Cryopreservation of Cells for Immunological Typing of Non-Hodgkin's Lymphomas

Adri A. Bom-van Noorloos, Agaath A. M. van Beek, and C. J. M. Melief

Department of Tumor Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, and Laboratory for Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands

ABSTRACT

The influence of cryopreservation on markers and functions of cells from lymph node, spleen, and peripheral blood of patients with non-Hodgkin's lymphoma was investigated. For the markers and functions tested, there were good correlations between the values obtained with fresh cells and those obtained with cryopreserved cells, provided that cryopreserved cells were incubated for at least 3 hr at 37° prior to testing.

INTRODUCTION

Recent classifications of NHL's are based on new knowledge of cell markers and functions of cellular components of the normal immune system. Usually, the malignant cells retain at least some immunological characteristics of the corresponding normal cells. Consequently, reliable assay of immunological markers and functions is often indispensable for a precise diagnosis. For such an analysis, it is necessary to obtain a suspension of viable cells from a representative fragment of tumor, and tests should be performed within a few hr after preparation of the suspension. Because samples of tissue or blood from patients for immunological investigation tend to arrive at irregular times in the laboratory, it is desirable to preserve the lymphoma cells so that the immunological tests may be performed at convenient hours. In addition, preservation allows the testing in one experiment of cell samples from different patients, preserved at different times, thus excluding technical variability between different experiments. Moreover, preservation allows retesting and use of standard reference cells. Normal lymphocytes can be successfully preserved with intact markers and functional properties by controlled freezing with dimethyl sulfoxide as a cryoprotectant.

The same method was applied for the preservation of viable cell suspensions obtained from lymph nodes, spleen, and blood from patients suffering from NHL's. The results of immunological characterization of cryopreserved lymphoma cells were virtually identical with those obtained with fresh lymphoma cells.

MATERIALS AND METHODS

Cells. Tissue specimens from lymph nodes and spleen from patients suffering from NHL were collected in sterile RPMI 1640-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid medium (Flow Laboratories, Irvine, Ayrshire, Scotland) supplemented with 20% v/v FCS (Grand Island Biological Co., Grand Island, N. Y.). Mononuclear cell suspensions were obtained by mincing the tissue and squeezing it through tantalum gauze. Cell suspensions from tissue fragments were always prepared within 4 hr of biopsy because later processing resulted in poor viability of the cells.

Mononuclear cells from defibrinated peripheral blood of healthy volunteers (controls) and patients were isolated by centrifugation on Ficoll-Isopaque (density, 1.079 g/ml). The viability of the cells was tested by exclusion of trypan blue. The mononuclear cells were frozen at various concentrations, never exceeding 100 x 10^6 cells/ampul.

The histological diagnosis on the tissue specimens from the patients was made by Dr. P. van Heerde (The Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands) and based on the classification according to Rappaport.

Freezing. For freezing, the cells were resuspended in RPMI Medium 1640 supplemented with 20% FCS and cooled on melting ice. An equal volume of the same medium admixed with 20% dimethyl sulfoxide was then slowly added to the cell suspension under continuous agitation. This mixture was transferred into glass ampuls which were sealed and placed in a Cryoson B.V. automatic biological freezer [Lery-(Eure), France]. The cooling rates were 1.4°/min until a temperature of -30° was attained and 6 to 7° min until -100° (7). The ampuls were then stored directly in liquid nitrogen (-196°).

Thawing. Ampuls were placed in a water bath at 37° and agitated until the ice had melted. The suspension was then slowly diluted, under continuous mixing, with a 10-fold excess of cold RPMI Medium 1640 supplemented with FCS. The cells were then spun down, washed once, and resuspended in medium.

