Assessment of mismatch repair function in leukaemic cell lines and blasts from children with acute lymphoblastic leukaemia

Elizabeth C. Matheson and Andrew G. Hall

The LRF Molecular Pharmacology Specialist Programme, Cancer Research Unit, Newcastle upon Tyne, UK

1To whom correspondence should be addressed: Cancer Research Unit, Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK

Email: a.g.hall@ncl.ac.uk

Defects in the DNA mismatch repair (MMR) pathway have recently been shown to be associated with resistance to several of the cytotoxic drugs used in the treatment of children with acute lymphoblastic leukaemia (ALL). We have assessed the MMR status of a range of leukaemic cell lines using an in vitro repair assay and correlated this with protein expression of the best characterized components of the system. We have also assessed MMR in leukaemic blasts from a limited panel of children with ALL and related this to Ki67 expression as a measure of proliferative capacity. Out of nine leukaemic cell lines tested, five of the seven lymphoid lines showed little or no repair using the in vitro assay and had low MMR protein expression. In three (NALM-6, Reh and MOLT 4) MMR defects have not been previously reported. Immunohistochemistry of clinical samples showed a wide range of expression of MLH1, MSH2 and Ki67 in nine cases studied at presentation, with a highly statistically significant correlation between MLH1 and Ki67 expression ($r^2 = 0.96$, $P < 0.0001$, Pearson correlation). Western blotting demonstrated high expression of MLH1, PMS2, MSH2 and MSH6 proteins. In vitro analysis of G.T repair using lymphoblast cytosol from the same patients showed a wide range of proficiency, which was markedly reduced in one case studied at relapse. These results suggest that MMR defects are more common in leukaemic cell lines and acute lymphoblastic leukaemias than previously thought.

Introduction

A complex post-replicative mismatch repair (MMR) system has evolved in order to detect and correct the small number of mispairs which occur during normal cell division and to thereby prevent the introduction of mutations into the genome (1,2). In eukaryotic cells repair is triggered by binding to the mispair of a heterodimer formed between MSH2 and MSH6, (the MutSβ complex). A second heterodimer, formed between MLH1 and PMS2 (the MutLα complex), is then recruited and excision and replacement of the mispaired base in the daughter strand is initiated.

In addition to the detection of base–base mispairs, the MMR system is also capable of detecting small (1–3 bp) insertion/deletion loops (IDLs) within DNA. Recognition of these structures occurs through the MutSβ complex formed between MSH2 and MSH3. IDLs commonly occur during normal replication following polymerase slippage (3). This is particularly frequent in stretches of DNA containing multiple small repetitive units. Because of occasional failures of the proofreading ability of the polymerase and MMR systems, these microsatellites are generally highly polymorphic within a population whilst retaining stability within an individual.

Germline mutations in MSH2 and MLH1 are associated with premature development of malignancy, predominantly within the colon where they can be detected in over 50% of cases of hereditary non-polyposis colon cancer (HNPCC) (4). Analyses of microsatellites in these cases generally reveal marked differences between the numbers of tandem repeats between normal and tumour DNA, a phenomenon known as microsatellite instability (MSI). MSI is also seen in tumours (chiefly lymphomas) that arise in mice with germline biallelic deletions of MLH1 or MSH2. However, MSI is not as prominent in the rare cases of HNPCC associated with MSH6 mutation or in mice with deleted MSH6, supporting in vitro observations that MSI is a measure of MutSβ rather than MutSα function (5,6).

In addition to HNPCC, MSI has also been reported in occasional cases of a range of sporadic malignancies including some which arise in the ovary, endometrium (7), breast, cervix, lung (8) or pancreas (9). These observations have led to a search for MSI in haematological malignancies. With the possible exception of leukaemias arising secondary to previous chemotherapy (10) and mantle cell lymphoma, most authors have demonstrated that MSI is rare in lymphomas or leukaemias (11) and from this have inferred that MMR defects are also uncommon. However, this inference may be incorrect in cases with MSH6 deficiency and consequent loss of MutSα function. As microsatellite alteration can only occur at cell division, cases with MutSα deficiency may also be overlooked in malignancies such as leukaemia which tend to be diagnosed relatively early in their clinical course, as these will have undergone fewer cell divisions and consequently fewer microsatellite changes prior to diagnosis than more cryptic tumours, such as those arising in the colon.

