RAPID COMMUNICATION

Deletion of Chromosome 20q in Myelodysplasia Can Occur in a Multipotent Precursor of Both Myeloid Cells and B Cells

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Deletions of the long arm of chromosome 20 are associated with several myeloid malignancies and, in particular, with myeloproliferative disorders and myelodysplastic syndromes (MDS). Together with deletions of chromosome 5q and chromosome 7q, chromosome 20q deletions have previously been thought to be restricted to myeloid cells in patients with MDS. We report here that deletion of chromosome 20q in MDS can arise in a multipotent precursor of both myeloid cells and B cells. Clonal Epstein-Barr virus (EBV)-transformed cell lines, both with and without a 20q deletion, have been isolated from a patient with MDS. Moreover, these cell lines have been shown to provide a useful physical mapping tool and have been used to confirm the interstitial nature of the 20q deletion. Microsatellite polymorphisms have been used to show heterogeneity of progenitor cell involvement in different patients with MDS. We report here that deletion of chromosome 20q in patients with MDS and in some patients with acute myeloid leukemia, but only rarely in association with lymphoid malignancies, is more common in the myeloproliferative disorders, myelofibrosis or other myeloproliferative disorders. Allossomes have subsequently been found in 10% to 15% of patients with peripheral blood granulocytes and monocytes but not in B cells or T cells. Clonality of the different lineages followed the same pattern as the 20q deletion. This represents the first report in which a chromosome abnormality associated with MDS has been immortalized in an EBV-transformed lymphoblastoid cell line. Furthermore, our data show that patients with apparent myeloid restriction of a chromosome deletion may in fact have a disease arising in a multipotent cell with both myeloid and lymphoid potential.

MALIGNANCY.3

MELODYPLASTIC syndromes (MDS) represent a heterogeneous collection of acquired clonal disorders characterized by cytopenia, morphologic dysplasia, and ineffective hematopoiesis. Several lines of evidence suggest that these disorders result from clonal expansion of abnormal multipotent hematopoietic progenitor cells. Early studies of two G6PD heterozygotes showed that a single isoenzyme was expressed in multiple hematopoietic lineages.1,2 These results have been extended by cytogenetic or molecular analysis of chromosome markers5-7 and more recently by various molecular techniques, including identification of RAS point mutations5,6 and the use of X-linked polymorphisms.8-12 Peripheral blood granulocytes and monocytes have been shown to be part of the neoplastic clone in most patients in whom these have been studied. Erythroid cells and platelets have been studied in far fewer cases but, where studied, they have also been found to be derived from the abnormal clone.1,4

By contrast, the involvement of lymphoid cells is much more controversial. Several reports have suggested that peripheral blood lymphocytes,6,11 B cells,1,13 or T cells5,13-15 may be part of the malignant clone. However, deletions of chromosomes 5, 7, and 20 have all been reported to be restricted to myeloid cells.3,5,7 Moreover, X-linked polymorphisms have been used to show that lymphoid cells are polyclonal in some cases.11,12 The reasons for these contrasting results are unclear, but it has been suggested that they may reflect heterogeneity of progenitor cell involvement in different patients with MDS.6,12

Deletion of an F-group chromosome associated with polycythaemia rubra vera was first described by Kay et al15 and subsequently identified as chromosome 20. Deletions of 20q have subsequently been found in 10% to 15% of patients with PRV16-18 and in a variable number of patients with myelofibrosis19,20 or other myeloproliferative disorders.20 Although more common in the myeloproliferative disorders, deletions of 20q are also found in approximately 5% of patients with MDS and in some patients with acute myeloid leukemia,21-24 but only rarely in association with lymphoid malignancy.23

