each of the types of cells more prevalent in filtered marrow (lymphocytes, normoblasts, and granulocytes, Table 2) indicate that only small and medium "lymphocytes" promoted the same amount of I^{125}-UdR incorporation in recipients of unfiltered and of filtered marrow. The I^{125}-UdR uptake values for other cell types (for example, myeloblasts, metamyelocytes, and erythroblasts) were not computed although such cells were present in small amounts in the filtered marrow, since neither in this experiment nor in earlier studies (*9*) has the number of such cells in the marrow been found to parallel its proliferative capability.

Recipients of filtered marrow were also observed for periods longer than 5 days to establish whether or not such marrow could afford long-term survival to mice which had been exposed to lethal radiation and whether or not the regenerated hemopoietic tissue would contain all of the known classes of mature hemic cells. A detailed account of the latter study will be given elsewhere; however, it can be stated that a large proportion of the irradiated recipients infused with minimal numbers ($< 10^5$) of filtered marrow cells survived more than 30 days, and that after 10 days their spleens contained hemopoietic nodules with megakaryocytes, mature granulocytes, and granulocytoblastic and erythroblastic elements.

It seems reasonable to infer from these and earlier data (*9*) that the marrow "lymphocyte" of the mouse is capable of continued proliferation and is probably a pluripotent stem cell. Since, however, the transplanted "lymphocytes" of these experiments were not labeled with a marker recognizable in the descendent hemic cells, an alternative interpretation involving trophic or stimulating functions of the donor "lymphocytes" on host hemopoiesis cannot be conclusively excluded. Nevertheless, correspondingly good regeneration of functional hemopoietic tissue in comparably irradiated mice has been induced after irradiation only by the successful transplantation of competent hemopoietic stem cells (*2*). The properties of this marrow "lymphocyte" appear to differ markedly from those of the lymph and of the lymph node–lymphocyte in several ways, for example, hemopoietic competence and distribution among myelopoietic and lymphopoietic sites of recipients exposed to lethal irradiation (*9, 15*), duration of life span (*11*), and labeling with tritiated thymidine (*1, 8–12*). The marrow "lymphocytes" may not be a homogeneous population, however, and all of them may not be endowed with stem cell activity, as has been suggested (*4*). The transplanted mouse marrow "lymphocyte" responsible for repopulation of recipient spleens may, for example, be equivalent to the "primitive free cell" of Sabin (*16*).

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Serologic Typing of Human Lymphocytes with Immune Serum Obtained after Homografting

Abstract. *Serums obtained from paired humans following reciprocal fourth-set skin homografts, or from a prolonged series of reciprocal intradermal injections of leukocytes, displayed potent cytotoxic activity against lymphocytes of certain individuals and no activity against lymphocytes of other persons. Study of one family suggested that lymphocyte reactivity may be transmitted according to simple Mendelian genetics. It is probable that this reactivity reflects and can be used to identify specific histocompatibility antigens in man.*

It was previously shown that antibodies reactive with human white blood cells develop after skin-homografting in the human (*1, 2*). Reactivity in both human and guinea pig serums after grafting was readily demonstrable with homologous lymphocytes and poorly or not at all with neutrophils (*2, 3*). Antibodies reactive with lymphocytes of the donor strain have also been found in a number of other species after homografting, including mice and chickens (*4*). It has therefore been our view that the lymphocyte may be the "key" cell to use in developing typing methods for human transplantation antigens (*5*). Our earlier investigations with human serums were accomplished by means of a technique in which a purified, I^{131}-labeled antibody to globulin was used. Because of its complexity, this technique has not been found practical for large-scale typing experiments, but has been useful for identifying antibody-containing serums after homografting and for selecting certain donors whose leukocytes reacted strongly with these serums. By using highly purified lymphocyte suspensions, we have now found a simple method for demonstrating in selected human serums, obtained after homografting, po-

tent cytotoxic activity against lymphocytes of selected human donors. This method is applicable to large-scale typing experiments. Our studies strongly suggest the existence of definable lymphocyte groups in man.

Two pairs of humans, all of blood group AB and Rh-positive, received reciprocal 1.5 by 1.5 cm skin homografts (full-thickness) at intervals from 4 to 6 weeks. One pair received a total of four grafts; the other pair, two grafts. Three separate pairs of AB, Rh-positive individuals received multiple, reciprocal intradermal injections of leukocytes at weekly intervals according to the technique of Friedman *et al.* (*6*). This procedure has been shown to sensitize the recipient to subsequent skin grafts from the same donor, as evidenced by accelerated "second-set" rejection (*6*). Two of the pairs received injections only twice, and the third pair for a period of 7 weeks. Strongly reactive serums were obtained from four of these ten individuals, specifically from those receiving skin homografts from the same donor for the fourth time—that is fourth-set homografts, and from those injected with leukocytes over the 7-week period. These four serums were used in the study described here.

