Osmotic Behavior of Normal and Leukemic Lymphocytes

By S. Ben-Sasson, R. Shaviv, Z. Bentwich, S. Slavin, and F. Doljanski

The response of normal human peripheral blood lymphocytes to a hypotonic environment may be divided into two phases: the cells first exhibit rapid osmotic swelling, followed by a slower shrinking phase, during which they regain their initial physiologic volume. This osmotic behavior is characteristic of most mammalian and avian nucleated cells so far examined. The normal human blood lymphocyte, however, shows the most rapid recovery phase (5 min). Lymphocytes from chronic lymphatic leukemia patients, in comparison, show a strikingly slower rate of return to their initial isotonic volumes. The mechanism underlying osmotic cell volume regulation and its significance are discussed.

Recent studies on the osmotic response of cells to a hypotonic environment have demonstrated that a variety of mammalian and avian cells do not behave according to the classic osmometer model, as do mammalian erythrocytes, but are able to regulate their volume under a wide range of hypotonic but not hypertonic conditions. When normal chicken blood lymphocytes, cultured neoplastic murine lymphoid cells, or duck erythrocytes are exposed to hypotonic media, they first swell as expected within 1–3 min, but immediately afterwards shrink back more gradually to their initial isosmotic volume. The velocity of the second regulatory phase is characteristic for each cell type.

In the present paper, cell volume regulation of human peripheral blood lymphocytes obtained from normal or nonleukemic donors is compared to that of lymphocytes obtained from patients with chronic lymphatic leukemia (CLL). One case of acute lymphatic leukemia (ALL) is also described. Striking differences have been found in the kinetic pattern of volume regulation between normal and leukemic lymphocytes.

MATERIALS AND METHODS

Lymphocytes

Human peripheral blood mononuclear cells (usually >90% lymphocytes) were isolated from 5–10 ml heparinized venous blood as described by Harris and Ukaejiofo with the following modifications: the blood was diluted with phosphate-buffered saline (PBS) 1:1, layered onto 2 ml Ficoll-Hypaque, and centrifuged at 250 g for 30 min; the isolated cells were washed once in PBS and resuspended in 1 ml autologous plasma (diluted 1:1 with PBS). This represents the stock cell
suspension. The lymphocytes can be maintained in this environment at room temperature for several hours without showing any change in their osmotic behavior. Commercial heparin should not be used, since chlorocresol present in such preparations may alter the size distribution pattern as well as the osmotic behavior of lymphocytes. A lymphocyte population enriched with B-lymphocytes was prepared according to Bentwich et al. 7

**Cell Volume Determinations**

These were carried out using the electrical method of cell sizing developed by Coulter. 8 We have recently proposed the theoretical basis for the electrical sizing of particles in suspensions 9 and have described in detail the instrumentation developed in our laboratory for accurate cell sizing. 10 The conditions required for lymphocyte cell-size analysis have also been reported, 11 and the procedures in the present investigations have been carried out accordingly.

**Suspending Media**

Phosphate-buffered saline (PBS) was prepared according to Dulbecco and Vogt. 12 Phosphate-buffered potassium (PBK) is identical to PBS except that sodium and potassium are interchanged and the calcium concentration is halved in order to prevent turbidity. These media are iso-osmotic with human plasma (relative tonicity, 1.0).

**Osmotic Shock**

One to four drops of the stock cell suspension were introduced into 20 ml PBS or PBK of 0.6 relative tonicity and volume measurement started within 30 sec; 0.6 tonicity was well within the range where a straight line relationship was found between volume and the reciprocal of the osmotic pressure of the medium.

The time required for a sizing of about 15,000 cells was 20 sec. Since human blood lymphocytes, like chicken blood lymphocytes, 1 show a homogeneous response to the osmotic shock, the mode of the cell volume distribution curve was used as the cell parameter representing the volume distribution of the cells. This criterion was used rather than mean volume, since the former could be read directly from the sizing device without any additional calculations.

**RESULTS**

**Volume Regulation in Normal Lymphocytes**

When normal human lymphocytes are introduced into an environment of low osmolarity, they first swell and then begin to shrink back to their initial volume. Figure 1A and Table 2 depict the time course of the changes in cell volume of lymphocytes obtained from ten apparently healthy donors. The osmotic response of lymphocytes taken from different individuals is remarkably

