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Ratio of blood and marrow-derived cells in bone marrow transplantation

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SUMMARY The content of the cell suspensions used in bone marrow transplantation can vary by at least twelvefold in the ratio of bone marrow-derived to blood-derived cells. The ratio is somewhat higher in young donors but is similar for male and female donors and is not closely correlated with the incidence of graft *v* host reactions.

In bone marrow transplantation in man, a mixture of blood and marrow is aspirated from the donor and injected intravenously to the recipient. To obtain enough cells (usually considered to be $\geq 2 \times 10^8$/kg body weight) the volume of aspirate must approach one litre, of which it can be assumed that 98% or so is actually blood and less than 2% is derived from the marrow itself. This cell mixture is in sharp contrast with that used in marrow transplants in mice, where femoral marrow cells form an inoculum which is virtually free of blood contamination. Furthermore, it is an old observation that in mice graft *v* host (G*v*H) reactions are relatively uncommon and difficult to induce by such marrow suspensions, whereas the addition of blood, lymph node or spleen cells greatly enhances the incidence and severity of G*v*H reactions.[1] Similar observations in dogs and other species confirm the general validity of the hypothesis that it is cells from the blood—presumptively T lymphocytes—which are the precipitating cause of G*v*H disease.[2 3]

What is not known is the actual composition of the blood-marrow mixture in human transplants and whether it is related to G*v*H disease. In particular, the increased susceptibility to G*v*H disease if the donor is female—irrespective of the recipient sex[4]—might result from differences in the content of the donor inoculum perhaps because the volume of accessible marrow was smaller in women and hence dilution with blood cells was greater.

Accordingly, we have analysed the components of the marrow-blood mixture as given to 42 patients in the last 2½ years.

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Material and methods

Marrow is aspirated under general anaesthesia from the iliac crests, anterior and posterior, and from the sternum of all donors. Occasionally other sites, such as the superior portions of the ala of the sacrum, the greater trochanters and tibias have been aspirated. The donors have all had normal blood counts and in most instances, the red cells and plasma from one unit of donor blood, taken one week earlier, are retransfused to restore, at least partially, the blood volume. Donors receive heparin 1500 U/m² and the aspirate is collected in bags with 3000 U heparin. These bags, usually three, are weighed to indicate the volume collected and an aliquot is taken for counting total nucleated cells in a Coulter F model counter.

Aspiration is continued until enough cells have been removed to give at least 2×10^8/kg of the recipient's body weight. In a few instances it has been difficult to procure enough cells, but in general, since all except three donors have been aged 13 yr or over, the possible disproportion in donor and recipient size has not been a problem. All but six of the donors have had blood counts at the Royal Marsden Hospital before the donation; serial counts throughout the donation in a sample of donors have shown no significant change so that in the other six it has been possible to calculate the data from a blood count performed after the donation.

A few attempts were made to measure the lymphocyte content of the aspirates from May-Grünwald-Giemsa stained films but the accuracy of this procedure is very low. In addition to smudge cells the marrow contains many ambiguous cells, possibly

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lymphocytes, possibly precursor cells or cells of the erythropoietic series—this makes a precise analysis almost impossible. Knowing the volume of marrow-blood aspirate and the blood leucocyte count, it is possible to estimate the proportion of blood-derived lymphocytes; thence by subtraction from the total nucleated cell content the number of bone marrow cells can be calculated. Separate analysis of each bag shows some variation between bags with maximum marrow cell yields in the first bag and with higher concentrations of marrow cells from the sternum as compared to the iliac crests.

Results

Tables 1 and 2 show the data from 42 donors aged 5-52 yr, together with information relating to marrow engraftment and GvH disease etc. All the recipients, except three (with chronic granulocytic leukaemia), have had acute myeloid leukaemia in either first, second or third remission. All but the three sets of identical twins were treated with cyclosporin A (CsA) as immunosuppressant. Some mismatched patients also received three doses of methotrexate immediately after transplant.

It will be seen that there is a fairly wide range of marrow cells aspirated but it must be realised that the volume and yield is to some extent determined by the size of the recipient and by knowledge of the yield, bag by bag, as the aspiration proceeds. In a few cases—for example, case Nos 82 and 102, suboptimal cell numbers were obtained leading to high volume donations. However only one marrow failed to take, case No 71. This was a low-yield aspirate but a second donation (of even lower yield) was successful.