The observation that MSI may be an unreliable marker of MMR in cells with defective MutSα function or in tumours which are diagnosed early in their clinical course prompted us to review the analysis of MMR proficiency in haematological malignancy using a more specific functional repair assay and an assessment of protein expression by western blotting and immunohistochemical techniques. We have also studied the relationship between MMR proficiency and proliferative capacity, as judged by Ki67 expression.

Materials and methods

Cell lines

The leukaemic cell lines and those used as positive and negative controls are summarized in Tables I and II. Cells were cultured in RPMI 1640 with 1-...
Table I. Characteristics of the leukaemic cell lines studied

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
<th>Genetic abnormality</th>
<th>P53 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raji</td>
<td>B-ALL</td>
<td>t(8;14)(q24;q32)</td>
<td>MYC-IGH</td>
<td>Mutant</td>
</tr>
<tr>
<td>DAUDI</td>
<td>B-ALL</td>
<td>t(8;14)(q24;q32)</td>
<td>MYC-IGH</td>
<td>Mutant</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>T-ALL</td>
<td>del(1)(p32)</td>
<td>SIL-TAL1</td>
<td>Mutant</td>
</tr>
<tr>
<td>Molt 4</td>
<td>T-ALL</td>
<td>2der(7)(7pter;7p15q11)</td>
<td>Not defined</td>
<td>Wild-type</td>
</tr>
<tr>
<td>JURKAT</td>
<td>T-ALL</td>
<td>45,X</td>
<td>P15 and P16 deletion</td>
<td>Mutant</td>
</tr>
<tr>
<td>NALM-6</td>
<td>Pre B-ALL</td>
<td>t(5;12)(q33.2;13.2)</td>
<td>PDGFRB-TEL</td>
<td>Wild-type</td>
</tr>
<tr>
<td>REH</td>
<td>Early B-ALL</td>
<td>(12;21)(p13q22)</td>
<td>TEL-AML1</td>
<td>Wild-type</td>
</tr>
<tr>
<td>K562</td>
<td>AML</td>
<td>t(9;14)(q23;q11)</td>
<td>BCR-ABL</td>
<td>Wild-type</td>
</tr>
<tr>
<td>HL60</td>
<td>AML</td>
<td>Complex abnormalities</td>
<td>Not defined</td>
<td>Altered</td>
</tr>
</tbody>
</table>


Table II. Characteristics of control cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>MMR status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hela</td>
<td>Ca cervix</td>
<td>Proficient</td>
</tr>
<tr>
<td>TK6</td>
<td>Lymphoblastoid</td>
<td>Proficient</td>
</tr>
</tbody>
</table>
| SW48      | Ca colon | Deficient in MLH1 due to promoter methylation
| LoVo      | Colorectal carcinoma | Deficient in MSH2 due to a homozygous deletion
| Hec-1-A   | Endometrial carcinoma | PMS2 gene mutation and a 199 base deletion in MSH6
| HCT15     | Colorectal carcinoma | Deficient in MSH6 due to homozygous mutation