Molecular characterization of 20q deletions has been very limited. Le Beau et al25 showed retention of the SRC gene in two patients with deletions of 20q, but this gene was deleted in two additional patients reported by Morris et al.26 These data probably reflect breakpoint heterogeneity, a concept which accords with the variable deletion size observed using both classical cytogenetics21 and fluorescence in situ hybridisation (FISH).27 Therefore, chromosome 20 deletions are unlikely to result in activation of a proto-oncogene located at a common breakpoint. Instead, the observations point to the presence of a tumor suppressor gene, the deletion or inactivation of which plays a role in myeloid malignancies. FISH studies have recently defined a segment from 20q11.2 to q12 that was deleted in 18 of 19 patients with 20q deletions.27 Several potential candidate genes with roles in growth regulation have been mapped to this region including HCK,28 phospholipase C,29 phosphotyrosyl phosphatase 1,30 EBP β,31,32 and phosphotyrosyl phosphatase 1B.33

In this article we report that a deletion of chromosome 20q in a patient with MDS arose in a progenitor capable of giving rise to both myeloid cells and B cells. In addition, we have generated Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines that carry the 20q deletion and have shown their usefulness for mapping studies. Analysis of purified cell populations obtained from peripheral blood

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suggested that both B cells and T cells were polyclonal and did not carry the deletion. Therefore, these data show that chromosome abnormalities may occur in a multipotent progenitor of lymphoid and myeloid cells, despite molecular evidence of myeloid restriction.

MATERIALS AND METHODS

Patient history. A 72-year-old woman presented in 1979 with tuberculous endometriosis. A blood count before treatment showed hemoglobin, 11.8 g/L; nucleated cells, 4.0 × 10⁷/L; platelets, 80 × 10⁹/L. Antithuberculosis treatment was initiated but was stopped after 4 months because of persistent neutropenia and thrombocytopenia. A bone marrow (BM) aspirate showed dyserythropoiesis and left-shifted granulopoiesis. Mild neutropenia and thrombocytopenia persisted for several years. In 1989 she re-presented with an acute abdomen and was found to have illal tuberculosis. After initiation of antituberculosis therapy she was noted to be anemic and neutropenic; hemoglobin 10.5 g/L; neutrophils 0.8 × 10⁹/L; platelets 270 × 10⁹/ L. At this time serum B12, folate, and ferritin were all normal. Morphologic examination of a BM aspirate showed dyserythropoiesis and monoclonal micromegakaryocytes. Cytogenetic analysis of BM metaphases showed a 20q deletion in all metaphases examined [46 XX, del 20(ql1.2-q13.1)]. The neutropenia and anemia subsequently improved despite continuation of antituberculosis therapy for 18 months. In March 1992 a full blood count showed the following: hemoglobin, 12.8 g/L; neutrophils, 2.0 × 10⁹/L; platelets, 450 × 10⁹/L. A repeat BM aspirate showed persistent dyserythropoiesis and micromegakaryocytes but, in addition, showed granulopoiesis to be left-shifted with hypogranular myelocytes. Cytogenetic analysis demonstrated persistence of the 20q deletion in all metaphases.

Cell separation. One hundred milliliters of venous blood was taken into EDTA and processed within 24 hours of collection. Granulocytes and mononuclear cells were separated using density gradient centrifugation with Histopaque 1077 (Sigma, St Louis, MO). Granulocytes were isolated from the pellet using an alkaline lysis or centrifugation with Histopaque 1077 (Sigma, St Louis, MO). Aliquots of 1,000 cells were deposited aseptically in RPMI 1640 using wire mesh. Lymphocytes were isolated from the mononuclear fraction by rosetting with 2-aminoethylisothiouronium bromide hydrobromide (AET)-treated sheep erythrocytes. The purity of fractions was 90% or greater in both cases, as evaluated morphologically using a rapid Field's stain (BDH, Poole, UK) or cytoxin preparations. High molecular weight DNA was extracted from both fractions using standard techniques.