Analysing the adult donors separately (age 22 yr and over) it can be seen that the ratio of bone-marrow cells to blood-derived cells varies from 0·4 to 5·1 and that the mean ratio for 10 females is higher (2·32) than for 22 males (2·02). The ratio for younger donors (<22 yr) is somewhat higher (2·85) as might be expected from the greater cellularity of young bone-marrow and the rather smaller quantity of aspirate.

There is no close correlation in this series between

Table 1 *Data relating to 29 male donor aspirates. All figures × 10^8 unless otherwise stated*

Case no	*Age (yr)*	*Vol (ml)*	*Total cells*	*Cells/kg recipients body weight*	*Donor*		*Blood-derived cells (BDC)*	*BDC/kg recipients body weight*	*Blood derived lymphs (BDL)*	*BDL/kg recipients body weight*	*Bone marrow cells (BMC)*	*BMC/BDC*	*Type of graft**	*GvH (etc)*†
					WBC (× 10^9/l)	*Lymphs (%)*								
88	5	946	227	3·3	4·4	46	42	0·61	19	0·28	185	4·4	C	Mild
49	9	478	157	3·0	7·7	28	38	0·72	10·2	0·19	120	3·2	C	0
67	14	468	104	3·7	5·7	32	27	0·95	8·5	0·3	77	2·8	C	Mild
96	15	956	204	2·8	4·7	52	45	0·61	23·4	0·32	159	3·5	C	Moderate
94	15	758	251	3·3	5·8	39	44	0·58	17·1	0·22	207	4·7	M	Severe (fatal)
82	15	1840	193	2·2	6·7	59	123	1·4	72·0	0·82	79	0·6	C	Moderate
60	16	782	203	3·6	6·4	27	50	0·89	13·5	0·24	153	3·05	C	0
61	17	958	207	3·2	5·0		48	0·74			159	3·3	C	Mild
74	19	1033	209	3·0	6·2	31	64	0·92	20·0	0·29	145	2·25	M	ARDS
54	20	980	207	3·2	8·9		87	1·34			119	1·35	C	Mild, late mod
86	22	1075	190	2·6	3·8	42	41	0·56	17·1	0·23	149	3·65	T	0
83	22	678	115	3·1	8·8		60	1·6			55	0·92	M	0
75	26	996	160	3·3	6·3		63	1·3			99	1·6	C	0
63	28	780	199	3·3	9·5		74	1·23			125	1·7	C	Mild
91	28	947	156	2·8	5·2	23	49	0·88	11·2	0·20	107	2·2	C	Mild
76	29	838	172	3·0	8·5	14	71	1·24	9·9	0·17	101	1·4	T	0
58	30	402	93	4·1	5·9		24	1·05			69	2·9	M	Severe (fatal)
99	32	600	125	3·2	6·7	27	40	1·00	10·8	0·27	85	2·1	C	Mild
71	33	934	144	2·7	10·2	30	95	1·79	28·5	0·53	49	0·5	C	Mild
89	36	1058	231	2·9	7·6	21	80	1·0	16·8	0·21	151	1·9	C	Fatal
65	37	652	99	3·5	10·6	21	69	2·45	14·5	0·51	30	0·4	M	Severe (recovery)
53	38	746	114	2·4	8·6		64	1·35			50	0·77	C	0
80	38	873	252	3·5	7·3		60	0·84			192	3·3	C	Acute renal failure
102	39	1554	151	2·3	3·9		61	0·92			90	1·5	C	Cardiac failure
93	40	1040	235	3·2	5·2	38	54	0·73	20·5	0·28	181	3·3	M	Moderate-severe
66	42	820	184	3·5	6·5	26	53	1·0	13·9	0·26	131	2·45	C	Chronic
85	42	774	204	3·6	6·8	22	53	0·93	11·5	0·20	151	2·9	M	Severe (fatal)
78	46	952	206	3·7	4·6	40	44	0·78	17·6	0·31	162	3·7	M	Mild
73	49	1244	175	2·6	6·5	31	80	1·19	47·4	0·70	95	1·2	C	Moderate

*Type of graft: T = identical twin
C = compatible HLA-identical sibling
M = haplotype-identical mismatch.
†The gradation of GvH in patients on cyclosporin A is difficult owing to variation in treatment. In general, mild = grade 1; moderate = grade 2; severe = grade 3 or 4. Fatal indicates death partly attributable to GvH disease.
ARDS = adult respiratory distress syndrome.