Table III. Details of patients studied

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Presentation</th>
<th>Sex</th>
<th>Age (years)</th>
<th>WCCb (×10⁶/l)</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>L458</td>
<td>Presentation</td>
<td>F</td>
<td>2.8</td>
<td>58</td>
<td>48,XX,+X,+21c(10)/47,XX,+21c(10) trisomy 21 consistent with Downs</td>
</tr>
<tr>
<td>L430</td>
<td>Presentation</td>
<td>F</td>
<td>3.5</td>
<td>17.1</td>
<td>52,XX,XX,+4,+6,+14,+21,+21(2)/52,ident(17)(q10)(3)/46,XX(1)</td>
</tr>
<tr>
<td>L466</td>
<td>Presentation</td>
<td>F</td>
<td>16.6</td>
<td>17.6</td>
<td>46,XX(25)</td>
</tr>
<tr>
<td>L444</td>
<td>Presentation</td>
<td>M</td>
<td>1.9</td>
<td>82.5</td>
<td>t(9;11)(11q23) rearrangement</td>
</tr>
<tr>
<td>L480</td>
<td>Presentation</td>
<td>M</td>
<td>4</td>
<td>36.5</td>
<td>G-banding normal 46,XY(13)</td>
</tr>
<tr>
<td>L486</td>
<td>Presentation</td>
<td>M</td>
<td>4.6</td>
<td>173</td>
<td>46,XY,add(6)(q17p14)/46,XY(16)</td>
</tr>
<tr>
<td>L467</td>
<td>Presentation</td>
<td>M</td>
<td>7.1</td>
<td>106.1</td>
<td>Near haplode, two copies of chromosome 21/25XY,+21(9)/46,XY(2)</td>
</tr>
<tr>
<td>L496</td>
<td>Presentation</td>
<td>M</td>
<td>7.8</td>
<td>31.5</td>
<td>46,XY,del(12)(p+),inc(3)</td>
</tr>
<tr>
<td>L433</td>
<td>Presentation</td>
<td>M</td>
<td>8</td>
<td>22</td>
<td>54-58,XY,+X/dup(1)(q22q32)+4,+5,+6,+8,+10,+710,+17,+18,+21,+21?mar,inc(cp19)/46,XY(2)</td>
</tr>
<tr>
<td>L450</td>
<td>Presentation</td>
<td>M</td>
<td>14</td>
<td>11.6</td>
<td>Normal</td>
</tr>
<tr>
<td>L4</td>
<td>Relapse</td>
<td>M</td>
<td>5</td>
<td>4.9</td>
<td>90-92,XXYYinc/46,XY</td>
</tr>
</tbody>
</table>

All patients studied had common ALL and >90% blasts in the marrow.

WCC, white cell count.

Leukaemic blasts

A total of 10 patient samples were analysed for expression of MMR proteins by western blotting, immunohistochemistry and for functional MMR. Nine samples were from children with presentation ALL (seven males, age 22 months to 14 years and three females, age 32 months to 16 years) and one from a 5-year-old male child with relapsed ALL. Clinical details of the patients studied are given in Table III. The normal bone marrow trephines had all been shown to have no bone marrow involvement by microscopy. All bone marrow/peripheral blood samples were obtained according to local ethical guidelines.

All bone marrow samples were processed within 2–3 h of collection. Lymphoblasts were separated by density gradient centrifugation over Lymphoprep (Nycomed UK, Birmingham). Separated cells were washed in phosphate buffered saline (PBS), counted and the percentage of blasts determined by cytological examination after May Grunwald–Giemsa staining. All samples had >90% blasts. Bone marrow trephine biopsies, taken at the same time as bone marrow aspirates, were fixed in formalin, subjected to formic acid-based decalcification and processed for paraffin embedding. Three-micron sections were mounted on slides coated with aminoalkylsilane to assist adhesion.

Cytosol preparation

Cytosolic extracts were prepared from cell lines and lymphoblasts using a minimum of 1 × 10⁶ cells. Cells were washed in ice-cold isotonic buffer (20 mM HEPES pH 7.9, 5 mM KCl, 1.5 mM MgCl₂, 0.2 mM PMSF, 1 mM DTT, 250 mM sucrose, one complete EDTA-free protease inhibitor tablet to 50 ml buffer (Boehringer Mannheim)) followed by ice-cold hypotonic buffer (as above but without the sucrose) then resuspended in twice the pellet volume of ice-cold hypotonic buffer. Cells were lysed mechanically using a BioSpec Mini-Beadbeater (Stratech Scientific, Luton, UK), checking the progression
of the lysis microscopically. When around 85% of the cells were lysed, nuclei were pelleted and the supernatant cleared by centrifugation at 12,000 g for 7 min. Aliquots of the lysate were stored at −80°C prior to analysis. Cytosolic protein concentration was measured using the bicinchoninic acid protein assay kit (Pierce and Worthington, Chester, UK).

