Technique for Human Marrow Grafting

By E. DONNALL THOMAS and RAINER STORB

A technique is described for marrow aspiration from living human donors and for processing the marrow for intravenous infusion to marrow graft recipients.

SINCE 1956, A LARGE NUMBER of human marrow grafts have been attempted using hemopoietic tissue from cadavers, fetuses and living donors. The general lack of success in these earlier transplantation efforts discouraged further attempts. Recent developments have caused a general resurgence of interest in clinical marrow grafting. Studies of marrow grafting after lethal irradiation in outbred dogs have demonstrated clearly a correlation between histocompatibility, graft survival and severity of the graft-versus-host reaction. These observations in an outbred species suggest that the recent rapid advances in human histocompatibility typing may be applied to the marrow grafting problem in man. It has also been demonstrated in rodents, dogs, monkeys and man that allogeneic marrow grafts can be achieved after doses of cyclophosphamide. Thus investigators have a means of studying marrow grafting after chemotherapy, as well as after whole body irradiation.

Since histocompatible donors will most likely be found from among members of the patient's own family, living volunteers are at present the principal source of donors for marrow grafts. Accordingly, it seemed worthwhile to report in detail the method of obtaining, preparing and administering marrow that has been developed in this laboratory over the last 15 years. These techniques have been successful in dogs, monkeys and man and the procedure can be carried out without harm to the donor or to the recipient.

MATERIALS AND METHODS

Heparin and TC-199

Some of the preservatives used in heparin solutions may be harmful to marrow cells. Accordingly, we have used a heparin that contains no preservatives (Connaught Laboratories, Toronto, Canada). Four ml. of a sterile solution of heparin containing 1000 units/ml.

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are added to each 100 ml. of a tissue culture medium (TC-199, Difco Laboratories, Detroit, Michigan).

**Beakers and Holder**

Stainless steel beakers of various sizes are used in order to eliminate the possibility of breakage during marrow collection. Prior to use, the beakers are treated with silicone (Siliclad, Clay-Adams, Inc., New York, N. Y.). The beaker containing marrow is kept in a holder in order to prevent an accidental spill.

**Needles**

Rosenthal needles, 2.5 cm., 16 gauge (Becton-Dickinson, Rutherford, N. J.) are used for aspiration of the sternum and may also be used for aspiration of tibiae and iliac crests in small children. In adults, the yield of marrow is improved by using a larger needle for aspirating the iliac bones. The Westerman-Jensen biopsy needle (Becton-Dickinson) may be used but the tip is easily plugged when the needle is advanced without the stylus in place. The Bierman needle,\(^1\) with a side opening, avoids this problem but the yield of marrow is not always satisfactory. Accordingly, we have devised a needle with a 45\(^\circ\) bevel at the end that represents a compromise between a straight bore and a side opening. This needle gives an excellent yield of marrow and only rarely becomes plugged. It is made of stainless steel. The length of the needle ranges from 6 to 8 cm. The internal diameter is 1.5 mm. and the external diameter is 3.0 mm. A large handle provides an adequate grip for the operator, and a deep locking notch prevents dislodgment of the stylus during insertion. This needle is available from the medical instrument shop of the University of Washington School of Medicine.

**Syringe and Screens for Filtration**

Repeated passage of marrow through any small bore orifice, such as a needle, or the use of a loosely fitting Potter-Elvehjem homogenizer will destroy many of the cells. Simple filtration through gauze or nylon results in a considerable loss of cells in the particles that remain on the filter. This problem was solved\(^1\) by using a cut-off 20 ml. syringe covered with a stainless steel screen. The marrow cell suspension is poured from the beaker into the barrel of the syringe where it falls through the screen into a second beaker. The screen becomes covered with marrow particles and flow diminishes. When this occurs, the plunger of the syringe is inserted into the barrel and very gentle pressure is applied after which the plunger is worked up and down slowly two or three times. The reverse flow from the second beaker back into the syringe promptly dislodges any particles that obstruct the screen. The gentle working of the plunger causes rapid breaking up of the particles. The filtration is continued in this manner until all of the marrow suspension has been passed through the first screen which has a 0.307 mm. width opening. The procedure is then repeated with a second screen of 0.201 mm. width opening. At this point, examination of the marrow cell suspension shows predominantly single cells with occasional clumps of only a few cells. Additional screening through smaller screens can be carried out but has not been found to be necessary. With screen sizes below 0.100 mm. width opening, loss of cells may occur. The cut-off 20 ml. syringe can be obtained from the Cardiovascular and Special Instrument Division, Becton-Dickinson & Co. The holders used to keep the screens over the end of the syringe with a tight seal can be obtained from the medical instrument shop of the University of Washington School of Medicine, and stainless steel screens in a wide range of sizes can be obtained from the W. S. Tyler Woven Wire Screen Co., Cleveland, Ohio.