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Table 2 *Data for 13 female donor aspirates. All figures* $\times 10^8$ *unless otherwise stated*

Case no	*Age (yr)*	*Vol (ml)*	*Total cells*	*Cells/kg recipients body weight*	*Donor*		*Blood-derived cells (BDC)*	*BDC/kg recipients body weight*	*Blood-derived lymphs (BDL)*	*BDL/kg recipients body weight*	*Bone marrow cells (BMC)*	*BMC/BDC*	*Type of graft**	*GvH (etc)†*
					WBC ($\times 10^9/l$)	*Lymphs (%)*								
68	7	410	106	3·5	8·8	43	36	1·20	15·5	0·51	70	1·9	C	Mild
92	13	710	163	3·1	6·5	33	46	0·87	15·4	0·29	117	2·55	C	Mild
98	14	629	137	3·1	6·1		38	0·87			99	2·6	C	0
87	22	633	163	3·4	9·3		59	1·23			104	1·77	C	0
62	22	938	183	3·1	6·5	20	61	1·03	12·2	0·21	122	2·0	C	Mild
56	24	570	168	3·1	4·8	11	27	0·50	3·0	0·05	141	5·1	C	0
101	33	943	161	2·5	5·0	21	47	0·72	9·8	0·15	114	2·4	C	0
100	36	1126	176	3·0	7·0	42	79	1·36	33·1	0·57	107	1·35	C	Fatal
97	37	938	237	3·3	7·6	30	71	1·0	21·4	0·30	165	2·3	T	0
84	38	896	207	2·6	7·0	35	63	0·79	22·0	0·28	144	2·3	C	Mild
57	43	941	146	2·2	8·3		78	1·18			68	0·87	C	Mild, late mod
64	43	1350	104	2·2	5·2		70	1·48			34	0·5	M	ARDS
90	52	776	172	3·1	4·0	38	31	0·56	11·8	0·21	141	4·6	M	ARDS

*†See footnotes to Table 1.

the incidence of GvH disease in compatible transplants with either the total number of blood-derived cells or the bone-marrow to blood-derived cell ratio. Possibly the total number of lymphocytes bears some relation since all five who received $0·5 \times 10^8$ or more blood lymphocytes from the donor had some degree of GvH disease but the converse does not hold since case No 89, receiving only $0·21 \times 10^8$ lymphocytes, developed fatal GvH disease. In the mismatched transplants the importance of blood leucocytes in the genesis of GvH disease or the acute mismatch syndrome is not apparent, although one major cause of death in such patients—adult respiratory distress syndrome (ARDS)—might be immunologically mediated. It is seen in patients having cyclosporin-A and appears to be much commoner after mismatched transplants than after fully matched transplants.

Discussion

These results show that bone-marrow aspirates as used in transplantation vary by as much as twelvefold in their relative content of marrow-derived and blood-derived cells. This variation may not affect the proportion of graft rejections and takes, provided the total number of cells is adequate since it was shown by Deeg *et al*[5] that in dogs viable lymphocytes from blood or thoracic duct, if added to suboptimal numbers of allogeneic marrow cells, could contribute to the acceptance of the graft—but at a cost in the incidence of subsequent GvH disease. What might be expected is that GvH disease would more frequently follow grafts with low marrow to blood cell ratios, but that has not been our experience. Possibly the lack of correlation is due to the small number of cases and the infrequency of GvH disease in this series. Furthermore, the propensity of marrow from female donors to cause GvH disease[4] cannot be attributed to any systematic difference in the ratio of marrow to blood cells and that phenomenon remains unexplained. One possible explanation is that female marrow contains more lymphoid cells, for which there is evidence. Rywlin *et al*[6] analysed the proportion of marrow aspirates containing lymphoid nodules and found that 53% of female marrows contained them, compared with only 41% of male marrows—these in addition to patients with nodular lymphoid hyperplasia (without lymphoma) where there were eight females against two males. Admittedly, the sample was mostly from an older age group than that from which bone marrow donors are recruited but it suggests a possible basis for the incidence of GvH disease in relation to donor sex.

In our study an attempt was made to count lymphoid cells in smears from the marrow suspensions but owing to morphological ambiguities this is an inaccurate procedure. The figures suggested that more lymphocytes were present than could be accounted for by the blood component but an analysis by section of particles or by specific lymphocyte markers would probably be more accurate. Current methods of marrow transplantation using antilymphocyte sera or lectins to remove lymphocytes might also indicate the basis of the sex difference in marrow transplants.

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