 1996-2000 with AML, myelodysplastic syndrome (MDS) or myeloproliferative disorder (MPD) including chronic myeloid leukemia (CML). Patients who showed alterations in blood group antigens by flow cytometry had their transfusion records scrutinized for transfusion with a different blood group.

Preparation of formalin-fixed spherical red blood cells

The method for preparation of formalin-fixed spherical red blood cells was adapted from Langlois et al.20 All solutions were filtered through 0.45 μm membranes to remove particulate matter that might interfere with flow cytometry. One hundred μL whole blood or 50 μL packed red blood cells was added to 1 mL of routine staining buffer (RSB) (Isoton II [Coulter, Hialeah, FL], 1% fetal calf serum, 0.1% NaN₃, pH 7.2) containing 0.005% sodium dodecyl sulfate (SDS). After 60 seconds, 10 mL of a second solution comprising 3% formaldehyde and 0.001% SDS in RSB was added. The tube was then incubated on its side for 90 minutes at room temperature, after which 0.7 mL formaldehyde was added and the tube laid on its side for fixation overnight at room temperature. After resuspension by inversion, the red blood cell spheres were pelleted and then washed at 200 g in spherical staining buffer (SSB) (0.15 M NaCl, 0.0072 M Na₂HPO₄, 0.0028 M NaH₂PO₄, 5 mg/mL BSA, 0.01% Noyes P40, pH 7.2) and finally resuspended in 5 mL SSB. Spherical red blood cell counts were performed on the Coulter STKS.

Flow cytometric analysis of ABH antigens

Flow cytometric analysis was performed on a Coulter Epics Profile II. 30,000 gated events were collected from a sample volume of 100 μL with a flow rate of 30 μL/min. Log fluorescence data were gated on a linear forward scatter versus linear side scatter dot plot.

Labeling of 30,000 formalin-fixed red blood cell spheres with the appropriate lectins or antibodies was performed in a final volume of 100 μL SSB for 15 minutes at room temperature. Lyophilized Ulex Europaeus lectin (UEA-I, detecting H antigen) conjugated to fluorescein isothiocyanate (FITC) (Sigma, St Louis, MO) was resuspended in RSB to make a 0.5 mg/mL stock solution. Lyophilized Helix Pomatia lectin (detecting A antigen), conjugated either to FITC or biotin (Sigma), was resuspended to make a 0.25 mg/mL stock solution. Streptavidin phycoerythrin PE (Becton Dickinson, San Jose, CA) was the secondary reagent for the biotinylated helix. The B antigen was detected using a mouse monoclonal antibody (Novacline anti-B blood grouping reagent; Dominion Biologicals, Dartmouth, NS, Canada) and goat F(ab')2 antimouse immunoglobulin G (IgG) (H+L) PE conjugate (Caltag, Burlingame, CA). The antibody was diluted 1:10 in SSB. The cells were washed with SSB prior to incubation with each antibody/lectin and washed 2 times with SSB before flow analysis. Lectin and antibody concentrations were optimized using serial dilutions to find optimal fluorescence with minimal agglutination for each batch.

Normal samples for relevant blood groups were included in each run of patient samples as controls for variations in the fluorescence that was seen as the lectin stock solutions aged, particularly the Ulex. The normal samples were used as references to determine if there was a change in the patient sample from the normal expected pattern.

Genotyping of the ABO locus

The new methodology presented here is similar in principle to that previously reported by us and others in that alleles are determined by using restriction enzyme digestion.21-23 Digestion of 2 polymerase chain reaction (PCR) products was used to genotype the ABO locus. The first PCR product spanning exon 6 was used to identify the O1 allele. The primers used were sense 5'-ttggagtcgcatttgcctctggtt-3' and antisense 5'-actgcctgtgaggatgct-gatgt-3' to give a 263-bp PCR product (nucleotides 1400-1662 of Genbank accession no. AC000397). The PCR conditions were 9 minutes at 94°C followed by 10 touchdown cycles (30 seconds at 96°C, 1 minute at 70°C - 1°C per cycle, 45 seconds at 72°C) followed by an additional 30 cycles (30 seconds at 96°C, 1 minute at 60°C, 45 seconds at 72°C). O1 alleles have a deleted G compared with the other alleles at nucleotide 261 of the coding sequence. This creates a KpnI restriction enzyme site that is at position 196 of the PCR product. All other alleles cut with BstEI at this site.