Rosette Tests. T-cells were detected by rosette formation with SRBC (5). In brief, 0.2 ml of mononuclear cells (2 x 10^6/ml) in Earle's balanced salt solution supplemented with 5% FCS was mixed with 0.2 ml of SRBC (1 x 10^9/ml), spun down, and incubated for 1 hr on melting ice or for 2 to 4 hr at 4°. A cell surrounded by 3 or more SRBC was scored as a rosette. The percentage of rosette-forming cells was determined by counting 200 cells. Cells bearing a receptor for the Fc portion of IgG were detected by the EA-rosette test (17). In brief, 0.2 ml of mononuclear cells (2 x 10^6/ml) was mixed with 50 μl of human type O Rh D-positive erythrocytes (1.2 x 10^8/ml) sensitized with anti-Rh(D) IgG antibodies. The mixture was spun down and incubated at room temperature for at least 15 hr.
min. Rosettes were scored as described above.

**Immunofluorescence.** B-cells were detected by the direct immunofluorescence test for membrane immunoglobulin (10). Mononuclear cells (0.5 to 1.10⁶) in 0.1 ml PBS were incubated for 30 min at 4°C with 0.1 ml fluorescein isothiocyanate-labeled, polyvalent, antimmunoglobulin serum (Batch SH 17-1-F1, Central Laboratory Match Blood Transfusion Service) diluted 1:10 for 30 min at 4°C with 0.1 ml fluorescein isothiocyanate-labeled, polyvalent, antimmunoglobulin serum (Batch SH 17-1-F1, Central Laboratory Match Blood Transfusion Service) diluted 1:10 in PBS containing 0.1 M sodium azide (pH 7.2). This serum detects human immunoglobulins of all (sub)classes. The fluorescence microscope. The fluorescence microscope. was determined by counting 200 cells in a Leitz Orthoplan microscope.

**Mononuclear cells (0.5 to 1.10⁶) in 0.1 ml PBS were incubated for 3 hr at 37°C before Ficoll-Isopaque centrifugation. Viability of cryopreserved cells was tested after incubation.**

**Lymphocyte Culture.** Short-term lymphocyte cultures were performed as described by du Bois et al. (2) using a microtechnique. Cultures stimulated with phytohemagglutinin and concanavalin A were harvested after 72 hr of incubation, and those stimulated with pokeweed mitogen and allogeneic lymphocytes were harvested after 144 hr of incubation.

**Density Gradient Centrifugation.** Linear density gradients were prepared as described by Loos and Roos (7). The gradients were prepared in siliconized glass tubes (diameter, 1.4 cm; height, 12 cm) from 2 stock solutions of Ficoll-Isopaque with densities of 1.050 and 1.085 g/ml, respectively. The total volume of the gradient was 30 ml. About 12 x 10⁶ cells were layered on top of the gradient, and isopyknic equilibration was obtained after a 15-min centrifugation (2200 x g) at 4°C. After centrifugation, the gradient containing the cells was collected in 24 or 14 fractions. The number of cells in each fraction was determined by electronic cell counting (Coulter Model ZF counter).

**RESULTS AND DISCUSSION**

**Removal of Nonviable Cells and Selective Cell Loss.** Studies Performed on Lymphocytes from Healthy Donors and Patients with NHL. Suspensions of cryopreserved normal peripheral blood lymphocytes prepared by Ficoll-Isopaque centrifugation contain about 10% nonviable cells (3, 4, 12, 13). Mononuclear cells isolated from the blood and spleen tissue of patients with NHL after cryopreservation also contained about 10% nonviable cells (mean ± S.D., 8 ± 5.2; n, 16). The percentage of nonviable cells found in suspensions prepared from lymph nodes of NHL patients was larger in both freshly isolated cells (mean ± S.D., 21 ± 14.6; n, 7) and cryopreserved cells (mean ± S.D., 19 ± 15.1; n, 24).