The contents of the sterile kit and accessory items are listed below:

**Bone Marrow Kit Contents**

- six or eight large needles of varying lengths (Fig. 1)
- five or six Rosenthal needles of varying lengths (Fig. 2)
- three or four Bierman needles of varying lengths
- one long, blunt 14 gauge needle
- one 250 ml. stainless steel beaker
- two 600 ml. stainless steel beakers
two 1200 ml. stainless steel beakers
one beaker stabilizer with adapter rings
two 50 ml. syringes
six or eight 20 ml. syringes with bored-out
lumen to fit the large needles
four cut-off syringes—two with 0.201 mm.
screens (88 mesh), two with 0.307 mm.
screens (62 mesh) (Fig. 3)
one curette
one hemostat
two EDTA bottles
two extra screens of 0.201 mm. width of
opening
two extra screens of 0.307 mm. width of
opening
Westerman-Jensen biopsy needle, if needed

Other Items Not in Sterile Kit
one bottle of Connaught heparin
two bottles of TC medium 199
one 5 ml. syringe, needle and alcohol wipe
for the heparin
eight 600 ml. Fenwal transfer bags
one marking pen
one culture tube

Fig. 1.—Contents of sterile kit for marrow aspiration.

Fig. 2.—The large needle used for aspiration of the iliac bones.
Procedure

The details of the marrow aspiration procedure are explained to the volunteer donor on at least two occasions, preferably on different days and with family members present. Specifically, we inform the donor of the potential risks of general anesthesia, of the fact that the aspiration site may be sore for a few days and of the possibility of infection. When the criteria for informed consent have been satisfied, the donor signs an appropriate consent form.

In a vigorous attempt to secure a maximum number of bone marrow cells, the volume of blood aspirated will be several hundred ml. Accordingly, we customarily store one unit of blood from the marrow donor a few days before the procedure and this blood is given back during the procedure. The use of an autotransfusion does not expose the patient to the normal risks of blood transfusion.

The marrow aspiration is carried out under sterile conditions and under general anesthesia in the operating room. Prior to aspiration, the aspirating syringe is rinsed with TC-199–heparin solution but the solution is expelled from the syringe. Sternal aspirations, done only on adults, are carried out first. The needle is inserted into 10–12 sites at approximately 1 cm. intervals over the length of the sternum. Through each hole in the skin,
some three or four aspirations are done with the needle point being moved a few millimeters for each aspiration. The needle point is inserted into the marrow cavity and vigorous suction is applied while the needle is rotated. Usually 1–3 ml are aspirated since aspiration of larger volumes from a single location increases the amount of blood taken but does not significantly increase the number of marrow cells. The marrow aspiration is performed quickly (2–8 seconds). The syringe, needle and marrow are then handed to the nurse-assistant who expels the marrow into a beaker containing 40 ml of TC-199-heparin solution. The syringe is then rinsed in a second beaker containing 20 ml of TC-199-heparin and returned to the operator. In transferring the marrow to the beaker and in rinsing the syringe, care is taken to avoid foam formation. After a number of rinses, the rinse beaker will have enough blood in it so that the barrel of the syringe will begin to stick. When this occurs, the rinse solution is transferred to the primary beaker and another 20 ml of TC-199–heparin is added to the rinse beaker.

When aspirations are completed on the sternum, the anterior iliac crests are prepared and draped and aspirations carried out in multiple sites from the top of the crests and also laterally using the large needles. While the patient is being turned to the prone position and prepared for posterior crest aspiration, the pool of marrow obtained from the sternum and anterior iliac crests is processed.

Posterior iliac crest aspirations are then carried out using the large needles. The volume aspirated with each pass of the needle is much larger on the posterior crest, usually around 20 ml. However, as this volume is being aspirated, the needle is constantly rotated and advanced into the marrow cavity. If the needle point is left in one place, there is a rapid fall-off of the number of marrow cells contained in the aspirated blood. On the posterior crests some 8–10 sites are used on each side, being careful to redirect the needle to a new area each time.