The second PCR product within exon 7 allowed for the differentiation of the other major ABO alleles: A1, A2, B, and O2. The primers used were sense 5'-tgctgagcatgctgtccttc-3' and antisense 5'-ctgctgagctgtgagct-gctca-3' to give a PCR product of 527 bp (nucleotides 41-567 of Genbank accession no. X84752). The same conditions were used as for the first PCR except that 5% dimethyl sulfoxide (DMSO) was included. Cutting with AluI, PvuII, and BstUI allowed for the differentiation of the A1, A2, B, and O2 alleles;21-23 BstUI was used instead of the previously used BstHI to type nucleotide 526 of the coding sequence which differentiates O2 and B from the other alleles.22

Results

Patterns seen in healthy individuals

To study the changes in patients with leukemia, it was necessary to understand the patterns in healthy individuals. We studied 180 healthy adults (34 A1, 8 A2, 2 A3, 4 Ax, 35 B, 29 A1B, 15 A2B, 51 O, and 2 Bombay).

Red blood cells from healthy O individuals fluoresced brightly with FITC-conjugated Ulex Europaeus lectin. Red blood cells from the Bombay individuals tested showed background Ulex fluorescence (Figure 1). Red blood cells from O individuals did not react with FITC-conjugated Ulex Europaeus lectin.
with the Helix Pomatia lectin or the anti-B antibody (data not shown).

When red blood cells from healthy A individuals were labeled with Helix and Ulex, the A1, A2, A3, and Ax phenotypes could be clearly distinguished (Figure 1). A1 individuals showed strong fluorescence with Helix and low levels of fluorescence with Ulex. Variation in A1 patterns was seen and was found to be dependent on the genotype. A1/A1 individuals showed very low Ulex fluorescence with A/A2 having distinctly more Ulex and A/O more again. All genotypes had minor populations of cells with negligible A fluorescence which were increasingly more frequent as one progressed from A/A1 to A/A2 to A/O.

For the A2 individuals (all presumed to be A/O), the H antigen was stronger than in A1 individuals but slightly weaker than in O individuals (Figure 1). The A antigen had a wide range of expression. However, even the most intensely staining cells had less Helix fluorescence than A/O cells and nearly 50% had no discernible reactivity with Helix. Interestingly, all cells had a similar level of H expression, regardless of how much A was present.

Both A3 individuals showed a pattern that resembled an A2 pattern except that the Helix-positive cell population comprised less than 5% of the cells and the Ulex fluorescence of the Helix-negative population approached that of O red cells (Figure 1). The 4 Ax individuals who were examined showed an identical pattern to O individuals with no apparent Helix staining and bright Ulex staining (Figure 1). A 50:50 mixture of red blood cells from an O individual and an Ax individual showed only a single Ulex peak, indicating that the vast majority of H residues were unconverted (data not shown).

In all the B individuals, there was more variation in the level of B antigen than was seen for the A antigen in A1 individuals and there was a population of 3% to 10% of O-like cells (Figure 1). Ulex staining was brighter than for A1 cells. There was a slight tendency for Ulex fluorescence to increase in those cells with low B fluorescence.

For the AB individuals, the use of the 2-color flow cytometer necessitated the analysis of 3 two-parameter dot plots: A versus H, B versus H, and A versus B (Figure 2). For A1B individuals, the A staining was always intense with the Helix/Ulex pattern being very similar to that of an A/A1 individual. B staining was markedly weaker than in BO individuals with a significant proportion of cells expressing negligible levels of B. However, in A2B individuals, the B expression closely resembled that of a BO individual whereas A expression was generally very low with the Helix/Ulex pattern resembling that of an A3 individual.

Patterns seen in patients with leukemia

Sixty-one patients with myeloid disorders were analyzed by flow cytometry for alterations in ABO antigens. There was a variant red blood cell population in a substantial proportion of the patients (Table 1). This variant ranged from a minor to a major proportion of the cells. In a few cases, there was no normal population of cells. The descriptions that follow will deal with the variant population. All patients with a variant population were genotyped.

Four patients with alterations were omitted from the analysis because they had received transfusions. Blood transfusion can give rise to 2 discrete cell populations because patients, especially A patients, can be transfused with blood of a different genotype. Figure 3 shows the patterns for 2 patients before and after transfusion. Transfusion thus has the potential to obscure some blood group alterations.

