Papers

Configuration of immunoglobulin and T cell receptor β and γ genes in acute myeloid leukaemia: Pitfalls in the analysis of 40 cases

L Parreira, C Carvalho, H Moura, A Melo, P Santos, J E Guimarães, A Parreira

Abstract

Aims: To evaluate the overall incidence of immunoglobulin (Ig) and T cell receptor (TCR) β and γ gene rearrangements in a series of 40 cases of acute myeloid leukaemia (AML) and to determine whether structural modifications of these genes could be correlated with the abnormal expression of lymphoid markers in malignant cells.

Methods: All cases were classified according to the criteria of the FAB group and immunophenotyped with a panel of monoclonal antibodies reactive with myeloid and lymphoid differentiation antigens. DNA analysis was performed by the method of Southern using probes for the Ig J\textsubscript{H}, TCR-C\textsubscript{β}, and TCR-J\textsubscript{γ} regions.

Results: Phenotypic analysis showed that in addition to myeloid markers, 10 cases expressed lymphoid antigens: CD7 in seven (of which three were TdT positive, one CD2 positive, and one CD19 positive) and CD19 in three. Southern blot analysis showed that bands with sizes different from the germ line control were present in the TCR β genes in 11 cases: in six of 30 with pure myeloid phenotype and in five of 10 of those expressing lymphoid markers. A close observation of the size and patterns of those bands, however, showed that they could be artefactual. Indeed, further analysis showed that they were either due to resistant Eco RI|Hind III sites at the β locus or to plasmid contamination. Rearranged genes were eventually found in only two of the 40 cases: at the Ig J\textsubscript{H} region in one of the 30 with pure myeloid phenotype (3.3%) and at the TCR γ genes in one of 10 with lymphoid markers (10%).

Conclusions: These observations showed that Ig/TCR gene rearrangements were rare in this AML series (overall incidence of 5%) and that they were not significantly more common in cases with aberrant expression of lymphoid markers. The size and pattern of the potential non-germline bands that can be found in these loci must be carefully evaluated.

The immunological analysis of leukaemic cells in acute myeloid leukaemia (AML) has been particularly helpful in the characterisation of poorly differentiated cases where the assignment of malignant cells as myeloid is not easy using conventional morphology and cytochemistry. On the other hand, it has shown that leukaemic cells with otherwise unequivocal myeloid features, such as myeloperoxidase expression, may often exhibit nuclear or membrane antigens that in normal haemopoiesis are restricted to B or T lymphoid lineages. Expression of terminal deoxynucleotidyl transferase (TdT), a nuclear enzyme known to be involved in the generation of diversity of Ig and TCR genes, has been reported to occur in 5–10% of AML cases and to be associated with the morphologically immature subtypes M0 and M1. A similar incidence and morphological correlation has also been found for the expression of CD7, a 40 kilodalton protein of unknown function, present on the membrane of normal thymocytes, mature T lymphocytes, T lymphoblastic leukaemias and also in a pluripotent progenitor cell capable of multilineage differentiation.

The biological importance of these findings is unknown despite the considerable debate it has raised—namely the cellular origin of these leukaemias and their clinical behaviour. The results on the analysis of Ig or TCR genes in these cases have also been controversial. Several reports point to a high incidence of Ig or TCR rearrangements in TdT positive AML. Other studies have failed to show such a correlation between the expression of TdT or CD7 and rearrangements at these loci. The reasons for this discrepancy are not clear but may be related to analysis of different patient groups (adults rather than children), stringency of criteria for the immunological diagnosis of AML, or even misinterpretation of Southern blot results.

Methods

Forty patients (31 adults and nine children) with newly diagnosed acute leukaemia were studied. The morphological diagnosis of AML was based on the examination of bone marrow and peripheral blood aspirate stained with May-Grunwald-Giemsa and the cytochemistry reactions, Sudan Black B or myeloperoxidase, and α-naphthyl-acetate esterase. All cases were classified according to the revised criteria of the FAB classification and were distributed as follows: M1-7; M2-7; M3-1; M4-6; M5a-12; M5b-2; M6-1. In four patients whose leukaemic blast cells were unreactive with SBB, MPO, and ANAE (cases 8, 18, 19 and 29), AML (M0) was diagnosed, based on the reactivity with the myeloid antibodies CD33 or CD13, and the absence of
lymphoid markers other than TdT or CD7. 24
Case 17 was classified as M6 by the presence of typical morphology and the reactivity with a glycophorin antibody in 37% of the blast cells.