Mismatch repair assay

The ability of cytosolic extracts to repair DNA mismatches was assessed as described previously (12). Briefly, 5 ng of an M13mp2 phage heteroduplex substrate, prepared containing a G-T mispair and a nick in the strand containing the T, was incubated with 50 µg of cell lysate and the repaired/unrepaired heteroduplex substrate purified and electroporated into MMR deficient E. coli. Transformed bacteria were plated out with the α-complementation E. coli strain CH59 onto minimal agar plates supplemented with isopropyl β-D-thiogalactopyranoside and X-Gal (Sigma, Dorset, UK). The resulting plaques were scored as pure blue, mixed burst or clear. Reduction in the percentage of mixed plaques and increase in pure blue or clear plaques is indicative of MMR. Repair efficiency was calculated as 1 – (%) mixed plaques in test sample divided by (%) mixed plaques in a water only control. Complementation assays were performed using human recombinant MutLα (190 ng) and/or MutSβ (100 ng) protein, kindly provided by Dr Gaincarlo Marra (Institute for Medical Radiobiology, Zurich, Switzerland). The M13mp2 phage derivatives and E. coli strains were kindly provided by Dr Thomas Kunkel (National Institute of Environmental Health Sciences, Research Triangle Park, NC).

Western blotting

Cell lysates were prepared as described above, diluted in Laemmli sample buffer to a protein concentration of 0.5 mg/ml and heated at 100°C for 5 min. Ten µg of protein were loaded onto a 4–20% precast polyacrylamide gel (Invitrogen, Paisley, UK) and electrophoresed. Proteins were transferred onto Immoblot PVDF membrane (Bio-Rad, Hemel Hempstead, UK) by electroblotting at 30 volts overnight in CAPS buffer (10 mM CAPS, pH 11 with 10% methanol). After blocking in 5% skimmed milk in TBS/Tween (10 mM TBS, 100 mM NaCl, pH 7.5 plus 0.05% Tween 20) for 1 h, membranes were probed with either anti-MLH1, anti-PMS2, or anti-MSH2 mouse monoclonal antibodies at 2 µg/ml (BD Pharmingen, Oxford, UK), or with anti-MSH6 rabbit polyclonal antibody at 1:3000 (kind gift from Prof. Jiricny, Zurich). Primary antibodies were made up in 5% skimmed milk powder and incubated with the blots for 1 h at room temperature.

Each blot was also probed for actin as a protein loading control, using mouse monoclonal anti-actin at 1:10 000 (Oncogene, CN Biosciences, Nottingham, UK). The secondary antibodies used were horseradish peroxidase conjugated anti-mouse Ig (BD Pharmingen) used at 1:1000 and horseradish peroxidase conjugated anti-rabbit Ig (Amersham Pharmacia Biotech, Little Chalfont, UK) used at 1:2000. The immune complexes were visualized using an enhanced chemiluminescence reagent, ECL-Plus (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

Immunohistochemistry

Immunohistochemistry was performed on bone marrow trephines for the detection of MLH1, MSH2 and Ki-67, using a streptavidin–biotin technique coupled to peroxidase, as previously detailed (13). Briefly, slides were deparaffinized in HistoClear (Fisher Chemicals, Loughborough, UK) and rehydrated through alcohol to water. After removal of endogenous peroxidase activity by incubation in 0.1% hydrogen peroxide for 20 min, slides were submitted to antigen retrieval by microwaving for 15 min in 10 mM sodium trichloracetate buffer. The following primary antibodies were used (all mouse monoclonal); anti-MLH1 (clone G168-15, BD Pharmingen), anti-MSH2 (AB-2, Oncogene) and anti-Ki-67 (BD Pharmingen). A negative control was run for each slide using an irrelevant, isotype antibody (MOPC-31C, BD Pharmingen). The Vectastain Elite ABC kit (Vector Laboratories, Peterborough, UK) was used to detect immune complexes, according to the manufacturer’s instructions. Slides were lightly counterstained with haematoxylin and eosin, dehydrated in alcohol, cleared in Histoclear and mounted in DPX (Fisher Chemicals). Positive and negative controls (summarized in Table II) were included on all slides. Slides were run for each slide using an irrelevant, isotype antibody (MOPC-31C, BD Pharmingen). The Vectastain Elite ABC kit (Vector Laboratories, Peterborough, UK) was used to detect immune complexes, according to the manufacturer’s instructions.

