The Presence of Deoxyribonucleic Acid (DNA)
Synthesizing Cells in Patients with Refractory Anemia

By I. A. Cooper and B. G. Firkin

Following incubation with H³ Thymidine and using autoradiography Bond et al.¹ have demonstrated the presence of a small number of circulating labeled mononuclear cells in specimens of blood obtained from normal persons under in vitro conditions. This presumably demonstrates the capacity of these cells for DNA synthesis, division and proliferation.

Rubini et al.² have described the incidence of labeled cells in the peripheral blood of patients with various hematologic disorders including one case of aplastic anemia which was possibly due to chloramphenicol toxicity.

The present communication is a report of the in vitro labeling in blood leukocytes of patients with refractory anemias including eight cases with aplastic anemia, two with hemolytic anemia, four with chronic renal failure. Three normal persons were included in this study as controls.

METHODS

The tritiated thymidine labeled DNA is detected by using a microautoradiographic technic, described by Bryant et al. (1960), a modification of a method originally described by Pelc.³

In vitro mixtures consisted of 8 ml. of venous blood, 2 ml. of Dextran (6 per cent), 5 drops of liquid heparin and 0.2 ml. of a solution containing 20 µci of tritiated thymidine.* This mixture was incubated in sterile McCartney bottles at 37 C. for 1 hour. At the end of incubation, the white cell rich supernatant was centrifuged at 1500 rpm for 10 minutes. The buffy coat was then aspirated into 5 drops of plasma. Smears of the buffy coat were then made on glass slides, dried and fixed in methanol for 12 hours. Auto-radiograms were made using Kodak AR-10 stripping film. The autoradiograms were exposed for 12-14 days at 4 C. and then developed, and stained with Wright's stain.

The labeling index was obtained by counting the number of labeled leukocytes in 3000. Cells that had 10 or more grains over the nuclei were considered labeled. The gross labeling index was established by estimating the number of white cells labeled per 10,000.

A differential and total count of the leukocytes was performed in all cases.