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>No. Leukocytes (cu mm)</th>
<th>% Lymphocytes</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>48</td>
<td>269,000</td>
<td>96</td>
<td>Leukoran</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>86</td>
<td>46,000</td>
<td>93</td>
<td>No treatment</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>78</td>
<td>73,000</td>
<td>92</td>
<td>Meticorten</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>65</td>
<td>36,000</td>
<td>95</td>
<td>No treatment</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>65</td>
<td>106,000</td>
<td>97</td>
<td>Meticorten</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>66</td>
<td>49,000</td>
<td>99</td>
<td>Leukoran</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>80</td>
<td>19,200</td>
<td>81</td>
<td>No treatment</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>61</td>
<td>19,800</td>
<td>85</td>
<td>No treatment</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>58</td>
<td>73,000</td>
<td>95</td>
<td>Endoxan then meticorten</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>64</td>
<td>44,000</td>
<td>94</td>
<td>No treatment</td>
</tr>
</tbody>
</table>
Fig. 1. Changes in cell volume of peripheral blood lymphocytes after hypotonic shock in 0.6 PBS. (A) Healthy donors. (B) Nonleukemic patients. The striped area is the range of volume changes of lymphocytes in isotonic PBS.
Table 2. Cell Volume and Time of Cell Volume Regulation in Normal and Leukemic Lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Healthy Donors</th>
<th>Nonleukemic Patients</th>
<th>CLL Patients</th>
<th>Values of t Test Between the Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Modal volume (cu μ)</strong></td>
<td>198 ± 15</td>
<td>216 ± 14</td>
<td>170 ± 32</td>
<td>(p &gt; 0.02)</td>
</tr>
<tr>
<td><strong>Time of return to the original volume after hypotonic shock (min)</strong></td>
<td>4.2 ± 1.2</td>
<td>5.2 ± 4.0</td>
<td>35.4 ± 15.3</td>
<td>(p &gt; 0.5)</td>
</tr>
<tr>
<td><strong>Time of return to 1.1 of the original volume after hypotonic shock (min)</strong></td>
<td>2.9 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>25.2 ± 13.6</td>
<td>(p &gt; 0.5)</td>
</tr>
</tbody>
</table>

*Hypotonic shock in 0.6 PBS.
†This parameter was underestimated in some of the leukemic cells since not all of the leukemic cells return to the original volume and some remain static at about 1.1 of the initial volume.

similar. The osmotic behavior of lymphocytes obtained from ten nonleukemic patients (Fig. 1B) does not differ from that of the lymphocytes of healthy donors. These patients included cases of aplastic anemia, polycythemia, rheumatoid arthritis, chronic bronchitis, regional ileitis, and three cases of cardiac disorders. Three patients received massive cortisone treatment. Figure 2 and Table 2 summarize the time course of the response of leukemic cells obtained from ten CLL patients. Each curve, numbered from 1 to 10, represents

![Diagram](image-url)

**Fig. 2.** Changes in cell volume of peripheral blood lymphocytes from CLL patients and one ALL patient, after hypotonic shock in 0.6 PBS. The striped area represents the range of the response of normal blood lymphocytes according to Fig. 1A. · --- · --- · ALL patient.
one of the patients whose leukocyte counts are summarized in Table 1. It is clear that the volume regulatory phase is much slower in such cells.

Only one case of ALL was examined (Fig. 2): a female, age 15. The lymphocytes were obtained during an acute stage of the disease (8200 leukocytes per cu mm, 95% lymphocytes, 45% lymphoblasts) and during remission following treatment (3200 leukocytes per cu mm, 47% lymphocytes, 2% lymphoblasts). It was clear that lymphocytes obtained during the acute phase exhibited a marked inhibition in their ability to regulate their volume, even more pronounced than in CLL lymphocytes. During remission a normal response occurred.

The Effect of Serum on the Osmotic Behavior of Lymphocytes

The striking difference observed in the velocity of volume regulation between normal and leukemic lymphocytes may be due to serum factors. In order to examine this possibility, leukemic lymphocytes were incubated in serum from healthy donors for 3 hr at room temperature, and normal lymphocytes were treated similarly with serum from CLL patients. It was found that this treatment did not alter the characteristic time course of volume regulation of each cell type.

The Response of Lymphocytes to Hypotonic Environment in the Presence of \( K^+ \) as the Dominant Cation in the Suspending Medium

When normal or leukemic lymphocytes are introduced into a hypotonic medium in which the dominant Na\(^+\) ion is replaced by K\(^+\), they continue to swell beyond the initial osmotic equilibrium volume reached within the first minute. Figure 3 depicts the process during the first 6 min of osmotic adaptation. After this period the cells continue to swell still further, but their characteristic and constant size distribution pattern becomes distorted. It is observed that the leukemic cells swell at a lower rate than normal lymphocytes. This difference is, however, less striking than the difference between the behavior of these two cell types during the shrinking phase in hypotonic PBS.

Size Distribution and Volume Regulation of T- and B-lymphocytes

Since the relative frequency of T- and B-lymphocytes in the blood of CLL patients is different from that of nonleukemic ones, it was important to determine whether or not any differences in the size distribution and osmotic behavior could be detected between these two types of lymphocytes. No significant difference could be detected in the size distribution pattern of normal blood lymphocytes (80%, T-lymphocytes) and a B-lymphocyte cell population (60%, B-lymphocytes as determined by sheep red blood cell rosette formation).