Results

Western blots

Western blots for the cell lines studied are shown in Figure 1. The two myeloid lines (K562 and HL60) demonstrated expression of all four MMR proteins studied, as did two of the seven lymphoid lines (Raji and Daudi). The five remaining lymphoid lines studied showed defects in one or both components of the MutSα (NALM-6 and JURKAT) or MutLα complexes (Reh, CCRF-CEM and MOLT 4). In NALM-6 neither component of the MutSα complex could be detected. In each of the four cell lines with defective expression, one member of either the MutSα or the MutLα complex was absent and the other markedly reduced in expression. These results indicate that a defect probably exists in one of the proteins, leading to the instability and partial degradation of its corresponding heterodimeric partner as has been previously suggested (14,15).

Western blots obtained from patient samples are shown in Figure 2, which includes samples from the 10 cases obtained at presentation and the one, non-paired sample, taken at relapse (arrowed). Expression MLH1, PMS2, MSH2 and MSH6 was clearly demonstrated in all cases studied.

Immunohistochemistry

Staining for MLH1, MSH2 and Ki-67 proteins gave strong nuclear positivity with wide inter-individual variation in the percentages of positive cells. An example is shown in Figure 3 (A–D) of a sample at diagnosis. Correlation of the percentage of positive cells demonstrated a highly significant correlation.
between MLH1 expression and Ki-67 expression with a $P$ value of $<0.0001$ (Figure 4). Correlations were not seen between numbers of cells positive for MSH2 and Ki-67 nor MLH1 and MSH2 (data not shown). Analysis of the trephine obtained at relapse showed it to have a higher ratio of MLH1/ Ki67 expression than the presentation cases.

Analysis of normal bone marrow trephines from five children with non-haematological malignancies (Figure 3, E-H) showed that expression was largely confined to less differentiated cells of the erythroid and myeloid lineages as previously reported (16).

In vitro assessment of MMR proficiency

In order to validate the functional MMR assay in our laboratory, analysis of G.T repair was carried out on a range of cell lines with previously documented MMR defects or proficiency.

Results are illustrated in Figure 5. In each case studied in vitro results correlated with western blot results. The MMR proficient positive controls, TK6 and HeLa, gave repair efficiencies of 90% and 75% respectively for G.T substrate. The cell lines defective in MLH1 (SW48), MSH2 (Lovo), PMS2 (Hec-1-A) and MSH6 (HCT15) all showed little or no repair. Addition of recombinant MutLα protein to the SW48 cytosol restored repair fully. Similar results were obtained using MutSα in the Lovo and HCT15. In the case of the Hec-1-A cell line, the addition of MutLα protein alone only partially restored repair, as there is also a reported defect in the MSH6 gene (17). Full complementation of this cytosol was obtained by the addition of both MutLα and MutSα proteins (Figure 5).

Results obtained using the panel of leukaemic cell lines
analysed by western blotting are shown in Figure 6, with good concordance between the functional repair results and the western blot data. Out of the nine cell lines tested, five showed little or no repair. Defects in MMR genes have previously been reported in two of these cell lines. CCRF-CEM has been shown to have a mutation and loss of expression of the MLH1 gene (18) and in JURKAT, both alleles of the MSH2 gene have been inactivated (19). Cell lines with expression of all of the MMR proteins (HL60, K562, Raji and Daudi) showed >50% repair efficiency. Interestingly, the proficient cell line with the lowest MSH2 expression (HL60) also had the lowest level of in vitro repair proficiency. Repair proficiency in the five lymphoid cell lines with defects in expression of one or more of the MMR proteins studied were all below 30%. Complementation experiments were performed to determine if defects were confined to either the MutS or MutL heterodimers. Results (shown in Figure 7) show restoration of function in each case.

In vitro analysis of G.T MMR in cytosolic extracts from lymphoblasts showed a wide range of proficiency (Figure 8). Although there was not a statistically significant correlation between repair proficiency and MMR protein expression, as judged by the percentage of positive cells on immunohistochemistry (data not shown), too few cases were studied to exclude a relationship. The lowest level of proficiency was recorded in the sample obtained at relapse. However, in this case, there was clear expression of each of the four MMR proteins studied as judged by western blotting and immunohistochemistry.

