Changes in T and B blood lymphocytes after splenectomy

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SUMMARY  The blood lymphocytes of 37 splenectomised patients were analysed by means of T and B lymphocyte surface markers. Sixteen patients had had a splenectomy for non-haematological and 21 for haematological reasons. The results show that 15 had normal numbers of T and B cells; decreased T cells were found in two patients, raised B cells in seven, raised T and B cells in eight, and raised T cells in five patients. Increased numbers of 'null' cells were observed in some patients, especially in those with raised B cells. Follow-up studies indicate that raised levels of T and B cells can be established by one to three months post-splenectomy and may persist, although in some patients the cells fall to normal levels. The lymphocyte proliferative response to phytohaemagglutinin and Concanavalin A in vitro was normal in eight out of nine patients with raised T cells and was depressed in one patient, possibly due to an intrinsic cell defect.

A raised blood lymphocyte count in patients after splenectomy has been reported by several groups of workers, for example, Ek and Rayner (1950), Lipson et al. (1959), Pedersen and Videbaek (1966), and McBride et al. (1968). From these reports it appears that, whatever the reason for splenectomy, a persistent lymphocytosis is found in more than half the patients. However, the increased numbers of blood lymphocytes in splenectomised persons have not, to our knowledge, so far been studied using surface marker tests for T and B lymphocytes. This report presents the results of a study of 37 splenectomised patients in whom the percentage and absolute numbers of T and B and non-T, non-B ('null') blood lymphocytes were measured. Some of these patients were followed up, and blood samples were analysed at intervals after splenectomy.

Patients

Altogether 37 splenectomised patients were studied; they were divided into two groups:

GROUP 1
This group comprised 16 patients (11 males and 5 females) splenectomised for a non-haematological reason (splenic rupture or during the course of a surgical operation such as gastrectomy or pyloroplasty). Patients with malignant disease were excluded.

GROUP 2
This group comprised 21 patients (4 males and 17 females) splenectomised because of a haematological disorder. The reason for splenectomy was idiopathic thrombocytopenic purpura (ITP) in 15 patients, autoimmune haemolytic anaemia (AIHA) in two patients, congenital spherocytosis (CS) in two patients, and hypersplenism with pancytopenia (HP) in two patients.

Both groups are heterogeneous as regards age and time since splenectomy. The only real difference between the two groups is the preponderance of males in group I and of females in group II.

Controls

Forty-two subjects served as controls (13 males, 29 females; age range 15-88 years). These comprised fit laboratory personnel, inmates of old people's homes, and outpatients coming up for blood tests in whom there was no evidence of haematological, inflammatory, or malignant disease and who were not taking any drugs.
Methods

**ESTIMATION OF T AND B LYMPHOCYTES**

Lymphocytes from samples of heparinised venous blood were separated on Ficoll/Triosil, washed in PBS (pH 7·3), and processed for the sheep red cell rosetting test for T lymphocytes (E-rosettes) according to the method of Wybran et al. (1973). The samples were stored overnight at 4°C before being examined for rosette-forming lymphocytes. The percentage of E-rosettes was calculated, and the absolute number of T lymphocytes per litre of blood was determined.

Aliquots of washed lymphocytes were incubated at 4°C with fluorescein-conjugated polyvalent sheep antiglobulin (Wellcome Laboratories), washed in PBS, and examined by epifluorescence (SM-LUX Leitz fluorescence microscope, HBO 50W mercury UV lamp). The percentage of cells with surface immunglobulin (SIg) was calculated, hence the absolute number of B lymphocytes per litre of blood.