The osmotic behavior of the B-lymphocyte population could not be examined, since the procedures for their isolation markedly inhibited their osmotic response. It was possible, however, to assume that these lymphocytes were similar to T-lymphocytes as regards osmotic behavior, for if they possessed a different pattern of response this would be detected in the size distribution analysis of the original normal blood lymphocyte population. These distributions were based on a very large number of cells (> 300,000),
and if 20% of the population responded differently this would easily be detected. It was also of interest to note that one of the CLL patients had a T-lymphocyte-type leukemia; these cells exhibited an osmotic adaptation characteristic of the usual type of leukemia.

**DISCUSSION**

Recent studies on the osmotic behavior of cells have shown that different types of nucleated cells are able to regulate their volume in an environment of lower than normal osmolarity. Two phases characterize the response of cells to hypotonic conditions: an initial rapid phase of cell swelling and a second slower phase in which the cells shrink and regain their initial isotonic volume. This phase is the volume regulatory phase. The findings described in this paper demonstrate that normal human blood lymphocytes exhibit a much faster rate of volume regulation (3–4 min) than any other kind of cells examined so far.

Lymphocytes from CLL patients regulate their volume seven to eight times more slowly. The difference in the velocity is marked, and no overlapping values are found between the normal and the leukemic cells. It is of interest to note that in the one case of ALL studied the process has been extremely slow in the acute phase of the disease and within the normal range during remission.

Studies on the osmotic behavior of normal or leukemic lymphocytes are few and deal mainly with the response of these cells to hypertonic or extreme hypotonic conditions. In the latter case, it has been found that leukemic cells exhibit increased osmotic resistance. We have demonstrated that lympho-
OSMOTIC BEHAVIOR OF LYMPHOCYTES

lymphocytes do not exhibit volume regulation in a hypertonic environment, and in mediums of very low osmolarity the cells stop behaving as perfect osmometers. Therefore, the nature of the relationship, if any, between the increased osmotic resistance of leukemic cells and their decreased ability to regulate their volume is not clear.

As we have shown previously, the permeability of the plasma membrane to potassium plays a dominant role in the regulation of cell volume. The model we have suggested proposes that, as a result of osmotic swelling, i.e., entry of water into the cell, the plasma membrane becomes selectively permeable to K\(^+\) but not to Na\(^+\). The shrinking phase which starts immediately after osmotic equilibrium is reached is due almost exclusively to the electrochemical potential gradient for K\(^+\) forcing this ion out of the swollen cell together with associated anions and water, until the plasma membrane recovers its normal low K\(^+\) permeability. This point has been found to occur at the normal physiologic cell volume. If, however, the Na\(^+\) ions in the suspending medium are replaced by K\(^+\), the electrochemical potential gradient works in the opposite direction, causing the swollen cells to enlarge still further until they reach a volume of about 2.5 times larger than their iso-osmotic volume. Direct estimation of intracellular K content has shown that, as expected, cells lose this cation during the shrinking phase. Roti-Roti and Rothstein came to a similar conclusion from studying the osmotic behavior of mouse lymphoma cells. Furthermore, they have demonstrated that these cells retain their viability and proliferative capacity after regaining their physiologic volume in a hypotonic environment.

The cause of the very slow volume adaptation of the leukemic cells is not clear. It could be due to a lower than normal intracellular K\(^+\) content. This however, seems unlikely, since it has been shown that the K\(^+\) concentration of human leukemic cells is similar to or higher than that in normal lymphocytes. Furthermore, if leukemic cells have a lowered K\(^+\) content, then their expected swelling in PBK should be at a higher than normal rate. However, as depicted in Fig. 3, the contrary is observed. Therefore, it seems more plausible to conclude that the slow rate of volume regulation observed is due to the leukemic cells having a more limited selective increase in membrane permeability to K\(^+\) following osmotic swelling. This finding may be relevant to the observation that leukemic lymphocytes may have an elevated K\(^+\) content and at the same time show no increase in Na\(^+\) + K\(^+\) ATPase activity. The elevated K\(^+\) content may be due to a decreased plasma membrane permeability to K\(^+\) manifested not only under hypotonic conditions but also in an isotonic milieu.

The change in the permeability properties of the plasma membrane of leukemic cells may be a characteristic feature of these lymphocytes or it may also be characteristic of the normal lymphoid cells from which the leukemic one has evolved. Such normal precursor lymphocytes may be markedly different from the normal mature small blood lymphocytes. The present experiments make no attempt to discriminate between these two possibilities.

If this lowered membrane permeability to K\(^+\) is indeed a characteristic feature of the human leukemic lymphocyte, this may affect many cellular activities. Investigations on a wide variety of cell types indicate a possible link between
K⁺ and protein and nucleic acid synthesis and cell division. It is known that K⁺ plays an important role in the blastogenic response of lymphocytes to specific and nonspecific mitogens. Leukemic lymphocytes show a very marked reduction in their ability to respond to mitogens. It is suggested that this may be related to their decreased permeability to K⁺.

Whatever the biologic significance of osmotic volume regulation may be, it is clear that this cell property may be a useful indicator in the detection of leukemic cells. Its potential value in diagnosis and prognosis still has to be elucidated.

REFERENCES

OSMOTIC BEHAVIOR OF LYMPHOCYTES


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