Granulocytes and mononuclear cells, obtained as described above, were washed with phosphate-buffered saline and incubated with the appropriate fluorescein-conjugated monoclonal antibody (MoAb) (Dako, Glostrup, Denmark). Pure populations of granulocytes (CD15+), monocytes (CD14+), T lymphocytes (CD3+), and B lymphocytes (CD19+) were obtained using a FACS-Star Plus (Becton Dickinson, Mountain View, CA). Aliquots of 1,000 cells were deposited in 200 μL of autoclaved water, boiled for 5 minutes, and then stored at −20°C.

EBV transformation of B lymphocytes. Mononuclear cells were aseptically obtained from a 10-mL aliquot of venous blood using density gradient centrifugation with Histopaque 1119 (Sigma). The plates were fed at 4-day intervals with RPMI 1640 containing 5% FCS and supplemented with 25% human tonsil-conditioned medium. Positive wells were expanded from microtiter plates containing at least 70% negative wells and were screened for the presence of the deletion cytogenetically.

Human tonsils obtained from routine tonsillectomy were disaggregated aseptically in RPMI 1640 using wire mesh. Lymphocytes were separated using density gradient centrifugation with Histopaque 1119. The mononuclear cells were washed twice with RPMI 1640 before counting and diluting to a density of 1 × 10⁶ cells/mL in RPMI 1640, 20% FCS, penicillin, streptomycin, and gentamycin. They were then incubated for 48 hours at 37°C in the presence of 5% CO₂. Media were harvested by centrifugation, followed by filtration through a 0.2 μm cellulose filter and stored at −20°C.

Cytogenetic analysis. Cells were harvested by conventional methods 24 hours after the initiation of cultures and at intervals thereafter. Slides were G-banded with Wright's stain. High resolution chromosome banding analysis was performed with the aid of a computer-based image analysis system (SmartCapture; Digital Scientific, Cambridge, UK). The International System for Human Cytogenetic Nomenclature has been used.

Cytotoxicity and immunophenotype. Stains for acid phosphatase, periodic acid Schiff, and Sudan Black were performed using routine techniques. Direct immunofluorescence staining for all surface antigen expression was performed using standard methods before analysis using a fluorescence-activated cell sorter (FACS) (Coulter Electronics Elite; Coulter Electronics, Hialeah, FL). The following MoAbs were used: T9-10 (CD2), DK23 (CD5), SS2-36 (CD10), HD37 (CD19), H299 (CD20), 4KB 128 (CD22), ACT-1 (CD25), T16 (CD38), FMC7, R1/69 (IgM), A8 B5 (kappa), and N10/2 (lambda).

PCR analysis. Forty-nanogram aliquots of DNA from the cell line, granulocytes, and T lymphocytes were each amplified in 50 μL of a mixture containing buffer (50 mmol/L KCl, 10 mmol/L Tris HC1 pH 8.3, 1.5 mmol/L MgCl₂, 0.1% gelatin), dNTPs (250 μmol/L each), primers (50 pmol each), and Cetus Taq polymerase (0.5 U; Perkin Elmer Cetus, Norwalk, CT). In all cases 1/10 vol of the left-hand primer was end-labeled with ³²P dATP using polynucleotide kinase. Samples were amplified in an MG Research (Watertown, MA) programmable thermal controller for 35 cycles (40 seconds, 94°C; 30 seconds 55°C). The following primer pairs (sequences kindly communicated by J. Weissenbach, Genethon, Paris, France) were used to examine the cell lines: one above the centromeric breakpoint (AFM238vh6a, CTTGGGACTTGTCAGCCTC; AFM238-CCAGTCCCTTGCTGTCATAATA); one within the deletion (AFM238vh6a, CTTGGGACTTGTCAGCCTC; AFM238-CCAGTCCCTTGCTGTCATAATA); one below the telomeric breakpoint (AFM276xh1a, TTTTACTAAGGAGACTACAAAGG; AFM276xh1b, TGCGCAATGCTGCTGAA). The amplified products were visualized by denaturing polyacrylamide gel electrophoresis followed by autoradiography.