IMMUNOLOGICAL STUDIES
The mononuclear cell fraction of peripheral blood or bone marrow was isolated by density gradient centrifugation with Lymphoprep (Nyegaard). Cyto centrifuge slides were prepared for cytochemical reactions and immunocytochemistry.

The panel of monoclonal antibodies used to identify the phenotype of blast cells included the following markers reactive with haemopoietic precursors and myeloid cells: CD34 (My10); HLA-DR (GRB1); CD33 (My9); CD13 (My7); CD11b (OKM1); CD14 (My4); CD15 (CD3-1); CD41 (J15); CD61 (Y2/51); CD71 (BerT9) and anti-glycophorin. The lymphoid differentiation antigens included TdT (HT-1, HT-3, HT-4 — Dako); for the B lineage, CD19 (HD37), CD22 (To15), and CD10 (CALLA); for the T lineage, CD7 (3A1), CD2 (T9-10), CD3 (T3-4B5), CD1 (NA1/34). The assessment of membrane antigens was performed by conventional immunofluorescence methods. The detection of nuclear TdT and cytoplasmic antigens was carried out by immunocytochemical reactions (indirect immunoperoxidase or APAAP) as previously described in detail. 8 Each specific marker was scored as positive when in the presence of 20% or more reactive cells, except for TdT and CD19, where a cutoff value of 10% was used.

DNA STUDIES
DNA was extracted from the peripheral blood or bone marrow mononuclear cell fraction according to previously published methods. 25
In all samples tested more than 85% of the cells were morphologically malignant. Control germine DNA was obtained from normal peripheral blood granulocytes.

To investigate Ig heavy chain gene rearrangements, overnight digestions (100 IU enzyme/15 μg of high molecular weight DNA) were carried out with Eco RI, Hind III, Bgl II and Bam HI. TCR β gene rearrangements were analysed with Eco RI, Hind III, and Bam HI and TCR γ with Bam HI. After digestion, DNA was size fractioned in 0.7–0.8% agarose gel electrophoresis, denatured, neutralised and blotted on to nylon membranes by capillary transfer according to the method of Southern. 26