Whole human blood was freed of neutrophils by the method of Greenwalt (*7*), modified for handling small volumes. Lymphocyte suspensions were then obtained from this blood by means of sedimentation and hemolysis techniques, as described elsewhere (*8*). These suspensions were essentially free of erythrocytes and contained no more than 1 or 2 percent neutrophils or monocytes. Test or control serums, plus animal serum as "complement" source, were incubated with small quantities of lymphocytes according to the method of Terasaki and McClelland (*9*). We used 50,000 to 100,000 lymphocytes, 0.2 ml of test or control human serum, and 0.05 ml of complement. The complement had been previously adsorbed at 4°C with washed, packed human erythrocytes. After 2 hours of incubation at 37°C in small celluloid tubes, the reactants were centrifuged, the supernatants largely removed, and the lymphocytes again suspended in freshly prepared 0.2 percent trypan blue dye. The suspensions were pipetted onto glass slides, a coverslip was applied, and the percentage of stained cells was determined by microscopic examination.

Table 1. Cytotoxic activity of immune serums, obtained from humans after grafting and after injections of leukocytes, with lymphocytes of the graft donors and lymphocytes from the panel of 12 humans selected at random. Results expressed as the percentage of lymphocytes which failed to exclude trypan blue dye.

Lymphocyte donor	Serum source					
	After grafting		After injecting leukocytes		Normal donor	Normal donor
	Mo*	Bu	Ma	To		
Mo	0	100	80	0	—	—
Bu	98	1	82	0	—	—
DR	3	94	2	55	4	7
IW	68	3	68	0	3	1
BG	100	1	94	4	6	4
CF	0	90	65	8	3	2
NR	0	37	94	0	0	0
BL	1	2	90	0	5	2
BH	4	74	73	64	4	4
CT	5	92	4	6	2	2
HG	1	0	47	4	0	0
DP	0	96	2	82	0	2
Do.P	2	74	2	58	0	0
PM	1	42	100	78	0	1
No. positive out of the panel	2	8	8	5	0	0

* The subjects from whom the four most strongly reactive serums were obtained are designated by the first two letters of the sensitized individual's last name. These designations are not, however, intended to be permanent labels for specific antigens. Other individuals are designated by their initials.

Preliminary experiments with human and guinea pig serums as sources of "complement" gave negative results, even with serums and lymphocyte suspensions which had been shown, by means of the I^{131}-labeled antibody technique, to yield positive reactions. A survey for complement potency of serums from 17 different animal species was therefore undertaken; the cytotoxic test was used with a known, positive immune serum-lymphocyte combination. Animals studied included the rabbit, hamster, gerbil, rat, mouse, guinea pig, human, rhesus monkey (*Macaca mulatta*), cat, dog, sheep, goat, chicken, pigeon, frog, alligator, and goldfish. Their serums fell into several groups with regard to potentiating effect on cytotoxic activity. A number of them (especially the frog, alligator, and chicken) were themselves cytotoxic and therefore unsuitable for use in such a system. A second group (sheep, pigeon) were nontoxic, but wholly without potentiating effect. Most of the other serums showed variable, generally rather weak activity in the test system. Of the 17 animal serums examined, only rabbit serum proved entirely satisfactory. After adsorption with human erythrocytes in the cold, it was nontoxic for lymphocytes from 20 different human donors, yet displayed strong and consistent potentiating activity in the cytotoxic test. Further results in this study were achieved by using rabbit serum as complement source. Prior inactivation of rabbit serum by heating at 56°C for 30 minutes greatly diminished, but did not eliminate, its potentiating effect. The precise nature of the "complement" activity in this particular reaction must therefore await additional studies.

Table 2. Cytotoxic activity of immune serums, obtained after homografting and after injecting with leukocytes, with lymphocytes of a single family. The results are expressed as the percentage of lymphocytes which failed to exclude trypan blue dye.

Lymphocyte donor	Serum source					
	After grafting		After injecting leukocytes		Normal donor	Normal donor
	Mo	Bu	Ma	To		
WW	12	55	43	5	4	9
JW	30	86	0	34	0	0
MS	3	29	2	8	4	0
ES	2	63	8	2	0	6
RW	2	68	100	3	0	3
MW	8	28	8	3	2	7
LW	3	72	86	6	2	0
BW	0	57	64	4	6	0
PW	4	28	3	10	11	0

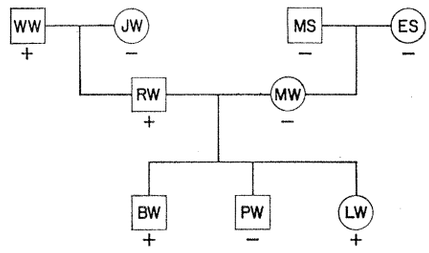

Fig. 1. Family relationships between the lymphocyte donors of Table 2. Reactions of lymphocytes of each individual with serum from Ma (Table 2) are indicated here as positive (+) or negative (−).