RESULTS

Table 1 summarizes the findings on the subjects included in this study. The age of the patients, hemoglobin values, white cell count and differential
Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age in Years</th>
<th>Hemoglobin in Gm./100 ml.</th>
<th>WCC/cu. mm.</th>
<th>Differential %</th>
<th>Total Cells Counted</th>
<th>Gross Labeling Index per 10,000 WBC</th>
<th>Ratio Labeled Cells: (L + M)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. R.</td>
<td>42</td>
<td>10.4</td>
<td>4,000</td>
<td>81</td>
<td>3,000</td>
<td>6</td>
<td>0.003</td>
<td>Aplastic anaemia†</td>
</tr>
<tr>
<td>I. L.</td>
<td>42</td>
<td>9.2</td>
<td>1,500</td>
<td>54</td>
<td>3,000</td>
<td>60</td>
<td>0.013*</td>
<td>Aplastic anaemia</td>
</tr>
<tr>
<td>E. C.</td>
<td>36</td>
<td>8.6</td>
<td>2,000</td>
<td>32</td>
<td>3,000</td>
<td>50</td>
<td>0.009*</td>
<td>Aplastic anaemia</td>
</tr>
<tr>
<td>N. P.</td>
<td>54</td>
<td>11.6</td>
<td>5,700</td>
<td>73</td>
<td>3,000</td>
<td>4</td>
<td>0.002</td>
<td>Aplastic anaemia†</td>
</tr>
<tr>
<td>J. O.</td>
<td>72</td>
<td>10.9</td>
<td>4,200</td>
<td>48</td>
<td>3,000</td>
<td>0</td>
<td>0.000</td>
<td>Aplastic anaemia†</td>
</tr>
<tr>
<td>B. M.</td>
<td>29</td>
<td>8.4</td>
<td>5,900</td>
<td>51</td>
<td>3,000</td>
<td>7</td>
<td>0.001</td>
<td>Aplastic anaemia†</td>
</tr>
<tr>
<td>F. P.</td>
<td>56</td>
<td>4.8</td>
<td>3,700</td>
<td>56</td>
<td>3,000</td>
<td>3</td>
<td>0.001</td>
<td>Aplastic anaemia†</td>
</tr>
<tr>
<td>E. A.</td>
<td>30</td>
<td>6.0</td>
<td>3,700</td>
<td>55</td>
<td>3,000</td>
<td>30</td>
<td>0.007*</td>
<td>Aplastic anaemia</td>
</tr>
<tr>
<td>A. H.</td>
<td>55</td>
<td>6.0</td>
<td>17,100</td>
<td>62</td>
<td>3,000</td>
<td>110</td>
<td>0.028†</td>
<td>Haemolytic anaemia</td>
</tr>
<tr>
<td>E. R.</td>
<td>52</td>
<td>7.8</td>
<td>21,000</td>
<td>50</td>
<td>3,000</td>
<td>80</td>
<td>0.017†</td>
<td>Paroxysmal nocturnal haemoglobinuria</td>
</tr>
<tr>
<td>K. McC.</td>
<td>49</td>
<td>9.0</td>
<td>12,800</td>
<td>70</td>
<td>3,000</td>
<td>0</td>
<td>0</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>J. M.</td>
<td>45</td>
<td>6.0</td>
<td>12,000</td>
<td>75</td>
<td>3,000</td>
<td>3</td>
<td>0.001</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>C. E.</td>
<td>28</td>
<td>6.8</td>
<td>6,900</td>
<td>72</td>
<td>3,000</td>
<td>3</td>
<td>0.001</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>P. L.</td>
<td>24</td>
<td>6.9</td>
<td>14,100</td>
<td>80</td>
<td>3,000</td>
<td>0</td>
<td>0</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>G. M.</td>
<td>26</td>
<td>13.2</td>
<td>7,200</td>
<td>63</td>
<td>3,000</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>B. F.</td>
<td>32</td>
<td>14.0</td>
<td>6,500</td>
<td>72</td>
<td>3,000</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>S. F.</td>
<td>28</td>
<td>13.4</td>
<td>7,500</td>
<td>70</td>
<td>3,000</td>
<td>2</td>
<td>0</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*These patients show a high ratio and labeling index.
†These patients gave a history of exposure to either benzol, chloramphenicol or carbon tetrachloride.
(DNA) SYNTHESIZING CELLS IN REFRACTORY ANEMIA

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together with the gross labeling index and diagnosis are included in the table.

(1) Patients with aplastic anemia: Of the seven patients with aplastic anemia and one patient with red cell hypoplasia, three demonstrated increased labeling. In each of the patients with increased labeling no history of any exogenous etiological factor leading to the anemia was obtained, whereas in four of the remaining five patients there was history of exposure to toxic agent factors.

(2) Patients with hemolytic anemia: One of the two patients had paroxysmal nocturnal hemoglobinuria with an associated positive direct Coombs' test (Beal et al.4). The other patient had an acquired hemolytic anemia of unknown etiology with a positive direct Coombs' test. Both demonstrated a markedly increased labeling index.

(3) Patients with chronic renal failure: The four patients in this group did not demonstrate any increase in labeling of leukocytes. The blood urea ranged between 120–280 mg./100 ml.

(4). Normal controls: These persons demonstrated 0–0.02 per cent labeling in keeping with findings of other workers, when examining blood from persons (Bond et al., 1958).