**RESPONSE TO MITOGENS**

Lymphocytes, separated on Ficoll/Triosil, were washed twice with RPMI 1640-medium supplemented with 10% autologus plasma, streptomycin, penicillin, and 1-glutamine, and buffered with NaHCO₃ (pH 7·6). Aliquots containing 6 × 10⁶ lymphocytes were distributed into wells of flat-bottomed microtitre plates (Sterilin Ltd). Phytohaemagglutinin (PHA) (Wellcome Laboratories) at 1/24 dilution and Concanavalin A (Con A) (Pharmacia Ltd) at 1 µg/well were added to five wells each, and five wells served as the unstimulated control. Three sets of wells were set up for harvesting at 60-72 and 84-96 hours. The plates were incubated at 36°C in a humidified CO₂ incubator with 5% CO₂ in air. After the incubation period the cells were pulsed for 4 hours with 2 µCi ³H-thymidine (specific activity 2 Ci/mM) and harvested on to glass fibre discs, which were counted in a liquid scintillation counter. The results were expressed as lymphocyte transformation index by dividing the average counts per minute of stimulated cells by the average of unstimulated counts.

Results

**ESTIMATION OF T AND B LYMPHOCYTES IN BLOOD SAMPLES**

The data from 42 control subjects are presented in Table 1. These figures are in reasonable agreement with data from other laboratories (Jondal et al., 1972; WHO/IARC Report, 1974; Hayward and Greaves, 1977). No differences between age groups were observed.

On the basis of the absolute numbers of T and B lymphocytes per litre of blood, the results in the 37 splenectomised patients can be classified as:

- Normal results (15)
- Low T lymphocytes (2)
- Raised B lymphocytes (7)
- Raised T and B lymphocytes (8)
- Raised T lymphocytes only (5)

**Normal results**

The numbers of T and B lymphocytes were within the normal control range in 15 patients (nos 1-15). Eight of these were non-haematological (group I) and seven haematological (group II). Of the latter, one had AIHA, one congenital spherocytosis, one hypersplenism, and four ITP. The interval since splenectomy ranged from one week to 17 years. As regards diagnosis, age, sex, and interval of time since splenectomy, these patients with normal levels of T and B lymphocytes are as heterogeneous as the entire group of 37 splenectomised patients.

**Low T lymphocytes**

Two patients (nos 16 and 17), both with ITP, showed low levels of T lymphocytes (0·43 × 10⁹/l and 0·35 × 10⁹/l), normal B lymphocytes, and raised 'null' cells in case 16. This patient had been treated with prednisone one month previously.

Table 1  Patients with increased numbers of B cells + control group

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Group</th>
<th>Time since splenectomy (yr)</th>
<th>Lymphocytes × 10⁹/l</th>
<th>T cells % × 10⁹/l</th>
<th>B cells % × 10⁹/l</th>
<th>'Null' cells % × 10⁹/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>73</td>
<td>M</td>
<td>I</td>
<td>10/12</td>
<td>3·98</td>
<td>28</td>
<td>1·11</td>
<td>1·11</td>
</tr>
<tr>
<td>19</td>
<td>56</td>
<td>M</td>
<td>II (HS)</td>
<td>4</td>
<td>4·86</td>
<td>18</td>
<td>0·87</td>
<td>2·07</td>
</tr>
<tr>
<td>20</td>
<td>77</td>
<td>M</td>
<td>II (ITP)</td>
<td>19</td>
<td>2·74</td>
<td>32</td>
<td>0·88</td>
<td>0·99</td>
</tr>
<tr>
<td>21</td>
<td>14</td>
<td>F</td>
<td>II (ITP)</td>
<td>7</td>
<td>3·10</td>
<td>52</td>
<td>1·62</td>
<td>1·12</td>
</tr>
<tr>
<td>22</td>
<td>11</td>
<td>M</td>
<td>II (ITP)</td>
<td>5</td>
<td>3·78</td>
<td>16</td>
<td>0·60</td>
<td>1·40</td>
</tr>
<tr>
<td>23</td>
<td>35</td>
<td>F</td>
<td>II (ITP)</td>
<td>25</td>
<td>3·45</td>
<td>6</td>
<td>0·21</td>
<td>1·24</td>
</tr>
<tr>
<td>24</td>
<td>34</td>
<td>F</td>
<td>II (CS)</td>
<td>4</td>
<td>4·88</td>
<td>40</td>
<td>1·95</td>
<td>2·59</td>
</tr>
</tbody>
</table>

*Mean values ± standard deviation.