In one group of patients, the H antigens were lost or diminished, creating a variant population of red cells. This is most clearly seen for the O blood group patients. Six of the 28 O patients studied

![Figure 2. Dot plots of healthy A1B and A2B individuals. The 2-color system used required 3 dot plots for the full analysis of AB individuals. HxUx refers to the dual staining with Helix on the LFL2 axis and Ulex on the LFL1 axis. BUx refers to the dual staining with anti-B on the LFL2 axis and Ulex on the LFL1 axis. BHx refers to the dual staining with anti-B on the LFL2 axis and Helix on the LFL1 axis.](image)

![Figure 3. The effects of transfusion on patient flow cytometry results. Dot plots for the dual staining of red blood cell spheres with Helix and Ulex are shown for an A1 patient before transfusion (A) and after an A2 blood transfusion (B). The A2 population is located within quadrant 1 and comprises 18% of the red blood cell population. An A2 blood group patient before and after transfusion with an A1 blood is also shown (C and D) with the A1 population comprising 16% of the red blood cell population after transfusion. The LFL1 axis shows Ulex fluorescence and the LFL2 axis shows Helix (A) fluorescence.](image)

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Patients were classified according to disease: acute myeloid leukemia (AML); myeloproliferative disorder (MPD), including chronic myeloid leukemia (CML); and myelodysplastic syndrome (MDS), including chronic myelomonocytic leukemia (CMML); NA (not applicable) refers to the O blood group where loss of A or B antigens could not be assessed.

**Table 1. Loss of blood group according to blood group and patient diagnosis**
showed loss of H. This was seen as a population of cells with diminished Ulex fluorescence in addition to the normal O population in an MDS patient (Figure 4A) or as a total reduction in Ulex fluorescence of the entire population in a patient with AML (Figure 4B). Loss of H was not seen in any of the 51 healthy O individuals.

Loss of H was the underlying mechanism for loss of A or B antigens in 5 out of 29 A, B, or AB patients. Figure 4C shows a patient with AML (genotype A0O) where loss of A due to H can easily be seen as the red cell population has less Ulex fluorescence causing it to resemble an A1A1 pattern. There is also a continuum of cells that have decreased Helix fluorescence while showing no increase in Ulex fluorescence. Another example of loss of A due to H can be seen in an A0O patient with chronic myelomonocytic leukemia (CMML) in whom the extent of H antigen loss is more extreme (Figure 4D). There are insufficient H sites remaining and the Helix labeling is considerably diminished from that seen in patient 4C. The entire population of cells is clearly different from a healthy A0O individual. Figure 4E shows a BO patient with MPD with loss of H. The fluorescent population is shifted to the left compared with a normal B pattern, indicating decrease of H. In this case, total B fluorescence was diminished due to the loss of H sites.

Loss of A or B without loss of H occurred in 8 out of 29 A, B, and AB patients. There was some compensatory increase in H in the cells where A or B antigens were decreased. Figure 5A shows a patient with AML (genotype A0O) who had previously grouped as an A showing complete loss of A. The patient showed the same abnormal pattern at the time of his death, a year later. Figure 5B shows the red blood cells of a patient with MDS (genotype A1A2) with 2 populations, a typical A1A2 population and an O-like population. There is also a continuum of cells with decreasing A reactivity and increasing H activity. Figure 5C shows an A1A2 individual with endstage CML with a second A2-like population of cells (about 15%) in addition to the normal A1 cells. Figure 5D shows the red cells from a patient with AML (genotype BO) with 20% of cells showing loss of B and an increase in H.

A third group (3/29) of patients appeared to have both loss of A/B and H. One A0O patient with AML had 2 populations of cells, a minor population resembling normal A0O cells and a major population with no A fluorescence. This population showed a continuum of H fluorescence ranging from no apparent expression to weak expression typical of most patients with loss of H (Figure 6A). This patient had several samples taken during the terminal stages of the disease with the normal A1 cells decreasing presumably as normal hematopoiesis shut down. Figure 6B shows an A0O patient with AML in whom loss of A and loss of H has occurred in 2 separate populations; one that has loss of H giving an A1A1 pattern and one that has partial loss of A in addition to loss of H. Hence, the first population has loss of H with no loss in A but the second population has loss of A with increasing H.

