Blood-Sample Processing for the Study of Age-Dependent Gene Expression in Peripheral Blood Mononuclear Cells

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Although most new biogerontological studies seeking to identify longevity candidate genes and factors involved in successful human aging are population based, and likely to involve the collection of blood from extremely old individuals, to our knowledge no unified protocols have yet been published to describe a methodology permitting the simultaneous generation of different kinds of biological specimens derived from a single source of a very small volume of peripheral blood. Here we describe a method permitting the simultaneous generation of plasma, RNA, DNA, protein, fixed lymphocytes, and frozen blood aliquots from a single 10- to 30-ml blood sample obtained from donors of any age (10–102 years old), and we show that the quality and quantity of DNA, RNA, protein, and fixed lymphocytes obtained do not vary significantly with age. As is frequently observed, the older individuals have higher plasma proportions.

METHODS

Between 10 and 30 ml of blood sample is collected from donors of any age in several 5-ml ethylenediamine tetra-acetic acid Vacutainers (Becton Dickinson, San Diego, CA). For each subject, one tube is set aside to generate frozen blood aliquots, whereas the others are used for PBMC extraction. The reserved Vacutainer is aliquoted into 1- or 2-ml samples under sterile conditions, supplemented with dimethyl sulfoxide to a 10% final concentration, and stored in a −150°C freezer.

The remaining blood-filled Vacutainers are centrifuged at room temperature for 15 minutes at 1100 rpm in a tissue-culture centrifuge to allow the separation of blood constituents. Following centrifugation, the plasma-containing layer is removed from each tube without disrupting the buffy coat, and it is stored in several 3-ml cryovials. After the removal of plasma, blood samples are diluted in 1× phosphate-buffered saline to double the original blood volume, and they are gently loaded onto several 3-ml Ficoll-Paque (Amersham–Pharmacia, Baie d’Urfé, Québec, Canada) cushions set up in 14-ml round-bottom tubes; PBMCs are extracted according to the manufacturer’s instructions (Amersham–Pharmacia). Following extraction, some of the PBMCs are resuspended in Trizol (Gibco, Rockville, MD), whereas
others are fixed with 1% paraformaldehyde. As more than one tube of PBMCs is obtained for each individual, it is necessary to ensure that one of these tubes is used to generate fixed cells and the rest are used for RNA, DNA, and protein isolation. RNA, DNA, and protein are extracted from PBMCs resuspended in Trizol, as described by Chomczynski (5) and as modified by Riol and colleagues (6).

**Results**

Using the following protocol, we processed blood samples obtained from 246 Chinese individuals aged 10 to 102 years, and we found that blood-plasma composition changes with age (Table 1 and Figure 2A). Thus, compared with very young individuals (10–19 years old), extremely old individuals (90+/1001 years) exhibit a twofold increase in the percentage of plasma present in their blood. However, despite the lower amount of plasma present in very young individuals, more than enough plasma can be obtained from all individuals to permit several laboratory tests, including enzyme-linked immunosorbent assays. When the total amounts of RNA, DNA, and proteins obtained per milliliter of extracted blood were calculated, it was found that the quantity of each species obtained from the different age groups does not change significantly (Table 2); the yield of RNA, DNA, and proteins obtained per milliliter of blood does not vary among age groups. As shown in Table 2, old individuals appear to have less protein in their samples; however, a t test reveals no significant difference from the other age groups. The lower average amount of protein present in older individuals results from the fact that most of the samples we have for this age group could not be quantified, because of damage incurred during shipment from Taiwan to Canada (samples were allowed to thaw, and they were left at room temperature for several days as a result of an in-transit delay of the shipment). No damage occurred to

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Very Young (10–19 y)</th>
<th>Young (20–39 y)</th>
<th>Intermediate (40–69 y)</th>
<th>Old (70–89 y)</th>
<th>Extreme Old (90+ y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Plasma in blood</td>
<td>26.94 ± 5.76*</td>
<td>39.13 ± 8.86</td>
<td>40.25 ± 8.82</td>
<td>43.07 ± 8.82</td>
<td>50.17 ± 6.07*</td>
</tr>
</tbody>
</table>

*Significant difference with all other age groups.

