

## Circulation of Donor Lymphocytes After Blood Transfusion in Man

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Atypical lymphocytes (ATL) appear in the circulation of a large proportion of patients during the first week following a blood transfusion. In order to determine the source of these ATL, cytogenetic studies were performed on the peripheral blood leukocytes of ten adult patients who received fresh blood from donors of the opposite sex. Nine of the ten patients had spontaneously dividing mononuclear cells of the recipient or host karyotype circulating during the latter part of the first week after transfusion. In two patients, the

spontaneously dividing cells were of donor as well as of host origin. Six patients had circulating phytohemagglutinin-responsive lymphocytes of the donor karyotype noted from 1–7 days after transfusion. These findings lend support to our hypothesis that the increase in circulating atypical lymphocytes seen 1 wk after transfusion represents the counterpart *in vivo* of the *in vitro* mixed leukocyte reaction. The dividing donor cells may represent a subclinical graft-versus-host reaction.

**D**URING THE FIRST WEEK after blood transfusion, there is a rise in the concentration of atypical lymphocytes (ATL) and DNA-synthesizing mononuclear cells in the circulation of patients who have received moderate to large amounts of blood.<sup>1</sup> Using *in vitro* <sup>3</sup>H-thymidine incorporation of the peripheral blood leukocytes and the enumeration of ATL as assays, we were able to show that the increases in these parameters were greatest in patients who were transfused with fresh blood. Patients who received blood stored for 5 days or more also had significantly increased leukocyte thymidine incorporation and ATL after transfusion. These responses were not detectable in patients who received leukocyte- and platelet-depleted red cells prepared by the glycerol-freezing method.<sup>1,\*</sup> The timing and the nature of this phenomenon suggested that it might be an analogue *in vivo* of the *in vitro* mixed leukocyte reaction<sup>2</sup> in which lymphoblasts develop when the leukocytes of HL-A non-identical or "mismatched" individuals are mixed.

In this study we have used the sex chromosome as a marker in an effort to detect the presence of any circulating donor cells in the transfused patient and to determine their role in the lymphocyte reaction seen after transfusion.

Our data indicate that donor lymphocytes which are phytohemagglutinin-

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\*We have since studied four additional patients who received glycerolized red cells. Their responses were identical to the three reported in the original study. (Schechter GP, Soehnlen F, Meryman H, McFarland W: Unpublished data.)

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responsive can be detected in the blood of patients up to 1 wk after transfusion. In some patients, donor cells dividing "spontaneously" have been found, as would be expected in a two-way mixed leukocyte reaction.

### MATERIALS AND METHODS

**Patients.** The experimental subjects were ten adult patients (eight males and two females) who underwent elective surgery and received known amounts of fresh blood (less than 24 hr of storage) from donors of the opposite sex. The blood from the opposite sex donors represented 40%–100% of the total amount of blood received during surgery and the early postoperative period. Nine patients had cardiopulmonary bypass procedures, and one patient had a total hip replacement. Although four of the patients had been transfused previously, none had received blood during the preceding year.

**Cytogenetic studies.** Blood was sampled for cytogenetic analysis from two to eight times (average five times) over a 2-wk period following transfusion. Most of these samples were taken during the first week (Table 2). Leukocyte suspensions were prepared from each blood specimen as previously described,<sup>3</sup> except that two sets of cultures, one with phytohemagglutinin (PHA) and one without PHA, were assembled from each specimen. Cultures containing PHA were harvested after 3 days incubation at 37°C and the cells in mitosis analyzed. The cultures without PHA were harvested after incubation for 1 day. The mitoses in the preparations from the cultures incubated for 1 day without PHA are referred to as spontaneous mitoses. Every evaluable mitosis on the prepared slides was counted and scored as to male or female karyotype.

**<sup>3</sup>H-thymidine incorporation.** Six of the ten patients had studies of leukocyte <sup>3</sup>H-thymidine incorporation after transfusion. Blood specimens were obtained prior to and 7 days post transfusion. Leukocyte suspensions were prepared and incubated with <sup>3</sup>H-thymidine for 3 hr at 37°C as previously described.<sup>1</sup> The trichloroacetic acid precipitable radioactivity of triplicate cultures was assayed by liquid scintillation counting.