For analysis of FACS-sorted cells, DNA was purified from each aliquot of 1,000 cells using standard phenol/chloroform extraction as described above and subsequently dispensed into 96-well microtiter plates precoated with mouse peritoneal macrophages (10⁶/well) obtained by peritoneal lavage of female BALB/c mice. Lymphoid cells were seeded at 10⁴, 10⁵, 10⁶, and 10⁷ cells per well. The plates were fed at 4-day intervals with RPMI 1640 containing 20% FCS and 25% human tonsil-conditioned medium (see below). Positive wells were screened for the presence of the deletion using polymerase chain reaction (PCR).

Cloning of cell lines. EBV-transformed cell lines were cloned by limiting dilution in round-bottomed 96-well microtiter plates precoated with mouse peritoneal macrophages (10⁷/well). A single-cell suspension was prepared from each cell line, checked microscopically, and dispensed at varying cell densities into 96-well plates. The plates were fed at 4-day intervals with RPMI 1640 containing 20% FCS and supplemented with 25% human tonsil-conditioned medium. Positive wells were expanded from microtiter plates containing at least 70% negative wells and were screened for the presence of the deletion cytogenetically.
followed by ethanol precipitation with 0.1 pmol glycogen as a carrier molecule. The DNA from each aliquot was resuspended in 50 μL of PCR solution and amplification was performed as detailed above, but with 60 cycles.

PCR analysis of the PGK gene was performed as described previously. DNA was purified from the cell lines, granulocytes, and T lymphocytes using standard phenol/chloroform extraction and ethanol precipitation techniques. Ten nanograms of DNA was then divided into two aliquots, one of which was digested with 50 U of the methylation-sensitive enzyme HpaII (Promega, Madison, WI) at 37°C for 3 hours. The DNA was then precipitated with ethanol and resuspended in 50 μL of a solution containing buffer (50 mmol/L Tris HCl pH 8.3, 1.5 mmol/L MgCl2, 0.1% gelatine), dNTPs (200 μmol/L each), 20 pmol of each primer, and Cetus Taq polymerase (0.5 U). Samples were amplified in an MJ Research programmable thermal controller for 30 cycles (1 minute, 94°C; 2 minutes, 58°C; 3 minutes 72°C). One tenth of this mixture was then added to a second reaction mixture containing primers 2A and 2B. Thirty cycles of amplification were performed using the above conditions. The buffer was then exchanged to allow digestion with BsrXI (55°C, 3 hours). PCR products were visualized by electrophoresis in a 2% agarose gel followed by staining with ethidium bromide.

For analysis of FACS-sorted cells, DNA was purified from each aliquot of 1,000 cells using standard phenol/chloroform extraction followed by ethanol precipitation with 0.1 pmol glycogen as a carrier molecule. The DNA was divided into two aliquots, one of which was digested with 50 U of the methylation-sensitive enzyme, HpaII, at 37°C for 3 hours. Both aliquots were then amplified as detailed above but with two 60-cycle reactions.

**RESULTS**

*Generation of a lymphoblastoid cell line carrying a 20q deletion.* Cytogenetic analyses of BM from this patient in 1989 and 1992 showed a del 20q (11.2-q13.1) in all metaphases examined (Table 1, Fig 1). No other structural or numerical chromosome abnormality was detected. To search for the presence of an occult translocation involving chromosome 20 that might mimic a deletion, FISH was performed on the patient’s BM metaphases using a chromosome 20 paint (Cambio, Cambridge, MA). A fluorescent signal was detected along the whole length of each chromosome 20 homologue and on no other chromosome (data not shown). This provided strong evidence against the presence of an occult reciprocal translocation involving chromosome 20.

Peripheral blood mononuclear cells were exposed to EBV and then immediately dispensed at a density of 10⁶ cells per well. Cell lines were obtained from four independent wells and their karyotypes were analyzed 10 weeks after transformation. As shown in Table 1, two of the lines (MH1B, MH1D) gave rise to significant numbers of metaphases containing a 20q deletion, whereas normal metaphases were observed in the remaining two lines (MH1A, MH1C).