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Raised B lymphocytes
The data from the patients with raised numbers of B lymphocytes are shown in Table 1. The upper limit of normal of B lymphocytes was taken as 0.8 × 10⁹/l. In two patients (cases 22 and 23) the number of T cells was depressed, the lower limit of normal being taken as 0.8 × 10⁹/l. None of the group had been taking steroids within the past four years. In six patients, high percentages and raised absolute numbers of ‘null’ cells were observed, taking the upper limit of normal of ‘null’ cells as 0.85 × 10⁹/l.

Raised T and B lymphocytes
The results from these patients are shown in Table 2. As with the other groups, this group is heterogeneous. The lymphocyte counts tend to be higher than in the previous two groups. In two patients (cases 27 and 28) the numbers of ‘null’ cells were increased.

In some cases the lymphocytes were cultured in serum-free medium TC 199 for 1 hour at 37°C before incubation with fluorescein-conjugated antilgulb. This was to eliminate any labile cytoplasmic γ-globulin which may have been adsorbed by the lymphocytes via the Fc receptor (Lobo et al., 1975; Winchester et al., 1975). The results show that there was little or no difference between the percentage of fluorescing cells with and without this culture step. Therefore few (and in some cases none) of the cells had adsorbed labile γ-globulin, and the calculation of B lymphocytes based on the fluorescence of non-cultured cells is substantially correct.

In one patient (case 32) a prespleenectomy sample was obtained two months before the operation. The lymphocyte count was 1.35 × 10⁹/l and the T and B lymphocyte counts were within normal limits. She was taking prednisone, which was discontinued one and a half months after splenectomy.

Six patients were followed up with serial blood samples (one to three samples from each case). As shown in Table 2, in four patients both T and B cells remained consistently elevated, and in two patients T or B cells fell to normal levels during the period of follow-up. Thus the level of T and B cells may be raised by one to three months post-splenectomy, and these cells either remain elevated or, in some cases, return to normal.

Raised T lymphocytes
The data from the five patients with raised T lymphocytes are given in Table 3. Four patients were followed up (with two to three serial samples each) and the increased numbers of T lymphocytes persisted in all four.

In several of the patients with raised T lymphocytes, large lymphocytes were observed in Jenner-Giemsa-stained blood films. These are cells with large nuclei of mature type, and pale, abundant cytoplasm with a few azurophilic granules. In cases 32, 33, 34, and 35, they comprised 40-70% of the lymphocytes. However, it was not possible to correlate the percentage of these large lymphocytes on blood films with the percentage of T lymphocytes found in separated lymphocyte populations. Cytocentrifuge preparations of sheep red cell rosetting cells showed that many large and some small lymphocytes formed rosettes. This indicates that these large lymphocytes are T cells, although some of the small lymphocytes are T cells as well.

<table>
<thead>
<tr>
<th>Table 2 Patients with increased numbers of T and B cells</th>
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<tbody>
<tr>
<td><strong>No.</strong></td>
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<tr>
<td>25</td>
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<tr>
<td>26</td>
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<tr>
<td>31</td>
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</table>

<table>
<thead>
<tr>
<th>Table 3 Patients with increased numbers of T cells</th>
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<tbody>
<tr>
<td><strong>No.</strong></td>
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<tr>
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</tr>
<tr>
<td>33</td>
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<tr>
<td>34</td>
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<td>35</td>
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<td>36</td>
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<td>37</td>
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Peripheral blood films from case 34 were stained for acid phosphatase by the method of Goldberg and Barka (1962). This cytochemical stain is a marker for cells of T-lymphocytic origin (Cavovsky et al., 1974). In this case the large lymphocytes comprised 70% of the total lymphocytes on blood films, and 80% of these large cells gave a positive reaction for acid phosphatase. This suggests that the majority of large cells are of the T-cell type.