In children under the age of 5, marrow can be obtained by aspiration of the upper one-third of the tibia in the general fashion described above.

Each batch of marrow is processed by passage through the screens as described above. Samples are taken for culture and for cell counting. The marrow suspension is aspirated through a large, blunt needle into a 50 ml syringe. The blunt needle is inserted into the needle adapter end of the tubing of the Fenwal bag and the marrow is run into the bag. The procedure is repeated until all of the marrow suspension is in the bag. Total volumes must be recorded with care. The known volume of the TC-199-heparin solution is subtracted from the total volume to give the volume of blood actually removed from the donor. As each batch of marrow is processed and placed in the Fenwal bag, it is taken immediately to the ward and administered intravenously to the recipient. No additional filter is used. About 1½–2 hours are required for the entire procedure.

RESULTS

Table 1 shows the results in four recent marrow aspirations. Two donors were adult males, one an adult female and one was a 3-year-old boy. The method of calculation is illustrated in the table. The number of nucleated peripheral blood cells in the aspirated marrow is calculated from the donor’s peripheral white cell count and the known volume of blood aspirated. These cells contributed from the peripheral blood are subtracted from the total nucleated cell number in order to give the actual number of marrow cells. In the case of the young adult donors, it is evident that about 17–24 × 10⁹ nucleated marrow cells can be obtained. The smaller number of cells obtained from the child (donor 4) was nonetheless adequate for an isogeneic marrow graft to his identical twin following 950 rads whole body irradiation. The marrow from donor 3 resulted in a successful allogeneic graft described elsewhere.¹²
Table 1.—Results of Human Marrow Aspirations

<table>
<thead>
<tr>
<th>Donor Age</th>
<th>Donor Sex</th>
<th>Weight Kg.</th>
<th>Aspiration Site</th>
<th>Total Volume</th>
<th>Volume of Blood</th>
<th>Nucleated Cells × 10^9</th>
<th>Peripheral Blood Cells × 10^9</th>
<th>Marrow Cells × 10^9</th>
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<tr>
<td>1 30 M</td>
<td></td>
<td>81.0</td>
<td>Sternum</td>
<td>50</td>
<td>25</td>
<td>1.68</td>
<td>0.18</td>
<td>1.50</td>
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<td></td>
<td></td>
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<td>Anterior crests</td>
<td>212</td>
<td>137</td>
<td>4.76</td>
<td>0.95</td>
<td>3.81</td>
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<td></td>
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<td>Posterior crests</td>
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<td>534</td>
<td>16.67</td>
<td>3.74</td>
<td>12.93</td>
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<td></td>
<td></td>
<td></td>
<td>All</td>
<td>896</td>
<td>696</td>
<td>23.11</td>
<td>4.87</td>
<td>18.24</td>
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<tr>
<td>2 28 M</td>
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<td>64.0</td>
<td>Sternum</td>
<td>142</td>
<td>112</td>
<td>3.23</td>
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<td>Anterior crests</td>
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<td>188</td>
<td>7.05</td>
<td>1.12</td>
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<td>Posterior crests</td>
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<td>465</td>
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<td>28.09</td>
<td>4.58</td>
<td>23.51</td>
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<td>3 35 F</td>
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<td>54.5</td>
<td>Sternum</td>
<td>64</td>
<td>24</td>
<td>3.36</td>
<td>0.14</td>
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<td></td>
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<td>61</td>
<td>4.24</td>
<td>0.35</td>
<td>3.89</td>
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<td>Posterior crests</td>
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<td>11.37</td>
<td>0.88</td>
<td>10.49</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>All</td>
<td>376</td>
<td>236</td>
<td>18.97</td>
<td>1.37</td>
<td>17.60</td>
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<tr>
<td>4 3 M</td>
<td></td>
<td>15.5</td>
<td>Tibia and anterior crests</td>
<td>60</td>
<td>35</td>
<td>3.1</td>
<td>0.3</td>
<td>2.8</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Posterior crests</td>
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<td>58</td>
<td>6.0</td>
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<td>5.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>All</td>
<td>143</td>
<td>93</td>
<td>9.1</td>
<td>0.8</td>
<td>8.3</td>
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DISCUSSION

Forty-two marrow aspirations have been carried out in the past year utilizing the technique described here in preparation for allogeneic, isogeneic or autologous marrow grafting. Invariably, the procedure has been well tolerated by the donor. Following recovery from anesthesia, the only complaint concerns some local soreness in a few, but not all, of the aspiration sites. The donor is usually discharged from the hospital the following day, but some have been able to go home on the day of aspiration following recovery from anesthesia. In two instances, some mild hypotension was observed toward the end of the aspiration procedure, but this has not occurred since the practice of autotransfusion was initiated.