Table 1. Cytogenetic Analysis of BM, Peripheral Blood Mononuclear Cells, and Lymphoblastoid Cell Lines

<table>
<thead>
<tr>
<th></th>
<th>BM</th>
<th>T</th>
<th>MH1A</th>
<th>MH1B</th>
<th>MH1C</th>
<th>MH1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total metaphases analyzed</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Metaphases with 20q deletion</td>
<td>25</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td>0</td>
<td>4</td>
</tr>
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</table>

Abbreviations: T, PHA-stimulated blood mononuclear cells; MH1A-D, independent EBV-transformed cell lines.

and MH1A were subsequently cloned by limiting dilution to give rise to MH1B14 and MH1A1. Cytogenetic analysis of the chromosome 20 homologs present in MH1B14 and MH1A1 clearly demonstrated a 20q deletion in the former cell line, which was morphologically identical to the 20q deletion present in the patient’s BM metaphases (Fig 1).

May-Grünwald-Giemsa staining of MH1B14 and MH1A1 showed lymphoblastoid morphology with no significant difference between the two cell lines. Both cell lines were positive for acid phosphatase and the periodic acid Schiff stain but were negative for Sudan Black. FACS analysis showed both cell lines to be positive for CD19, CD20, CD22, and CD38 but negative for CD2, CD5, CD10, CD25, FMC7, IgM, kappa, and lambda (data not shown). These results confirm the B-cell nature of both MH1B14 and MH1A1.

The 20q deletion is interstitial. To characterize the deletion at a molecular level, microsatellite PCR was used. Sev-
eral PCR primer pairs for polymorphic microsatellite repeats on 20q were tested using DNA from MH1A to determine whether they were informative in this patient. Three informative primer pairs were then used to amplify DNA from MH1B14 and MH1A1. The smaller allele detected by AFM238vh6 was clearly absent from MH1B14, thus localizing this locus within the deletion. By contrast, both alleles detected by AFM294zc5 and AFM276xh1 are retained in MH1B14 (Fig 2). Because AFM294zc5 and AFM276xh1 are centromeric and telomeric, respectively, with respect to AFM238 vh6 (Fig 2), these data show that the 20q deletion in this patient is interstitial. Moreover, they show that MH1B14 and MH1A1 will prove useful for the physical mapping of this 20q deletion.

**Lineage involvement of the 20q deletion.** To determine whether microsatellite PCR could be used to study the lineage involvement of the 20q deletion in purified peripheral blood cell populations, it was necessary to determine the sensitivity of microsatellite PCR in the presence of contaminating normal cells. Therefore, DNA was extracted from MH1B14 and mixed with varying amounts of DNA from MH1A1. As shown in Fig 3, the ratio of the intensity of the lower allele to that of the upper allele was clearly reduced, even in the presence of 30% normal cells.

Therefore, purified blood cell populations were prepared and analyzed using AFM238vh6 primers (Fig 4A). DNA from T cells separated by a sheep red blood cell rosetting method gave rise to alleles of equal intensity. By contrast, DNA from granulocytes purified by density-gradient centrifugation consistently produced a lower allele of reduced intensity. The residual lower allele amplified from granulocyte DNA was thought to reflect the presence of contaminating lymphocytes and/or the presence of a subpopulation of nonclonal granulocytes. To investigate this further, different cell populations were separated by FACS and analyzed using the same primer pair (Fig 4B). Granulocytes and monocytes clearly carried the deletion, whereas it was not detected in either B cells or T cells.