Notes: The percentage of plasma within the blood is significantly different when the extreme old age group is compared with all other age groups. A significant difference also exists between the percentage of plasma present within the blood of very young individuals and all other age groups.
the other samples, as samples from other age groups were shipped separately. Samples from our population have been studied more than 3 years after extraction and still show no signs of degradation. We find that degradation can be largely prevented by generating aliquots of working samples and thawing them as needed, thus preventing samples from undergoing several rounds of freeze–thaw cycles. Our RNA was used to conduct a quantitative reverse transcriptase chain reaction, whereas proteins were used for Western blotting; DNA was used for polymorphism studies. All biological species were of good quality (Figure 2B).

Paraformaldehyde-fixed PBMCs were kept at 4°C until they were used in flow cytometry experiments; as observed for the RNA, DNA, and protein, the quantity of fixed PBMCs obtained per milliliter of blood sample does not change significantly with age. Thus, except for the yield of plasma, the quantity of biological materials extracted per milliliter of blood remains constant with age. As many commercially available antibodies do not work with paraformaldehyde-fixed PBMCs, it is important to verify antibodies before conducting flow cytometry experiments, or to use alternative methods of fixation to avoid loss of antigenicity, while tolerating the less than ideal morphological preservation.

**Discussion**

The use of population studies in aging research has contributed to our understanding of age-related physiological changes and has permitted the identification of several factors involved in successful aging. However, population studies are very expensive, because the quantity of biological materials obtained per individual is very limited; we cannot collect large volumes of blood from extremely old individuals without significantly compromising their health. For this reason, most population studies currently underway are conducted with only one type of biological material, such as DNA from finger-pricked samples for genetic profiling. In this paper, we describe a methodology permitting the simultaneous extraction of several different kinds of biological specimens from a single source of 10–30 ml of peripheral blood. This protocol not only renders population studies more cost effective, but also allows for in-depth biological investigation. It is important to obtain different kinds of biological material from each individual, because the analysis of each type contributes to different aspects of aging studies. Furthermore, our population confirms the common observation that blood composition is not identical among different age groups, as the blood of extremely old individuals contains proportionately more plasma than that of younger individuals. Because our data indicate that the volume of PBMCs per milliliter of blood does not significantly change with age, it seems that other cellular blood components, specifically red blood cells, decrease in extremely old individuals, resulting in decreased hematocrit levels, as is often reported (7,8). The implementation of this protocol will facilitate the identification of novel factors involved in successful aging, and it will render population studies more cost effective. The present report describes a standardized method for extracting DNA, RNA, protein, plasma, and cells from modest blood samples, thus allowing for studies of genetic profiling at the level of single nucleotide polymorphism and gene products, as well as regulation of functions, and it provides a comprehensive approach to studying factors involved in aging.

**Acknowledgments**

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**References**


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**Table 2. Amounts of Fixed Cells, RNA, DNA, and Protein Obtained per Milliliter of Blood**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young (20–39 y)</th>
<th>Intermediate (40–69 y)</th>
<th>Old (70–89 y)</th>
<th>Extreme Old (90 + y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed cells (×10^6)</td>
<td>1.02 ± 0.62</td>
<td>1.55 ± 0.59</td>
<td>1.11 ± 0.63</td>
<td>1.25 ± 0.57</td>
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<tr>
<td>RNA (μg)</td>
<td>1.73 ± 0.68</td>
<td>1.72 ± 0.55</td>
<td>1.57 ± 0.54</td>
<td>1.44 ± 0.65</td>
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<tr>
<td>DNA (μg)</td>
<td>8.16 ± 3.53</td>
<td>6.96 ± 3.13</td>
<td>7.66 ± 1.40</td>
<td>8.91 ± 1.99</td>
</tr>
<tr>
<td>Proteins (μg)</td>
<td>64.73 ± 32.98</td>
<td>57.13 ± 35.85</td>
<td>32.34 ± 29.7</td>
<td>76.75 ± 68.02</td>
</tr>
</tbody>
</table>

*Note: The amount of fixed cells, RNA, DNA, and protein obtained per milliliter of blood processed is similar for all age groups.*