Figure 4. Dot plots of patients with loss of H. Red blood cell spheres stained with Ulex (LFL1) from an O patient with MDS (A) and an O patient with AML (B) both with a normal population of cells and a population of cells having diminished H fluorescence. The red blood cell spheres from an A0O patient with AML (C) and an A0O patient with CMML (D) stained with Helix (LFL2) and Ulex (LFL1). The loss of H is more extensive in the patient with CMML and is affecting the levels of the A antigen as well. (E) Red blood cell spheres from a BO patient with MPD stained with anti-B (LFL2) and Ulex (LFL1).

Figure 5. Dot plots of patients with loss of A or B. Red blood cell spheres from an A0O patient with AML with 100% of cells showing no Helix staining (A), an A1A2 patient with MDS with 20% of red blood cells, located in quadrant 4, having no Helix staining (B), and an A1A2 patient with CML with 15% of cells with reduced Helix staining (C). (A), (B), and (C) were stained with Helix (LFL2) and Ulex (LFL1). (D) A BO patient with AML red blood cell spheres stained with anti-B (LFL2) and Ulex (LFL1) with 20% of cells, located in quadrant 1, having minimal B staining.

Figure 6. Dot plots of patients with loss of A and loss of H. Red blood cell spheres of an A0O patient with AML at 6 weeks, 4 weeks, and 1 week before death. The normal A0O population decreased from 25% at 6 weeks before death to 10% at 4 weeks before death before falling to 1% just before death. (B) The dot plot of an A0O patient with AML showing a population of cells (28%) with loss of H (quadrant 1) and a second population of cells with loss of A. The LFL1 axis shows Ulex fluorescence and the LFL2 axis shows Helix fluorescence.
Discussion

Alterations of ABH antigens on the surface of red blood cells of patients with hematologic malignancy have been a recurrent if infrequently reported finding. Most of the reports have been individual case reports, primarily in patients with myeloid disorders. The variant population of red blood cells in the patients with alterations is assumed to derive from the malignant clone of cells as normal cells rarely show variant populations or altered patterns. Consistent with this, combined immunophenotyping and cytogenetic analysis has shown that a substantial proportion of patients with AML show erythroid lineage cells derived from the malignant stem cells.16-19 In a few cases, loss of ABH antigens has been reported in patients with lymphoid malignancies, but these are likely to be pluripotent stem cell malignancies with a primarily lymphoid phenotype.

Alterations in ABH antigens have been considered to be rare in hematologic malignancies but few systematic approaches have been taken to determine their frequency. Indeed, some studies have indicated quite high rates of alterations,24,25 However, the less overt changes are likely to have been missed even by painstaking serologic methods. We set out to determine the frequency of loss of ABH antigens in a systematic fashion using 2-color flow cytometry of patients with myeloid malignancies. Flow cytometry is a sensitive method to determine loss of antigenic expression as it can resolve multiple populations, determine partial or full loss of expression, and can often quantify the number of cells involved. Several patterns were seen for the A1 blood group and it became apparent that these patterns were dependent on genotype (Figure 1). There was a noticeable dosage effect in that the presence of 2 A alleles led to more conversion of H sites than one alone. Thus, in healthy A1 individuals, there were patterns corresponding to the A1A1 and A1O genotypes, with the A1A1 individuals showing considerably less H fluorescence. Genotypes with a strong allele and a weaker allele (ie, A1B and A1A2) showed a range of H staining between the genotype of an A1A1 and that of an A1O.

A substantial proportion of red blood cells in the A1O individuals had negligible A fluorescence (Figure 1) although the A2 blood group does not give a mixed-field reaction when grouped. The A3 blood group does give a mixed field, characterized by small agglutinates surrounded by a mass of unagglutinated cells, and this is reflected by the flow cytometry pattern. The majority of A3 red blood cells look like O cells on flow cytometry, as do all the cells from the Ax individuals.

When the red cells from BO individuals were compared to those from A1O individuals, the red cells showed less intense fluorescence with B than the red cells from A1O individuals showed with A. This was not due to the different reagents used, as red cells from B individuals also showed more Ulex fluorescence, indicating less conversion of the H antigen. There was also a more heterocellular distribution of B fluorescence with 3% to 10% of cells showing no B fluorescence.