LYMPHOCYTE RESPONSE TO MITOGENS

Controls
The lymphocyte response in 10 control subjects was determined. These were healthy laboratory personnel (6 men, 4 women, aged 26-48 years).

The response to PHA was greatest at 60-72 hours. Considerable variation in the lymphocyte transformation index (LTI) was found between individual controls, although the LTI was always greater than 20 and, in some instances, was greater than 100.

As regards the response to Con A, in most (but not all) experiments this was greater at 84-96 hours than at 60-72 hours, and the maximum response from the controls always gave LTI greater than 12.

Patients
The following nine patients were studied on at least one occasion (and, in some cases, on two or three separate occasions): Cases with raised T and B lymphocytes: cases 25, 26, 27, 31, and 32. Cases with raised T lymphocytes: cases 33, 34, 35, and 36. The response to PHA and Con A was normal in all the patients (except case 33); that is, the LTI was greater than 20 for PHA at 60-72 hours and was greater than 10 for Con A at either 60-72 or 84-96 hours. As in the controls, the LTI varied considerably and in some cases was very high (>100). The magnitude of the LTI could not be correlated with the level of T lymphocytes in individual cases. Case 33: Cultures with PHA and Con A were set up on several separate occasions. The response to both mitogens was consistently poor compared with the controls. Thus the maximum LTI observed with PHA was 7-3. This patient, who had the highest number of circulating T lymphocytes (see Table 3), nevertheless gave the weakest response to the predominantly T-cell mitogens PHA and Con A. The response to tuberculin (PPD) in vitro was brisk (LTI 102), showing that some of his T cells, at any rate, are capable of mounting a specific immune response in vitro. Further experiments were carried out, culturing washed lymphocytes from case 33 and from a normal control with PHA or Con A, together with patient's serum or pooled AB serum or control serum. The results show that the defect lay in the cells themselves with no evidence for a serum inhibitory factor.

Discussion
This study is concerned with the effect of splenectomy on the numbers of the main blood lymphocyte populations, that is, the T, B, and 'null' lymphocytes. It demonstrates the main changes in these populations that can be observed after splenectomy, whether for a non-haematological or a haematological disorder. The commonest haematological disorder in this series is idiopathic thrombocytopenic purpura, which has failed to respond to steroids. The changes in the blood lymphocytes can be summarised as follows:

About 40% of patients have normal numbers of T and B blood lymphocytes.

The remainder show either increased numbers of B lymphocytes, or increased numbers of T lymphocytes, or increased numbers of both T and B lymphocytes. Approximately equal numbers of patients are distributed in each of these three categories. There is no correlation between being in any particular category and the reason for splenectomy (non-haematological or haematological).

The data suggest that the large lymphocytes frequently observed in the blood films of splenectomised patients are mostly T lymphocytes.

A minority of patients (4 out of the total of 37) have low levels of T lymphocytes. In one case this could be related to recent steroids.

In about 25% of patients there are increased numbers of 'null' lymphocytes. This is more likely to be associated with increased numbers of B lymphocytes than with other changes.

Follow-up data in a number of cases show that raised levels of T and B blood lymphocytes may be established by one to three months post-splenectomy and that these may persist. Several patients who had a splenectomy performed more than 10 years ago have persistently raised T or B lymphocytes (or both). In some cases, T or B lymphocytes (or both populations) increase and then subsequently fall to normal levels during the first year after splenectomy.

Most patients with raised T lymphocytes show a normal proliferative response in vitro to PHA and Con A. One patient showed a defective cellular response to both mitogens but a normal response in vitro to PPD.

One further point of some practical importance can be made. The persistent blood lymphocytosis in one patient (case 33) led to an erroneous diagnosis of mild chronic lymphocytic leukaemia; our results suggest that such a diagnosis should be made with caution in patients with a history of splenectomy.

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References


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