No major problem has been encountered in recipients of marrow infusion. Usually the first portion of marrow can be administered rapidly and without ill effect. However, since the volume may be rather large, caution is indicated. In a few instances, transient mild cyanosis with a tachycardia has been noted. Accordingly, it is customary to monitor the recipient carefully for vital signs and electrocardiogram with a physician always in attendance. In recipients that have subsequently died of their primary disease, a few micropulmonary emboli have been found. However, since initiating the screening procedure described here, no major problem with microemboli has been encountered as was described before the screening procedure was adopted.17

Several authors have attempted to estimate the number of allogeneic marrow cells necessary for successful engraftment in man.18,19 A reasonable estimate is that approximately 5 × 10^8 marrow cells/Kg. is an adequate number. The results shown in Table 1 indicate that the technique described here does not quite produce that number of cells. Nevertheless, this number of cells has been shown to be adequate for isogeneic and allogeneic grafts in man.12,13 Clearly, more marrow cells would be desirable. The rib has been used20 but
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does not appear to be a practical source of marrow from volunteer donors. We have aspirated the spinous processes of the vertebra, but the amount of marrow obtained is very small. On one occasion, rich marrow was obtained from the greater trochanter of a 6-year-old child. A similar aspiration on a 25-year-old man yielded only fatty marrow.

Previous studies have emphasized the importance of having some protein in the marrow cell suspending medium in order to avoid aggregation, stickiness and loss of cells. In the aspiration procedure described here, the patient's own plasma proteins provide this component. However, in applying this technique to spleen cells or to cadaver marrow, it has been found necessary to provide additional protein, either as plasma or albumin, in the suspending medium.

One of the principal advantages of the screening technique described here is that the marrow can be given intravenously with reasonable safety. Other routes of marrow administration have been explored. Recently, human patients have been given marrow intraperitoneally. As pointed out by van Bekkum et al., the number of marrow cells required for successful engraftment by the intraperitoneal route in rodents is approximately 70 times that required for the intravenous route. Since the number of marrow cells obtainable for human marrow grafts is already minimal, it would appear desirable to use the intravenous route.

The technique described here has been applied to the preparation of spleen cell suspensions and fetal liver hematopoietic tissue. For the spleen, we have found it best to cut the organ into 2–3 mm slices. The slices are placed in a large stainless steel tea strainer and are forced through the strainer using the gloved thumb. The cell suspension is then passed through a series of graded stainless steel screens (0.600–0.200 mm, width opening). The cell suspension is then centrifuged at slow speeds and the supernatant containing disrupted cells is discarded. Thus, the dangers of intravenous spleen cell administration can be avoided.

Recently, it has been suggested that it may be possible to separate a stem cell-rich fraction of the bone marrow using an albumin gradient technique, thus removing the immunologically competent cells that cause acute graft-versus-host disease. The cell suspension preparation technique described here could be used for that purpose. However, the separation technique requires prolonged exposure of the cells to room temperature and does involve a loss of cells which may be undesirable since the number of cells available may be minimal. In addition, a really effective removal of the lymphoid cell population might be undesirable particularly for the patient with immunological deficiency disease. The marrow grafting technique described here has been shown to be adequate for securing long-term canine chimeras, even in the face of known histoincompatibility. It remains to be seen whether or not the same long-term results can be achieved with a stem cell-rich fraction of marrow.

Marrow grafting is clearly indicated when a normal identical twin is available to serve as the marrow donor for certain patients with drug-induced or idiopathic bone marrow failure or accidental radiation exposure. Marrow
grafting may also be indicated for the patient with severe immunologic deficiency who has a histocompatible sibling to serve as donor.23,30 All other potential applications of marrow grafting are as yet highly experimental procedures. It appears likely that a better definition of the indications for marrow grafting in man will result from current studies in progress in several centers.

REFERENCES


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