Table 1: Clinical and haematological characteristics of 40 AML patients

<table>
<thead>
<tr>
<th>Case No</th>
<th>FAB</th>
<th>Sex</th>
<th>Age</th>
<th>White cell count</th>
<th>% Blast*</th>
<th>MPO</th>
<th>HLA</th>
<th>CD34</th>
<th>CD13/CD33</th>
<th>TdT</th>
<th>CD19</th>
<th>CD10</th>
<th>CD7</th>
<th>CD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M5a</td>
<td>M</td>
<td>1</td>
<td>600</td>
<td>90</td>
<td>0</td>
<td>91</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M2</td>
<td>M</td>
<td>3</td>
<td>120</td>
<td>90</td>
<td>90</td>
<td>38</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M1</td>
<td>M</td>
<td>35</td>
<td>25</td>
<td>90</td>
<td>55</td>
<td>65</td>
<td>23/50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M5a</td>
<td>F</td>
<td>58</td>
<td>19</td>
<td>60</td>
<td>30</td>
<td>72</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M1</td>
<td>F</td>
<td>21</td>
<td>9</td>
<td>90</td>
<td>18</td>
<td>82</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M5a</td>
<td>M</td>
<td>20</td>
<td>40</td>
<td>95</td>
<td>95</td>
<td>0</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M2</td>
<td>F</td>
<td>68</td>
<td>88</td>
<td>94</td>
<td>27</td>
<td>44</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M0</td>
<td>F</td>
<td>8</td>
<td>4</td>
<td>99</td>
<td>1</td>
<td>7</td>
<td>43</td>
<td>50/76</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M5a</td>
<td>F</td>
<td>3</td>
<td>70</td>
<td>90</td>
<td>20</td>
<td>50</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M5a</td>
<td>M</td>
<td>59</td>
<td>79</td>
<td>70</td>
<td>30</td>
<td>53</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M5a</td>
<td>M</td>
<td>19</td>
<td>40</td>
<td>80</td>
<td>0</td>
<td>55</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M5a</td>
<td>F</td>
<td>18</td>
<td>180</td>
<td>98</td>
<td>5</td>
<td>50</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>M5a</td>
<td>F</td>
<td>56</td>
<td>64</td>
<td>84</td>
<td>0</td>
<td>70</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M5a</td>
<td>M</td>
<td>19</td>
<td>48</td>
<td>85</td>
<td>0</td>
<td>75</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>M2</td>
<td>F</td>
<td>9</td>
<td>48</td>
<td>95</td>
<td>90</td>
<td>76</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>M1</td>
<td>F</td>
<td>66</td>
<td>37</td>
<td>93</td>
<td>90</td>
<td>57</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>M6</td>
<td>M</td>
<td>77</td>
<td>22</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>M0</td>
<td>F</td>
<td>69</td>
<td>5</td>
<td>60</td>
<td>0</td>
<td>70</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>M0</td>
<td>M</td>
<td>73</td>
<td>2</td>
<td>90</td>
<td>0</td>
<td>60</td>
<td>28</td>
<td>62/02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>M2</td>
<td>M</td>
<td>82</td>
<td>200</td>
<td>90</td>
<td>70</td>
<td>64</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>M2</td>
<td>F</td>
<td>83</td>
<td>20</td>
<td>90</td>
<td>79</td>
<td>79</td>
<td>20</td>
<td>20/56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>M5b</td>
<td>F</td>
<td>7</td>
<td>19</td>
<td>95</td>
<td>95</td>
<td>4</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>M2</td>
<td>F</td>
<td>54</td>
<td>65</td>
<td>87</td>
<td>65</td>
<td>75</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>M4</td>
<td>F</td>
<td>19</td>
<td>58</td>
<td>85</td>
<td>35</td>
<td>53</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>M5a</td>
<td>F</td>
<td>4</td>
<td>263</td>
<td>90</td>
<td>0</td>
<td>87</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>M4</td>
<td>F</td>
<td>65</td>
<td>25</td>
<td>75</td>
<td>25</td>
<td>80</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>M5b</td>
<td>F</td>
<td>76</td>
<td>3</td>
<td>70</td>
<td>0</td>
<td>82</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>M4</td>
<td>F</td>
<td>19</td>
<td>38</td>
<td>75</td>
<td>50</td>
<td>70</td>
<td>37/22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>M0</td>
<td>M</td>
<td>64</td>
<td>32</td>
<td>95</td>
<td>0</td>
<td>85</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>M1</td>
<td>M</td>
<td>71</td>
<td>122</td>
<td>70</td>
<td>9</td>
<td>62</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>M1</td>
<td>M</td>
<td>60</td>
<td>40</td>
<td>80</td>
<td>10</td>
<td>80</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>M1</td>
<td>M</td>
<td>64</td>
<td>37</td>
<td>88</td>
<td>12</td>
<td>87</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>M5a</td>
<td>F</td>
<td>32</td>
<td>90</td>
<td>72</td>
<td>0</td>
<td>90</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>M4</td>
<td>F</td>
<td>63</td>
<td>120</td>
<td>86</td>
<td>89</td>
<td>7</td>
<td>67/92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>M4</td>
<td>F</td>
<td>4</td>
<td>120</td>
<td>70</td>
<td>20</td>
<td>63</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>M1</td>
<td>M</td>
<td>72</td>
<td>50</td>
<td>90</td>
<td>15</td>
<td>80</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>M2</td>
<td>F</td>
<td>14</td>
<td>70</td>
<td>80</td>
<td>70</td>
<td>70</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>M2</td>
<td>M</td>
<td>79</td>
<td>40</td>
<td>95</td>
<td>40</td>
<td>40</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>M5a</td>
<td>F</td>
<td>54</td>
<td>70</td>
<td>48</td>
<td>3</td>
<td>48</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>M2</td>
<td>F</td>
<td>45</td>
<td>85</td>
<td>90</td>
<td>78</td>
<td>30</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bone marrow.
solution, 100 μg/ml denatured hsDNA, and
hybridised for 18 hours with 2 × 10⁶ cpm/ml
of labelled probe. After hybridisation, blots
were washed for 2 × 15 minutes in 2 x SSC,
0.1% SDS, 2 × 15 minutes in 0.2 SSC, 0.1% 
SDS at 65°C, and autoradiographed at - 70°C
with intensifying screens for four to seven
days.