### RESULTS

The number and type of mitoses evaluated for each patient during the post transfusion period are found in Table 1. Six of the ten patients were also studied prior to transfusion, and none of these had mitoses in the cultures incubated for 1 day without PHA (spontaneous mitoses). In contrast, spontaneous mitoses

**Table 1. Number of Mitoses and <sup>3</sup>H-Thymidine Incorporation of Post Transfusion\* Leukocyte Cultures From Ten Recipients of Opposite-Sex Blood**

Transfusion Recipient	Units†	PHA-induced Mitoses		Spontaneous Mitoses		Leukocyte <sup>3</sup> H-Thymidine Incorporation (DPS)‡
		Host Type	Donor Type	Host Type	Donor Type	
1. C.M.	10	299	2	48	3	700
2. C.S.	8	435	19	9	1	3125
3. T.T.	7	350	3	4	0	—
4. B.S.	4	226	2	9	0	1190
5. E.G.	6	405	2	1	0	—
6. B.B.	5	295	7	2	0	800
7. J.R.	11	206	0	10	0	775
8. J.G.	5	237	0	0	0	270
9. R.T.	4	63	0	2	0	—
10. W.B.	2	337	0	12	0	—
	Total	2853	35	97	4	

\*Six patients were studied prior to transfusion. No spontaneous mitoses were observed. Of 304 PHA-induced mitoses, one (from patient 6) was of the opposite sex type (see text).

†Units of blood received from donors of the opposite sex.

‡<sup>3</sup>H-thymidine incorporation of leukocytes sampled 7 days after transfusion. The mean pretransfusion incorporation was  $134 \pm 64$  disintegrations/sec (DPS).

**Table 2. Number and Time of Appearance of Mitoses of the Donor Karyotype in Leukocyte Cultures of Ten Recipients of Opposite-Sex Blood**

Transfusion Recipient	Before Transfusion	Day Post Transfusion							
		1/2	1	2	3	4	5	6	7
1. C.M.	0	—	—	—	—	0	3*	1	1
2. C.S.	—	6	1	4	0	3	3†	0	3
3. T.T.	0	—	0	1	2	0	0	0	0
4. B.S.	0	—	0	—	—	2	0	0	0
5. E.G.	0	—	2	—	—	0	0	0	0
6. B.B.	1	—	—	—	—	—	3	2	2
7. J.R.	0	—	—	—	0	—	—	—	0
8. J.G.	—	—	—	—	—	—	0	—	0
9. R.T.	—	—	—	0	—	—	0	—	0
10. W.B.	—	—	—	0	—	—	0	0	0

Except where indicated by \* and †, mitosis were PHA-induced. Dash indicates blood not tested.

\*Spontaneous mitoses were PHA-induced. Dash indicates blood not tested.

†One spontaneous, two PHA-induced mitoses.

were noted in nine recipients during the post transfusion period, the majority of these mitoses (97 of 101) being of the sex type of the recipient or "host." Two patients (1 and 2) were responsible for the four spontaneously dividing donor cells found in the 1-day cultures. These two patients and four others (3,4,5, and 6) also demonstrated 35 PHA-induced donor type mitoses out of over 2000 evaluated. The four remaining patients had only host karyotypes noted in the unstimulated and/or the PHA cultures.

A single opposite sex mitosis was found in the PHA-induced mitoses from the pretransfusion leukocyte cultures. This mitosis was found in patient 6 (Table 2), the mother of a 3-yr-old male child. She had not been transfused previously. She was, however, the only patient who had a significant percentage of cells showing chromosomal aberrations.

The thymidine incorporation of leukocytes sampled 7 days after transfusion was increased significantly in five of six patients so tested (Table 1). The levels were similar to those of our published series of patients receiving fresh blood.<sup>1</sup> Four of the five patients with elevated levels demonstrated circulating cells of the donor type following transfusion. The patient with the highest leukocyte thymidine incorporation had the greatest number of circulating donor cells; however, the number of studies performed was too small to allow a correlation between these two indices.

The PHA-induced donor-type cells were distributed evenly through the first 7 days following transfusion (Table 2). In contrast, the spontaneous mitoses, both host (data not shown) and donor type were noted only during the latter part of the week. No opposite sex mitoses, either PHA-induced or spontaneous, were noted in blood sampled during the second week. However, only one of the five patients who were studied during the second week had had donor mitoses noted earlier. Patients 1-4 and patient 6 were not studied beyond 7 days.

#### DISCUSSION

In this prospective study of ten patients who received moderate to large amounts of transfused blood, we were able to demonstrate circulating PHA-responsive donor lymphocytes in six patients for up to 1 wk after transfusion.