Absence of the deletion in purified T cells accorded with the cytogenetic analysis of PHA-stimulated peripheral blood mononuclear cells, which showed only normal metaphases (Table 1). However, although microsatellite PCR failed to detect the deletion in peripheral blood B cells, cytogenetic analysis of EBV-transformed cell lines showed two of four to contain metaphases with a 20q deletion. These cell lines were generated by dispensing 10⁶ cells per well, and so each independent cell line is likely

![Microsatellite PCR analysis of chromosome 20q in cell lines MH1A1 and MH1B14](image-url)
to represent multiple transformation events. The possibility of selection for or against lymphoblastoid cells carrying the 20q deletion in such polyclonal populations rendered it difficult to infer from these results the prevalence of peripheral blood B cells carrying the 20q deletion.

To address this question, EBV transformation was repeated and, on this occasion, the transformed cells were cloned immediately by limiting dilution. Cell lines were only expanded from microwell plates containing cells dispensed at a dilution that produced greater than 70% negative wells. Sixteen cell lines obtained in this manner were characterized by microsatellite PCR with AFM238vh6 primers. Interestingly, all 16 independent cell lines carried the deletion (Fig 5).

Taken together, these data show that the majority of EBV-transformed lymphoblastoid cells contained the 20q deletion even though the deletion was not detected in purified peripheral blood B cells. These observations raise the possibility that B cells derived from the myelodysplastic clone may be preferentially transformed by EBV or may survive better after transformation.

Clonality of different lineages. This patient was heterozygous for the BsuXI polymorphism of the PGK gene. Therefore, clonality of different purified cell populations was assessed by PCR. X-chromosome inactivation was unilateral and involved the same allele in peripheral blood granulocytes, monocytes, and the MH1B14 cell line. By contrast, a polyclonal pattern was clearly seen in B cells and T cells (Fig 6).

DISCUSSION
Our results show that a deletion of chromosome 20 can arise in a multipotent progenitor of both lymphoid and myeloid cells.
eloid cells despite apparent myeloid restriction of the deletion and the presence of polyclonal B cells and T cells. Moreover, to our knowledge this represents the first report in which a chromosome deletion from a patient with MDS has been immortalized in an EBV-transformed lymphoblastoid cell line.

Several groups have investigated the lineage involvement of chromosome abnormalities in MDS. Chromosome 7 deletions have been reported to be present in myeloid cells but not lymphoid cells as assessed by Southern blotting with polymorphic probes. Both Southern blotting and microsatellite PCR have also been used to show the presence of chromosome 5 deletions in myeloid cells but not in T cells or B cells. The lineage involvement of trisomy 8 has been studied by FISH in three patients with MDS. Trisomy 8 was seen in various proportions of granulocytes, monocytes, eosinophils, and basophils, but not in lymphocytes. We are aware of only one previous example of a chromosome deletion detected in B cells in MDS. Lawrence et al. reported two patients with acquired idiopathic sideroblastic anemia and a 13q deletion. The 13q deletion was detected in BM metaphases and also in a minority of metaphases derived from EBV-transformed cultures. However, no clonal cell line containing the 13q deletion was reported and so the nature of the dividing cells carrying the 13q deletion in the EBV-transformed cultures was not confirmed. In addition to chromosome markers, a number of other approaches have been used to assess whether lymphoid cells are derived from the neoplastic clone in patients with MDS. Wainscoat et al. reported absence of IgH and TCR rearrangements in peripheral blood lymphocytes as assessed by Southern blotting. More recently Culligan et al. detected CDR 3 rearrangements by PCR in a minority of MDS patients, but not in age-matched controls. However, there are several problems with these approaches. Even if lymphoid cells were derived from the malignant clone, they may well have undergone independent antigen receptor rearrangement events and thus appear polyclonal. Furthermore, immunologic abnormalities are well recognized in MDS and these may predispose to oligoclonal or monoclonal lymphoid expansions that are unrelated to the neoplastic clones. The use of X-linked polymorphisms represents a more informative approach but has produced conflicting results in patients with MDS. G6PD isoenzyme analysis in two heterozygous patients has shown the same isoenzyme in lymphoid and myeloid cells. Jansen et al. have also demonstrated the involvement of T and B cells in two patients with chronic myelomonocytic leukemia (CMML) using an assay for RAS gene point mutations. However, analysis of X-linked restriction fragment length polymorphisms has been reported to show T or B cells derived from the malignant clone in some patients but not in others.