**Results**

**IMMUNOLOGICAL PHENOTYPING**

The clinical and haematological features of the
40 AML cases are summarised in table 1. In 10
cases a significant proportion (20% or higher)
of blast cells expressed markers that in normal
haemopoiesis are regarded as specific for
lymphoid lineages. Of these 10 cases, five were
morphologically immature (two M0; three
M1), and in five the blast cells showed features
of maturation (three M2 and two M4). Seven
cases were CD7 positive, of which three were
also TdT positive (cases 5, 8, and 29). The
three TdT positive, CD7 positive AML cases
also expressed CD34 and CD13/CD33 except
for case 5 which was classified as M1 because of
typical morphology and 8% MPO positive
blasts; other lymphoid antigens including cyto-
plasmic CD3 were absent. In case 7, a typical
M2 with Auer rods, both CD7 and CD2 were
expressed in a high proportion of blasts. Cyto-
plasmic CD3 as well as TdT were negative.
The remaining CD7 positive cases (cases 3, 16
and 34) were negative for all other lymphoid
markers. A proportion of 21% CD7 positive
cells and 27% CD2 positive cells were also
observed in cases 35 and 36, respectively,
where the reactivity for these markers with
remaining normal T cells could not be ruled
out. A significant proportion of leukaemic cells
reacting with CD19 was found in three cases
(2, 15, and 35).

**DNA ANALYSIS**

The results on DNA analysis are summarised in
table 2. The Ig(JH) and TCR-β genes were
investigated in all cases and the TCR γ genes in all but nine cases.

Ig genes exhibited a germline pattern in all but case 1, a 3 year old child with M5a, which did not express any lymphoid marker. Two rearranged bands were found with three restriction enzymes. *Eco RI* and *Hind III* digests of this case are shown in fig 1. A faint germline band was observed in the *Hind III* digest, indicating that a minor population of cells retained a germline configuration despite the morphological and immunological homogeneity of the blast cells in this case.

The TCR β genes probed with C β1 showed germline bands in 28 cases. In the remaining 12 one or more bands with sizes different from the germline control could be observed—in six out of 29 AML cases without lymphoid markers (20%) and in six out of 10 cases expressing lymphoid antigens (60%). The new bands were observed only in *Eco RI* or *Hind III* digests and had the following characteristics: an 8 kilobase *Eco RI* band was found in five different cases (table 2 and fig 2). This band disappeared in different digests of the same samples (cases 2 and 3). Bands of 3-2 and 2-7 kilobases were present in case 1. The former was also visible in cases 5, 9, and 15. New *Eco RI* digests of cases 1 and 9 probed with radiolabelled cloning vector (pUC19) showed that those bands were the result of plasmid contamination (fig 2B). Cases 9 and 15 also had the 8 kilobase band; in the *Hind III* digests a 13 kilobase band was present in cases 2, 3, 12 and 18, 34, 36 and 37 (table 2 and figs 3 and 4). In all these cases the persistence of the 11 and 4 kilobase *Eco RI* and the 7-4 and 3-3 kilobase *Hind III* germline bands was difficult to reconcile with any pattern of β gene rearrangement. The findings with these two enzymes are compatible with partial digestions at the *Eco RI* site close to the 5' end of the C β2 region or at the *Hind III* site that lies within the first intron of C β2 (fig 2). The *Bam HI* digests (not tested in cases 3 and

---

**Figure 2A** *Eco RI* digests hybridised with Cβ1 probe. Bands with a different size from that of the germline control (lines) are indicated with an asterisk. Cases 2, 3, and 5 are shown in repeated digests. The partial restriction enzyme map at the bottom is based on previously published data. The germline fragments detected by *Eco RI* (R), *Hind III* (H), and *Bam HI* (B) are indicated by continuous lines below the map and fragments derived from partly resistant *Eco RI* and *Hind III* (R) by broken lines.