**Nonviable cells can be removed from cell suspensions by Ficoll-Isopaque centrifugation (14).** Indeed, when cryopreserved lymphoma cells from lymph nodes of NHL patients were centrifuged on Ficoll-Isopaque, the proportion of nonviable cells was reduced in all cases (Table 1). The mean percentage of nonviable cells after Ficoll-Isopaque centrifugation of cryopreserved lymph node cells was 9 ± 3.6 (mean ± S.D.). Before Ficoll-Isopaque centrifugation, this percentage was 37 ± 12.8 (mean ± S.D.). This difference was significant (p < 0.001; Student's t test).

**Table 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cell source</th>
<th>Fresh</th>
<th>Cryopreserved before F-I</th>
<th>Cryopreserved after F-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymph node</td>
<td>28</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Lymph node</td>
<td>39</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Lymph node</td>
<td>21</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Lymph node</td>
<td>38</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Lymph node</td>
<td>NT</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Lymph node</td>
<td>NT</td>
<td>55</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>Lymph node</td>
<td>NT</td>
<td>50</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>Lymph node</td>
<td>NT</td>
<td>33</td>
<td>9</td>
</tr>
</tbody>
</table>

*a* F-I, Ficoll-Isopaque centrifugation; NT, not tested.

**Table 2**

<table>
<thead>
<tr>
<th>Cell distribution</th>
<th>Nonviable (%)</th>
<th>E-rosette-forming (%)</th>
<th>Surface immunoglobulin-bearing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>13 ± 8.0</td>
<td>45 ± 2.6</td>
<td>24 ± 12.0</td>
</tr>
<tr>
<td>Interphase after Ficoll-Isopaque centrifugation</td>
<td>75 ± 10.7</td>
<td>5 ± 3.6</td>
<td>39 ± 10.9</td>
</tr>
<tr>
<td>Pellet after Ficoll-Isopaque centrifugation</td>
<td>25 ± 11.1</td>
<td>32 ± 14.0</td>
<td>64 ± 13.1</td>
</tr>
</tbody>
</table>

*a* Mean recovery of this centrifugation, 52 ± 28.3%.

*b* Mean ± S.D. of 5 experiments with lymphocytes from 5 healthy donors.

*c* Percentage of the combined interphase and pellet fractions after Ficoll-Isopaque centrifugation.

*d* Significantly higher percentage of E-rosette-forming cells in pellet than in either original suspension (p, 0.03; Student’s t test) or interphase (p, 0.001; Student’s t test).

**Chart 1. Density distribution of cryopreserved lymphocytes (from 2 healthy donors) directly after thawing.**

**Figures 1 and 2.**

**Ficoll-Isopaque centrifugation** performed directly after thawing of frozen mononuclear cells from the blood of healthy donors.

**Cryopreservation and Immunological Typing of NHL**
Student's $t$ test). When the percentage of nonviable cells after cryopreservation of lymphoma cells was less than 20%, it was deemed unnecessary to remove the dead cells. This was the case in 37% of lymph node cell suspensions from NHL patients. Ficoll-Isopaque centrifugation could conceivably lead to a selection of subpopulation(s) in the case of cryopreserved cells. Experiments concerning this point were performed with cryopreserved peripheral blood cells from healthy donors. Table 2 shows the data of experiments with the lymphocytes from 5 healthy donors, indicating that a selection indeed occurs. E-rosette-forming T-cells are enriched in the pellet while immunoglobulin-bearing B-cells are enriched in the interphase. Since the selective loss of viable T-cells on a Ficoll-Isopaque gradient is probably the result of changes in the density of these cells caused by the preservation procedure, such changes might be reversed by allowing the cells to recover in culture at 37° (15).

The results of experiments designed to check this point are shown in Chart 1. Chart 1 shows that the density distribution of lymphocytes (from 2 healthy donors) directly after thawing is abnormal in that the sharp peak, usually shown in the density profile at 1.067 g/ml (15) in lymphocytes from healthy donors, is not observed. This change in density of the cells may be the result of the strongly changing toxicity conditions during freezing and thawing. The changes are reversible because upon incubation at 37° for 16 hr, the cells show a density profile comparable to that of freshly isolated lymphocytes (see Charts 2 and 3) as they also do after an incubation period of 3 hr at 37° (data not shown). Similar results were obtained with the lymphocytes from 2 additional healthy donors. These findings indicate that loss of lymphocytes by processing immediately after thawing can be avoided by prior incubation of the cells at 37°.