Discussion

MSI has primarily been reported in solid neoplasms and appears to be rare in acute leukaemias (11,20–22). However, loss of microsatellite stability has been reported in other haematological malignancies, including chronic myeloid leukaemia (CML) (23) where instability was found to be associated with transition to blast crisis, chronic lymphocytic leukaemia (24) where it was found in a subset of patients (7%), and therapy related leukaemia (10) where instability was detected in 94% of the patients studied. Most investigations of MMR competency in haematological malignancies have relied almost exclusively on the measurement of MSI. One notable exception is a recent report in which all 19 exons of the hMLH1 gene were screened for mutations in 97 cases of CML (53 in chronic phase and 44 in blast crisis) and 36 cases of AML (17 de novo and 19 secondary) (25). No mutations were detected in any of the samples screened. However, as hypermethylation of the promoter region of the hMLH1 gene has been reported as a cause of MMR defects in cell lines and solid tumours (26,27), and the MMR pathway involves several other components the lack of mutations in the hMLH1 gene cannot be accepted as proof of repair proficiency. Recently Zhu et al. reported that MSI and p53 mutations were associated with low levels of MSH2 expression, as judged by western blotting, in adults with acute leukaemia (AML), mainly AML in the elderly or following prior chemotherapy (28,29). Analysis of other components of the MMR system was not included in this report.

Although MSI has been clearly linked with MMR defects in HNPCC due to germline mutations in MSH2 or MLH1,
defects due to mutations in MSH6 are not detected by this method, which only assesses MutSβ and MutL function as these are involved in IDL detection and correction. As recently reviewed by Maehara et al., the assessment of MSI is also fraught with technical problems, affecting both the performance of the analysis and the interpretation of the results obtained (30). The authors point out that a clear distinction between MSI and loss of heterozygosity is often difficult to establish. Finally, the generation of altered microsatellites can only occur during cell division and, to be detectable, must be present in a significant percentage of cells. These conditions may not occur in a rapidly expanding malignancy such as acute leukaemia. In this context it is interesting to note that MSI has been detected in more indolent haematological malignancies such as chronic myeloid leukaemia (CML), some patients with chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma, as noted above.

In view of the limitations of relying exclusively on MSI as a measure of MMR proficiency we have used the in vitro repair assay originally described by Thomas et al. (12) to assess MMR function in a panel of cell lines developed from patients with haematological malignancies and in cells from children with ALL. Cell lines were chosen for inclusion in the panel to be studied in order to reflect different forms of childhood ALL (31) rather than because of previously described defects in MMR or drug resistance. Surprisingly five out of seven of the lymphoid cell lines tested were found to have a defect in the ability to detect and repair a G.T base–base mismatch, a frequency higher than that seen in colon cancer cell lines (32). In each of the five defective lines, loss of expression of one or more of the four best characterized MMR proteins was demonstrated on western blotting. As previously reported (14,18) defects affected both partners of either the MutS or MutL heterodimers, suggesting a primary defect in the expression of one gene with subsequent instability of the undimerized partner protein. Addition of cytosol from MutL defective cell lines (CEM, Reh and MOLT 4) to lysate from MutS defective JURKAT cells restored repair proficiency. Similarly, addition of cytosol from MutS defective NALM-6 cells restored repair activity to MutL defective MOLT 4 or Reh cells. These results suggest that defects in these cell lines are confined to either the MutS or MutL heterodimers. The defective lines are not confined to a single lineage; CCRF-CEM, MOLT 4 and JURKAT are all immature T-ALL cell lines (33), whereas NALM-6 and Reh are both precursor B-ALL lines. In a recent publication (34), MSI and BAX mutations were analysed in a large cohort of ALL cell lines. MSI was found in a higher proportion of lymphoid lineage cell lines than myeloid and furthermore, it was significantly more frequent in T-lineage than B-lineage. A very high incidence (82%) of BAX frameshift mutations was also observed in a subset of these cell lines with MSI. As CCRF-CEM, MOLT 4 and JURKAT were all derived from patients at relapse it is tempting to speculate that the defects in MMR that they display arose during the course of treatment in vivo. However, the defects may have been present at diagnosis or have arisen in culture.