**Figure 2B** *Eco RI* digests of cases 1 and 9 hybridised with 32P labelled pUC19 show the presence of plasmid contamination in these samples.
5) probed with Cβ1 eventually showed that all these cases were in germline configuration at the β locus, thus confirming the artefactual nature of those findings (figs 4 and 5).

The TCR γ genes were in germline configuration in all but case 8 (AML-M0, TdT, and CD7 positive), where two new bands were visible in Bam HI digests. This finding is compatible with a diallelic rearrangement for Cγ1 (fig 6). This sample was in germline configuration at the Ig and β genes. In case 1 a 2.7 kilobase band was visible, identical in size with a band found in the Eco RI digest of the same case, probed with Cβ1.

In summary, we found a total of 25 “non-germ-line” bands in 40 AML samples (table 2), of which 16 were present at the β locus in Eco RI or Hind III digests. None of these corresponded to a true rearrangement, as shown by Bam HI digests and as suspected by examination of the respective restriction map.39 32 One additional band at the TCR γ locus was probably the result of a cross-hybrisation artefact. Of the remaining eight bands, six were present at the Ig heavy chain locus in one case (detected by three different restriction enzymes), and two were seen at the TCR γ in another case. Only two out of 40 AML samples had rearranged genes: 1 of 29 of pure myeloid phenotype (3-3%) and 1 of 10 expressing lymphoid markers (10%), a difference which was not significant.

**Discussion**

The DNA results in this series could, at first
sight, be interpreted as evidence for the presence of rearrangements in TCR β genes in a significant proportion of AML cases expressing markers of lymphoid lineage. A closer analysis of the molecular data, however, would immediately pose the following problems of interpretation: (1) the presence of new Eco RI or Hind III bands in the β genes that had the same molecular weight in different DNA samples; (2) patterns of "rearrangement" with both enzymes that did not fit with each other.

The first finding would, on its own, be an unexpected pattern for rearrangements occurring in genes with great diversity, unless the "inappropriate" rearrangements in AML cells reflected the use of the same gene segments in different patients. A more likely explanation would obviously be technical artefact. In fact, an analysis of the size of those bands showed that five of 10 new Eco RI fragments were 8 kilobases, even in lanes with some electrophoretic distortion due to DNA overload, and all new Hind III bands were 13 kilobases. These sizes would be expected if a partial digestion at the Eco RI site 5' to Cβ2 region or the Hind III site within Cβ2 had occurred and the fragments from which they originate should not be interpreted as rearrangements. In cases 1, 5, 9 and 15 Eco RI fragments of less than 4 kilobases were found. In our view, Eco RI bands of less than 4 kilobases do not seem compatible with a rearrangement for Cβ1 as the distance between the Eco RI site 3' to Cβ1 and the more proximal J β1 segment is more than 4 kilobase in germline DNA. Alternative explanations would be rearrangements occurring through a non-looping out deletion mechanism, which has been reported in β genes, or a cytogenetic anomaly involving the β locus or Eco RI site polymorphisms. Nevertheless, it seems unlikely that any of the first two phenomena would produce similar bands in different samples. In fact we were able to show that those bands were not caused by true rearrangements as the Bam HI digests showed a germline configuration at the β locus in these patients. Although Eco RI polymorphisms cannot be ruled out in the absence of a germline control derived from each individual patient, polymorphisms for this enzyme do not seem to occur at this locus. As expected, a cross-hybridisation artefact due to plasmid contamination was shown in two samples.

An additional problem in those samples would be the coexistence of strong germline fragments with "non-germline" bands in the Eco RI and Hind III digests. The intensity of the 11 kilobase germline fragment containing Cβ1 in Eco RI digests, or the 7-3 kilobase germline fragment containing Cβ2 in Hind III digests made it unlikely that a monoallelic rearrangement for these regions had occurred in those patients (figs 2 and 3). Also unlikely would be the presence of a dual cell population, as all the samples analysed had more than 85%...
leukaemic blasts, the morphology of which seemed to be homogeneous.

We can conclude, then, that the β genes were in germline configuration in all these patients, despite the presence of restriction fragments that differed from the control DNA in two different digests.