There are probably several explanations for the conflicting data. First, extreme lyonization is now recognized to occur in a significant minority of normal women. Therefore, appropriate controls are vital before assuming that unilateral inactivation reflects monoclonality. Second, different techniques used to assess lymphoid involvement vary greatly in their sensitivity. Minor populations of lymphoid cells derived from the neoplastic clone may escape detection in many cases. Third, there may exist heterogeneity in the level of the multipotent progenitor involved in different patients with MDS. It has been suggested that the transformed cell may be myeloid restricted in some patients but in others be capable of giving rise to both lymphoid and myeloid progenitors.

Our data are particularly relevant to the latter two issues. In the patient reported here, peripheral blood B cells were polyclonal (Fig 6) and the 20q deletion was not detected in highly purified B cells (Fig 4B). Since mixing experiments showed that the 20q deletion was readily detectable (reduced intensity of lower allele), even in the presence of 30% normal cells (Fig 3), these results suggest that the 20q deletion is only carried by a minority of peripheral blood B cells. However, EBV transformation with immediate limiting dilution showed that 16 of 16 EBV-transformed lymphoblastoid cell lines carried the 20q deletion (Fig 5). These apparently paradoxical results could reflect preferential expression of CD21.

Fig 6. X-inactivation patterns of different lineages. Ethidium-stained gel showing PCR analysis of clonality as assessed by X-inactivation patterns (PGK). Cells were separated by density-gradient centrifugation (A) or by FACS sorting (B). +/−, with or without HpaII predigest; m, marker; G, granulocytes; T, T lymphocytes; M, monocytes; B, B lymphocytes; 1b, MH1B14.
DEL 20q IN MDS OCCURS IN MYELOID AND B CELLS

the purified B cells by T cells is also highly unlikely because almost all normal peripheral blood B cells express CD21. Contamination of the purified B cells by T cells is also highly unlikely because CD19 is expressed strongly by B cells and not at all by T cells. Therefore, we speculate that B cells derived from the myelodysplastic clone may have been preferentially transformed by EBV or may have exhibited enhanced survival after transformation.

Our data also clearly show that the 20q deletion in this patient arose in a progenitor capable of giving rise to granulocytes, monocytes, and B cells. However, we could find no evidence of the deletion in T cells by microsatellite PCR. Moreover, 50 PHA-stimulated metaphases were analyzed cytogenetically and none contained a 20q deletion. There are two broad explanations for these observations. First, the 20q deletion may have arisen in a progenitor capable of giving rise to myeloid cells, B cells, and T cells. In this case our inability to detect T cells carrying the 20q deletion may reflect one or more of the following: (1) the unresponsiveness to PHA of T cells carrying the 20q deletion; (2) the methods used were not sensitive enough to detect a very small proportion of T cells carrying the 20q deletion; or (3) the 20q deletion itself inhibits differentiation along the T-cell lineage. Second, the 20q deletion may have arisen in a progenitor capable of giving rise to myeloid cells and B cells but not to T cells. There is mounting evidence for a close association between B cells and myeloid cells. B-cell lines have been reported to generate macrophage-like cells. Furthermore, multipotent human and murine progenitors capable of giving rise to B cells and myeloid cells have recently been identified.

Therefore, our results suggest that at least some of the reported heterogeneity at the level of the transformed progenitor in different patients with MDS may be more apparent than real. In this patient both the 20q deletion and unilaterial X inactivation were apparently restricted to myeloid cells, and yet the neoplastic clone clearly arose in a multipotent cell capable of giving rise to B cells. It remains to be seen how many other patients, described as having unilateral X inactivation or a chromosome marker restricted to the myeloid lineage, in fact have a disease arising in a multipotent cell with both myeloid and lymphoid potential.

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