A1B red cells have been reported to often give an A2-like pattern with anti-A antibodies and A2B red cells have been reported to give an A3 pattern. However, substantial diminution of A was not apparent by flow cytometry in any of the 29 A1B individuals in this study (Figure 2). The Helix vs Ulex plot was similar to that of the A1A1 control. The B fluorescence in A1B individuals was considerably weaker than that seen in a healthy BO individual. On the other hand, the A2B samples gave results consistent with previous reports (Figure 2). The B fluorescence was virtually identical to that seen in a BO individual. The A fluorescence was considerably weaker than that seen in an A1O and gave an A3-like pattern with the majority of cells appearing negative for A. Thus, the activities of the 2 alleles in AB patients do not act additively. The reduction of B in A1B individuals may be due to direct competition of the A and B transferases as there is near saturation of the H sites. However, in an A2B individual, there are a substantial amount of free H sites which could be modified so there is no clear explanation why the A fluorescence is diminished.

The mosaicism seen for some of the blood groups confirms previous agglutination and electron microscopy studies.26,27 The mechanisms are unclear but are perhaps due to the weaker blood groups being associated with a different sequence in the upstream region. It has recently been shown that an upstream repeat element of the ABO gene, which varies in copy number according to the allele, has enhancing ability.28,29 Surprisingly, the enhancing ability of the single repeat associated with the A1 allele was reported to be weaker than that of the multiple repeat associated with the A2 allele in vitro studies.29 Recent studies show that enhancer-mediated gene expression operates at a stochastic level.30 We would then expect a weak enhancer to give a higher degree of null cells whereas A1 is associated with lower amounts of null cells than A2 and B. It may be necessary to consider the whole upstream region rather than isolated segments of it.

Sharon and Fibach reported a flow cytometry–based approach for studies of healthy individuals.31 They were able to differentiate the A1A1, A1A2, A1O, and A2O genotypes by analyzing the ratio of A-to-H and B-to-H fluorescence. We have confirmed that flow cytometry can be used to deduce these as well as other genotypes. Our methodology differs from that of Sharon and Fibach in important technical details such as the use of Helix Pomatia lectin rather than a monoclonal antibody to detect A and the use of red blood cell spheres to decrease agglutination problems. The use of red blood cell spheres has several other advantages, the principal one being the decreased scatter of the cells with better visualization of the entire population leading to ready gating to exclude agglutinated cells and debris. Also, formalin-fixed red blood cells are much more stable than unfixed red blood cells and may be analyzed for several months after fixation.

Moreover, we use visual analysis of the 2-parameter flow cytometry patterns. Pattern analysis allows us to recognize differences not only in healthy patients in whom the different patterns reflect the genotype, but more importantly in patients with leukemia in whom variant and normal red cell populations may coexist. This methodology is also applicable to other applications such as monitoring patients after allogeneic bone marrow transplantation.

We saw no variant patterns that were unrelated to the genotype in the panel of 180 healthy individuals. However, in a considerable number of patients with leukemia (see below) there was a variant red blood cell population. In almost all cases, the changes were not detected by routine serologic typing.

Genotyping of patients was an important aspect of this study as it was necessary to relate the changes to the normal pattern for that genotype. Moreover, A1O patients who showed a shift to an A1A1-like pattern due to loss of H would have been missed without genotyping analysis. It was also necessary to verify the genotype for other patients with complete or near complete loss of antigens. For example, Figure 4D shows a pattern which looks like it derives from an A2O pattern but is actually from an A1O patient with almost total loss of H.

The changes in patients with leukemia were of 3 different types. First, a group of patients that had decreased H expression to which
the loss of A or B antigens was secondary (Figure 4). Presumably, there is a loss of expression of one or both alleles of the gene coding for H antigen (FUT1 in erythroid precursors) although it is possible the loss may occur in a precursor of H or may even be due to masking of the H epitope. A second group of patients had an intrinsic decrease or loss of activity of A or B antigens. This can be seen by the increase of H activity, which is slighter in some cases than might be expected. A third group had decrease or loss of H and of A or B.

One common pattern was when A O patients showed a shift to an A1 A1-like pattern in that there was a markedly decreased amount of H (Figure 4C). This can most readily be explained by a reduction in H activity perhaps due to inactivation of one of the 2 FUT1 alleles. We consider that most of the losses of H we observed are compatible with the inactivation of one FUT1 allele as Ulex fluorescence is diminished and not lost. No patients showed a total loss of H in all cells but some showed a subpopulation of cells with total loss of H (Figures 4, 6).