The TCR γ genes were in germline configuration in all cases except case 8, where two faint bands were just visible in Bam HI digests. This patient, an 8 year old girl, had morphologically immature leukemia (M0); the blasts expressed TdT and CD7 antigens in a high proportion of cells. The Ig and TCR β genes were in germline configuration. The pattern of bands observed in this case could correspond to a diallelic rearrangement for Cγ1 but, due to the limited number of combinations of the TCR γ genes, does not necessarily indicate a monoclonal population.34 35 In normal T cells as well as in almost all T lymphoblastic leukemias, the β and γ genes are rearranged in the same cells, reflecting the fact that both genes rearrange at the same stage of differentiation in T cell ontogeny.25 31 36 37 The rearrangement of γ genes in the presence of germline β genes has nevertheless been reported in TdT positive AML,16 although that seemed to occur in cases where the Ig genes were also rearranged.

Rearrangement at the Ig heavy chain genes was observed in only one patient (case 9) of our series, a 3 year old child. It was an M5a AML without any lymphoid marker. An Eco RI or Hind III polymorphism, due to extremely variable rearrangement in the region 5′ to the J cluster,38 was unlikely as two rearranged bands were also visible in Bgl II digests in this case. The only two rearrangements found in this series occurred in children. In the series published by Foa et al,16 where a high incidence of Ig gene rearrangements was found in 10 patients with TdT positive AML, eight were children and there was a predominance of monocytic subtypes. In a series of AML in infants Koller et al39 found Ig rearrangements, with germline TCR β and γ genes, in 20% of the patients, although no relation with TdT expression or any particular FAB subtype could be established. It is conceivable that AML in children and infants might intrinsically have biological characteristics which differ from those found in adult AML. Rearrangements in Ig or TCR genes, regardless of the phenotypic profile exhibited by the leukemic cell, could be just one of these characteristics.

In summary, our results confirm recent reports stating that TdT or CD7 expression in AML blasts is not associated with a high incidence of rearrangements in Ig or TCR genes. These findings are not totally unexpected, especially in terms of CD7 expression in leukemic cells. The expression of this molecule is known to precede TCR gene rearrangement in T cell ontogeny, a fact that suggests that it is not necessarily related to the rearrangement of those genes.40 41 On the other hand, it has been shown that normal CD7 positive fetal liver cells are capable of differentiating either into lymphoid T or myeloid lineages.12 Kurtzberg et al3 showed the same phenomenon in a series of CD7 positive immature leukemias. One of the patients had an in vivo phenotypic transformation from T cell lineage to myeloid differentiation (with the myeloid blasts retaining the rearrangement of TCR genes). In this context CD7 expression in myeloid blasts in a few cases may represent just a relic of an ancestral phenotype. Those few cases of CD7 positive AML that exhibit TCR gene rearrangements might indeed correspond to the expansion of a clonalogenic cell that, having transiently passed throughout a T cell pathway of differentiation, has transmitted to its progeny the permanent scar of an irreversible molecular event.

We thank Dr T H Rabbits (Laboratory of Molecular Biology, Cambridge) for providing DNA probes and Dr Garrido Torres-Pujol (Granada) for the gift of several monoclonal antibodies.

Part of this work was performed in the Instituto Guibemhan de Ciencia under the supervision of Dr M C Lecher. This research was supported in part by grant No. 87576 from Junta Estatal de Investigacion Cientifica e Tecnologica and also by Instituto Nacional de Investigacion Cientifica.

We thank all those colleagues who provided clinical material used in the study.

18 Furley AJW, Chan LC, Mizutani S, et al. Lineage
specificity of rearrangement and expression of genes encoding the T cell receptor-T3 complex and immunoglobulin heavy chain in leukaemia. *Leukemia* 1987;1: 644-52.


Configuration of immunoglobulin and T cell receptor beta and gamma genes in acute myeloid leukaemia: pitfalls in the analysis of 40 cases.

L Parreira, C Carvalho, H Moura, A Melo, P Santos, J E Guimarães and A Parreira

doi: 10.1136/jcp.45.3.193

Updated information and services can be found at:
http://jcp.bmj.com/content/45/3/193

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/