The results shown in Table 3 indicate that a selective loss of T-cells in the pellet occurs upon Ficoll-Isopaque centrifugation of healthy donor lymphocytes immediately after thawing. However, incubation for 3 and 16 hr followed by Ficoll-Isopaque centrifugation leads to the selective removal of nonviable cells without the selective removal of T-cells. Analogous experiments performed with lymphoma cells gave comparable results.
± S.D., 41 ± 18.0; n, 24). Cell recovery did not affect immunological characterization, however, because no substantial difference was found between fresh and cryopreserved cells with regard to membrane markers and functional activities in short-term culture (Tables 4 and 5).

In a paired t test, no statistically significant differences were

<table>
<thead>
<tr>
<th>Time of incubationa</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>15</td>
<td>18</td>
<td>18</td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>18</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Interphase after F-1</td>
<td>79</td>
<td>96</td>
<td>66</td>
<td>79</td>
<td>96</td>
<td>66</td>
<td>79</td>
<td>96</td>
<td>66</td>
<td>79</td>
<td>96</td>
<td>66</td>
</tr>
<tr>
<td>Pellet after F-1</td>
<td>21</td>
<td>21</td>
<td>34</td>
<td>21</td>
<td>21</td>
<td>34</td>
<td>21</td>
<td>21</td>
<td>34</td>
<td>21</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>3 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>14</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Interphase after F-1</td>
<td>62</td>
<td>61</td>
<td>84</td>
<td>62</td>
<td>61</td>
<td>84</td>
<td>62</td>
<td>61</td>
<td>84</td>
<td>62</td>
<td>61</td>
<td>84</td>
</tr>
<tr>
<td>Pellet after F-1</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>16 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>20</td>
<td>5</td>
<td>16</td>
<td>20</td>
<td>5</td>
<td>16</td>
<td>20</td>
<td>5</td>
<td>16</td>
<td>20</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Interphase after F-1</td>
<td>88</td>
<td>94</td>
<td>86</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>88</td>
<td>94</td>
<td>86</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Pellet after F-1</td>
<td>12</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 3
Ficoll-Isoaque centrifugation of cryopreserved cells from 3 healthy donors after various periods of incubation at 37°C

Table 4
Membrane markers of fresh and cryopreserved lymphoma cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cell source</th>
<th>Histological diagnosis</th>
<th>Fresh</th>
<th>Cryopreserved</th>
<th>EA-rosette</th>
<th>Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>11</td>
<td>16</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>18</td>
<td>21</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>26</td>
<td>24</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>15</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>45</td>
<td>55</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>51</td>
<td>52</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>38</td>
<td>35</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>33</td>
<td>28</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>11</td>
<td>15</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>26</td>
<td>35</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>49</td>
<td>48</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>18</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>47</td>
<td>41</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>40</td>
<td>39</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>21</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>3</td>
<td>1</td>
<td>NT</td>
<td>20</td>
</tr>
<tr>
<td>22</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>80</td>
<td>68</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>20</td>
<td>22</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>24</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>15</td>
<td>30</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>26</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>27</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>9</td>
<td>4</td>
<td>NT</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>8</td>
<td>13</td>
<td>1</td>
<td>NT</td>
</tr>
<tr>
<td>29</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>36</td>
<td>49</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>30</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>55</td>
<td>54</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>32</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>11</td>
<td>11</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>33</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>25</td>
<td>14</td>
<td>8</td>
<td>NT</td>
</tr>
<tr>
<td>34</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>23</td>
<td>16</td>
<td>43</td>
<td>45</td>
</tr>
<tr>
<td>35</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>36</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>67</td>
<td>73</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>37</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>81</td>
<td>86</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>38</td>
<td>Lymph node</td>
<td>Follicular PDL1</td>
<td>67</td>
<td>64</td>
<td>27</td>
<td>19</td>
</tr>
</tbody>
</table>

a Cell recovery at 0 hr was 80 to 100%, and after incubation it was 50 to 76%.
b Percentage of the combined interphase and pellet fractions after Ficoll-Isoaque centrifugation.
c F-1, Ficoll-Isoaque centrifugation.