There were similar frequencies of loss of H in the O patients (6/28) and the A-or-B patients (8/29) giving an overall rate of loss of 25%. Loss of A or B was seen in 11 out of 29 (38%) of the A-or-B patients. Fifty-five percent (16/29) of A-or-B patients showed alterations at one or both of the loci. Considering A-or-B and O patients together, alterations were seen in 22 out of 57 (39%) patients compared to 0 out of 178 healthy individuals, with a 95% confidence limit of 26% to 52% of patients with loss. This indicates that a sizeable proportion of patients have loss of ABH antigens. If underlying molecular mechanisms are considered, the frequency is likely to be higher because O individuals are not informative for changes occurring at the ABO locus. Moreover, A and B individuals are most frequently AO and BO heterozygotes and changes inactivating the O allele would not cause a phenotypic difference.

In some patients, we were able to track changes during the course of their diseases. This was often hindered due to transfusions after presentation. Figure 6 shows a patient with AML (genotype A 0) in which the population of normal A cells continually decreased during the final months of his disease presumably due to continuing loss of normal hematopoiesis. Several other reports have also noted changes during the time course of the disease.5,6 Gold et al reported a patient with AML in whom 98% of the cells had loss of A at presentation. This fell to 35% in remission before rising again at relapse.5,6 The amount of variant red cells seen is clearly the result of a dynamic process being influenced by many factors including the intrinsic ability of the leukemic cells to differentiate to red cells, the balance of normal and malignant hematopoiesis, and tumor evolution.

We examined the association of antigenic loss with patient diagnosis, age at presentation, and survival after diagnosis. Table I shows loss data according to patient diagnosis and blood group. There was no significant difference in the proportion of patients with loss among patients with AML and the other patients (Fisher exact test). Subclass information was available for 26 of the patients with AML. There were 4/7 M1 patients, 3/8 M2 patients, 1/4 M3 patients, 2/4 M4 patients, 1/2 M5 patients, and 1/1 M6 patient showing variant cell populations. Loss does not appear to be restricted to particular subclasses although this requires further assessment because the numbers are low.

Thirty-six patients with AML had information regarding prior predisposing disease such as MDS or MPD. An identical frequency of patients with loss (36%) was seen for those with a predisposition (5/14) and those with no predisposition (8/22).

The high frequency of ABH alterations seen are not likely to be due to nonspecific aging as similar proportions of alterations are also seen in younger patients. There were losses in 5 out of 14 (36%) patients younger than 50 years old and in 17 out of 43 (40%) patients older than 50 years old. There were also no alterations in the 178 healthy individuals examined who ranged in age from 21 to 74. This supports the hypothesis that the alterations in the red blood cells are related to the disease process.

Kaplan-Meier analysis showed no significant difference in survival between patients with and without loss. Two analyses were done; considering all patients and considering patients with AML alone. There was, nevertheless, a tendency for patients with loss to show a shorter survival time. Patients with AML with loss showed a median survival after presentation of 5 months whereas patients with AML without loss showed a median survival of 11 months.

Loss of ABH may in some cases arise from loss of heterozygosity. The ABO gene is located on chromosome band 9q34 whereas the H (FUT1) gene is on chromosome band 19q13. There is a recurrent deletion of the 9q23-31 region in myeloid malignancy and this may extend to 9q34 in some cases.33 None of the patients with loss had 9q or 19q (or −9 or −19) deletions.

There were 5 patients with CML with the t(9;22) translocation, 2 in chronic phase, and 3 in blast crisis. Neither of the chronic phase patients showed blood group abnormalities whereas one blast-crisis patient showed abnormalities (Figure 5C). Loss of ABO has been reported in patients with CML in this study and in others,19 but clearly is not a direct consequence of the Philadelphia translocation as otherwise it would be much more commonly observed.

The results in the patients can best be explained by considering independent silencing mechanisms acting at the ABO and FUT1 loci. For each locus, one or both alleles may be silenced. Methylation of the genes is a possible silencing mechanism and has been reported for the ABO gene in human cancer cell lines.34 In line with an epigenetic explanation, it is noteworthy that in some cases, loss of expression of A, B, or H seems incomplete (eg, Figure 5). A decrease of gene activity may be occurring rather than a complete loss. Epigenetic mechanisms are also supported by parent of origin effects on loss of A and B alleles.35

We have used flow cytometry to show that red cells from healthy individuals show patterns of antigenic expression consistent with their genotype whereas red cells from leukemic individuals often show major disturbances of antigenic expression at the 2 major loci affecting expression of the ABO blood group. Whether these alterations represent epiphenomena or important signposts to fundamental alterations in leukemogenesis remains to be determined.

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Loss of red cell A, B, and H antigens is frequent in myeloid malignancies

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