DISCUSSION

This study has confirmed the findings of Bond et al.1 that small numbers of mononuclear cells capable of DNA synthesis are normally present in peripheral blood. Patients previously studied by this technic have consisted mainly of instances in which primitive cells might be expected to be found in the peripheral blood viz, acute leukemia, myeloid metaplasia, multiple myeloma, polycythemia vera, and infectious mononucleosis (Rubini et al.).2 Such primitive cells were not present in the blood of the patients included in this study, with refractory anemia, nor can the increase in labeling index be accounted for by a disproportionate increase in the number of mononuclear cells present. The cell type which has taken up the label has the morphologic characteristics of the young lymphocyte. The appearance of these cells bore no relation to recent blood transfusion. The fact that these labeled cells appear in increased numbers in some patients with idiopathic aplastic anemia and in two patients with hemolytic anemia (both with abnormal globulins) suggests two possible mechanisms:

(a) Aplasia may result from damage to the red cell stem cells or to the bone marrow matrix. It would follow that two types of aplasia may occur. In this study there were two groups of patients, the one with an increased labeling index, but the other with a normal labeling index (table 1). No definite conclusion can be drawn from these findings but the increased labeling index could be interpreted as being due to the influence of totipotential cells (Yoffey5) as a compensating mechanism to repopulate the depleted bone marrow.

(b) An alternative explanation would be that these cells are concerned with a process of auto-immunity (Burnet6). The production of young lymphocytes after antigenic stimulation has been described by Hall7 and the
presence of young lymphocytes in an anemia associated with a positive direct Coombs' test is not surprising. A similar increase in patients with aplastic anemia is of great interest, since this might support an auto-immune etiology in some cases of aplasia. This hypothesis is supported by the bone marrow findings in aplasia where infiltration with lymphocytes and plasma cells is common, in the recently reported association of positive Coombs' tests in some cases of aplasia (Havard\textsuperscript{8}) and in some instances, by the presence of thymomas of refractory anemia (Wintrobe\textsuperscript{9}).

The fact that corticosteroid therapy was being used in both groups of patients makes the possibility that this increase in labeling is due to corticosteroid therapy most unlikely. Indeed there is evidence to suggest that corticosteroids would have the opposite effect (Dougherty and White\textsuperscript{10}).

**SUMMARY**

An autoradiographic technic with tritiated thymidine to label cells in the peripheral blood undergoing DNA synthesis, has been utilized to study patients with refractory anemia.

Included in this study are patients with aplastic or hypoplastic anemia and acquired hemolytic anemia. Patients with anemia and chronic renal failure and normal persons as controls have been included.

The patients with aplasia, with no history of exposure to toxic agents, showed a marked increase in labeling index, whereas the patients with a history of exposure to toxic agents showed a normal labeling index.

The patients with refractory hemolytic anemia and positive Coombs' test revealed a marked increase in labeling.

The cells revealing the increased labeling have the morphologic characteristics of the young lymphocyte.

The hypotheses have been put forward that the cases of aplasia showing an increased labeling index together with the cases of refractory hemolytic anemia may be explained on an auto-immune basis or an increased number of circulating totipotential cells in response to the anemia.

**SUMMARIO IN INTERLINGUA**

Un technica autoradiographic, utilisante thymidina a tritium in marcir cellulas in le sanguine peripheric in stato de synthetisage de acido deoxyribonucleic (ADN), esseva applicate al studio de patientes con anemia refractori.

Includite in le studio es patientes con anemia aplastic o hypoplastic o con acquiritite anemia hemolytic. Es etiam includite patientes con anemia e chronic disfallimento renal e subjectos normal qui servi como gruppo de controlo.

Le patientes con aplasia sed sin le antecedente de exposition a agentes toxic monstrava un marcate augmento del indice de marcage, durante que le patientes con un tal antecedente monstrava un normal indice de marcage.

Le patientes con refractori anemia hemolytic e positivitate in tests de Coombs revelava un marcate augmento del indice de marcage.
SYNTHESIZING CELLS IN REFRACTORY ANEMIA

Le cellulas que exhibi le augmentate marcase ha le caracteristicas mor-
phologic de juvene lymphocytes.

Es presentate le hypothese que le casos de aplasia con augmento del indice
de marcase e etiam le casos de refractori anemia hemolytic pote esser explicate
a base de un mecanismo de auto-immunitate o a base de un augmentate
numero de cellulas totipotential in responsa al anemia.

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