Spontaneously dividing donor lymphocytes were also demonstrated in two of these six patients. These findings support our hypothesis that the early wave of atypical lymphocytes and  $^3\text{H}$ -thymidine incorporating leukocytes seen in transfused patients represented an *in vivo* mixed leukocyte reaction. Our previous study<sup>1</sup> of blood transfusion showed that 15 of 17 recipients of fresh or stored blood had marked increases in these two indices of cellular immunologic reactivity. In keeping with those results, the current study showed that nine of ten patients had spontaneously dividing cells at the end of the first week after transfusion, and five of six patients tested had significantly elevated leukocyte  $^3\text{H}$ -thymidine incorporation. The majority of the dividing cells were of the host karyotype. It seems likely that the cells in mitosis derive from the pool of ATL, although the proof is indirect. Autoradiographic studies previously reported<sup>1</sup> showed that the  $^3\text{H}$ -thymidine labeled cells seen post transfusion were similar to the ATL morphologically. Also, no spontaneously dividing cells were found in the pre transfusion blood specimens which had low leukocyte thymidine incorporation and few ATL.

The frequency and time course of the increased leukocyte thymidine incorporation in the earlier study,<sup>1</sup> with a maximum response 1 wk following transfusion, and its absence after transfusion of leukocyte- and platelet-depleted red cells suggested that an immunologic response to donor lymphocyte-defined antigens was the cause of the reaction. The present finding of prolonged circulation of donor lymphocytes which could provide those antigens is compatible with this explanation, while the demonstration of some spontaneously dividing donor cells indicates the two-way nature of this phenomenon.

That lymphocytes derived from transfusions might circulate for periods of time, in immunologically intact individuals, has been suspected since histocompatibility typing of transfused individuals has been noted to change 24 hr after open heart surgery.<sup>4</sup> It has been suggested that the postoperative period is associated with immunosuppression,<sup>5</sup> and this possibility may account for the circulation of the transfused lymphocytes in our patients. Normal neonates have been reported to have circulating donor lymphocytes persisting for 6–8 wk without ill effect after fetal or exchange transfusion with either maternal blood or unrelated bank blood.<sup>6</sup> Maternal cells have been demonstrated after intrauterine transfusion at 2 yr in four children and at four years in one child.<sup>6</sup> Fatal graft-versus-host disease associated with intrauterine and exchange transfusion has been reported in two infants with apparently normal immune systems. In one of these infants, 25% of the circulating lymphocytes were of donor origin, and spontaneously dividing bone marrow cells had the donor karyotype.<sup>7</sup> None of our patients, including the two patients with spontaneously dividing donor cells, had clinical findings which could not be attributed to their underlying condition or postoperative state. However, the presence of the spontaneously dividing donor cells does raise the question of the occurrence of a subclinical graft-versus-host process.

One of our patients (No. 6), a woman with a 3-yr-old male child, had a cell with a male karyotype demonstrated prior to transfusion. The source of this cell is uncertain. The finding of a significant amount of chromosomal aberration in the cells of this patient raises the possibility of a chromosomal anomaly mimicking a male karyotype and thereby creating a false-positive result, a situa-

tion previously suggested.<sup>8,9</sup> An alternative explanation is the persistence of male cells derived from a fetal-maternal transfusion during pregnancy. Fetal cells have been found in mothers up to 6 mo after delivery of male infants.<sup>3</sup> A number of women have been described who had circulating male cells possibly derived from pregnancies as long as 3 yr previously.<sup>3,10</sup> The PHA-responsive lymphocytes of patient 6 have been studied again, 1 yr after transfusion and 4 yr after the birth of her son. Of 100 cells analyzed, one cell had a male karyotype. The persistence of fetal cells in the circulation seems the most likely reason for this finding and may present a clue to the sustained high levels of lymphocytotoxic antibodies in the plasma of multiparous women.

The role of the host's atypical lymphocyte response in clearing the donor cells from the circulation is not known. It has been shown that specific cytotoxicity develops in the one-way mixed leukocyte culture against stimulator cells with serologically defined histocompatibility differences.<sup>11</sup> This process may represent a mechanism that allows clearance of circulating donor cells in the patient with normal immunologic function. The potential for clinical graft-versus-host disease from transfusion is known to exist for the fetus and the individual with immunologic deficits. It is conceivable that immunosuppressed patients receiving massive transfusion therapy, such as patients treated with chemotherapy for leukemia and other malignancies, may also be candidates for graft-versus-host disease from transfusion.

*Note:* Since this paper was submitted for publication, Hutchinson et al.<sup>12</sup> have confirmed the appearance of ATL post transfusion in a large series of patients receiving whole and dextran-sedimented blood. They also noted the absence of ATL and the failure to produce lymphocytotoxic antibodies after transfusion of frozen cells.

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