In a previous study of expression of MMR proteins in acute leukaemia by western blotting, we reported that MSH2 was detectable in all cases studied (50 children and 22 adults with ALL). MLH1 was expressed in all but three cases. PMS2 was absent in 29 cases and MSH6 in 16 (35). One aim of the current study was to determine if variations in protein expression were related to the number of positive cells present or reflected differences in levels in the entire blast population. Immunohistochemical analysis of trephine biopsies in a cohort of nine patients demonstrated clear differences in the percentage of positive cells present. There was a strong relationship between expression of MLH1 and proliferative capacity as determined by expression of Ki67. This was not seen for MSH2, which was expressed in over 40% of cells in all cases studied. In a study of peripheral blood lymphocytes, Marra et al. reported a 12-fold increase in the level of MSH2 after cells were stimulated to divide ex vivo (36). In contrast, another report has characterized MSH2 and Ki-67 expression in breast carcinomas and found upregulation of the MSH2 protein in tumour tissue compared with normal breast tissue, but found no correlation with Ki-67 expression (37).

In our previous study (35) western blot analysis of the mononuclear fraction of normal bone marrow demonstrated no MLH1 or PMS2 expression in the seven cases studied. MSH6 was detected in every case and MSH2 in four of the seven samples. Immunohistochemistry of normal bone marrow trephines suggests that MLH1 expression is confined to the less differentiated components of the bone marrow; cells that also express Ki67. These results suggest that in normal bone marrow, and in childhood ALL, expression of MLH1 is confined to cells which are actively dividing and may be lost in cells in the Go phase of the cell cycle or which have become terminally differentiated. In a review of the possible consequences of expression of MMR proteins in non-dividing cells, MacPhee argues that MMR in non-dividing cells may have a detrimental effect (38). As there is no mechanism to differentiate between DNA strands in Go cells there is a 50% chance that a mutation will become established in the genome rather than eliminated by the MMR pathway. Down-regulation of MLH1 in non-dividing bone marrow cells may therefore form an important protective mechanism against repair induced damage.

Analysis of MMR proficiency using the functional repair assay revealed a wide range of activity. Further cases will have to be studied to establish if this is related to expression of the MMR proteins. In one case studied at relapse there was absent G.T base–base repair. This was not associated with absent expression of any of the four MMR proteins studied as determined by either western blotting or immunohistochemistry. Absent repair ability in this case may be due to a defect in another component of the MMR system or a point mutation in the coding region of one of the MMR genes resulting in a non-functional truncated protein which, nevertheless, still retains the antibody epitope. This could account for the few HNPPC cases reported in the literature where normal expression of both MLH1 and MSH2 protein is associated with high MSI (39,40). As defects in base–base MMR repair are associated with drug resistance (41,42), this abnormality may be responsible, at least in part, for the emergence of relapsed leukaemia in this case.

In summary, using an in vitro repair assay and western blotting, we have demonstrated MMR defects in five of seven lymphoid cell lines. In cells obtained from patients with leukaemia we have shown that expression of MLH1 is closely related to proliferative capacity and that there is a wide range of repair proficiency. In one case studied at relapse, expression of the MMR repair proteins appeared normal in spite of a marked defect in the ability to repair G.T base–base mismatches in vitro. These results suggest that MMR defects may be more
common in acute lymphoid malignancies than previously suspected from studies that have relied exclusively on analysis of MSI.

Acknowledgements

The invaluable assistance of Professor J Jiricny and Dr G. Marra (Institute for Medical Radiobiology, Zurich, Switzerland) in establishing the MMR functional assay and of Dr M. Reid (RVI, Newcastle, UK) for the provision of clinical samples is gratefully acknowledged. This work was supported by grants from the North of England Children’s Research Fund and the Leukaemia Research Fund.

References


Received June 20, 2002; revised September 17, 2002; accepted September 19, 2002