Table 4
Membrane markers of fresh and cryopreserved lymphoma cells

Proportion of cells.
found \((p > 0.10)\) for the membrane marker values tested with cells from lymph nodes, spleens, or peripheral blood nor when the data on spleen, lymph nodes, and peripheral blood were pooled (E-rosette, \(p > 0.10\); EA-rosette, \(p > 0.10\); immunoglobulin, \(p > 0.10\)). The values for the different marker tests obtained with cryopreserved lymphocytes were very close to those obtained with fresh cells because the following coefficients of correlation were calculated: 0.97 for the E-rosette test; 0.83 for the EA-rosette test; and 0.98 for the fluorescence test with immunoglobulin. The relatively low coefficient of correlation found for the EA-rosette tests is caused by the values obtained with cells from Patients 19, 22, and 29 in this test. Cells from Patients 19 and 22 showed much lower values of EA-rosettes after cryopreservation, which may indicate that in these 2 instances Fc receptors for IgG were lost by the cryopreservation procedure. The somewhat higher percentage of EA-rosette-forming cells after cryopreservation of cells from Patient 29 remains unexplained, but it is probably the result of under-estimation of the percentage before cryopreservation.

Concerning the functional properties of lymphoma cells, 5 cell suspensions isolated from solid tissues and 5 suspensions isolated from peripheral blood were tested, both freshly isolated and after preservation. Again, no statistically significant differences \((p > 0.10)\) were found between fresh and cryopreserved lymphoma cells in the responses to phytohemagglutinin, concanavalin A, pokeweed mitogen, and the reactivity in the mixed lymphocyte culture (Table 5).

It is evident from Tables 1 and 4 that cryopreservation can be performed with lymphoma cells of different morphological types. The use of cryopreserved lymphoma cells has numerous advantages, such as the testing of samples from different patients in one experiment and storage of cells for other tests to be performed on a later date. In addition, the use of cryopreserved lymphocytes allows exchange of cells between laboratories for comparative studies and reference purposes.

**Table 5**

<table>
<thead>
<tr>
<th>Functional properties of fresh and cryopreserved lymphoma cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation by</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Spontaneous incorporation</td>
</tr>
<tr>
<td>PHA</td>
</tr>
<tr>
<td>Con A</td>
</tr>
<tr>
<td>ALS</td>
</tr>
<tr>
<td>PWM</td>
</tr>
<tr>
<td>Allogeneic cells</td>
</tr>
<tr>
<td>Stimulatory capacity in mixed lymphocyte culture</td>
</tr>
</tbody>
</table>

* Five cell suspensions were prepared from lymph nodes, and 5 were from peripheral blood.

† cpm/10,000 cells × 10^3.

‡ PHA: phytohemagglutinin; Con A, concanavalin A; ALS, antilymphocytic serum; PWM, pokeweed mitogen.

ACKNOWLEDGMENTS

We thank Dr. F. J. Cleton, Dr. J. A. Loos, Dr. W. P. Zeijlmaeker, Dr. A. E. G. K. van den Borne, Dr. A. Astaldi, and Dr. P. T. A. Schellekens for helpful discussions.

**REFERENCES**

Cryopreservation of Cells for Immunological Typing of Non-Hodgkin's Lymphomas

Adri A. Bom-van Noorloos, Agaath A. M. van Beek and C. J. M. Melief


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/8_Part_1/2890

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.