The four most strongly reactive immune human serums were tested for cytotoxic activity against lymphocytes of the two fourth-set skin donors as well as those of a panel of 12 individuals selected at random (Table 1). In none of the four immune serums was the presence of antibodies to erythrocytes demonstrated by direct agglutination or by methods in which antibodies to human globulin were used. Reactions with human lymphocytes were clear-cut, however (Table 1). Uptake of trypan blue dye by less than 10 percent of the lymphocytes of a suspension was regarded as a negative cytotoxic reaction; by more than 20 percent, as positive. Most results were well below or above these respective limits. The several values in the low 10 to 20 percent range are probably negative.

The antibody could be adsorbed from positive serums by previous exposure to a reactive lymphocyte suspension. It was present in the γ-globulin fraction of the serum. The unadsorbed serums obtained after grafting yielded positive reactions with lymphocytes from the specific donors, while serum specimens obtained before grafting gave negative reactions. The reaction of the immune serums appeared to differentiate between at least four distinct antigenic factors present on lymphocytes of various donors (Table 1). To date no correlation has been found between the lymphocyte reactivity and the ABO, Rh, MN, Kell, or Duffy blood group types of the lymphocyte donors. No agglutinins for the leukocytes (leukoagglutinins, which would react against neutrophils) were detected in the immune serums.

The results of a study of the lymphocytes of nine individuals, representing three generations of a single family, are presented in Table 2. The ways in which these family members are related are shown in Fig. 1. Serum from only one of the members reacted with the antiserum of Mo or To, all serums reacted with Bu, and four reacted with Ma. Results with Ma are also indicated in Fig. 1 and are consistent with a simple Mendelian hereditary pattern.

These results indicate that antibodies reactive with human lymphocytes, and demonstrable by means of a simple technique, can be produced by skin homografting of humans or by multiple intradermal injections of leukocytes into humans. Evidence from previous work with other animal species and from various laboratories, as reviewed elsewhere (*3*), suggests that such lymphocyte reactions reflect histocompatibility antigens. It is entirely possible that transplantation antigens are also present on other blood cells. Indeed, Payne showed that at least some antigens are shared by neutrophils and lymphocytes (*10*) and a correlation between leukoagglutinin tests and renal homograft survival in humans was claimed by Hamburger *et al.* (*11*). Nevertheless, neutrophil agglutination has been studied intensively since 1952 and has so far failed to define any histocompatibility systems in the human. It is our view that serologic analysis of human transplantation antigens may be accomplished by lymphocyte typing as here described.

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Knots in Leucothrix mucor

Abstract. *The filaments of* Leucothrix mucor *are able to form true knots under certain cultural conditions. Such structures have apparently not been previously seen in filamentous organisms. As the culture ages, the knots become tighter and eventually the cells in the knot region fuse and form a large bulb. The filament breaks on each side of the bulb, and two shorter filaments and a free bulb are produced. The free bulbs have not been observed to grow into new filaments.*

Leucothrix mucor is a large colorless marine bacterium related to the blue-green algae, and it was first studied in detail by Harold and Stanier (*1*) and Pringsheim (*2*). This organism shows several growth habits: multicellular filaments, unicellular gonidia, and rosettes, the latter being formed by aggregation of the gonidia. As shown by Pringsheim, the filamentous habit is more common in a relatively rich culture medium, and gonidia form by fragmentation when filaments are transferred to a medium more dilute in the organic constituents.

I have found this organism to be a common epiphyte of macroscopic algae in the Friday Harbor, Washington, area, and I was able to confirm the observations of Harold and Stanier and Pringsheim with the strain which I isolated in pure culture. In addition, I have discovered a structural feature previously undescribed by these authors, namely, knots (*3*).

I have found knots when the organism was grown in a variety of simple and complex culture media, the only requirements apparently being good aeration and a medium which induces heavy growth of long filaments. Figure 1 shows organisms grown in the following medium: NaCl, 11.75 g; $MgCl_2$, 2.5 g; Na_2SO_4, 2.0 g; $CaCl_2 \cdot 2\ H_2O$, 0.75 g; KCl, 0.35 g; $NaHCO_3$, 0.1 g; yeast extract, 1.0 g; tryptone, 1.0 g; water, 1000 ml. The yeast extract and tryptone can be replaced with monosodium glutamate (10 g/liter) and Na_2HPO_4 (50 mg/liter) with essentially the same results. In these media gonidial production and rosette formation are infrequent, and the organism grows mostly as long filaments. If the organism is transferred from an agar slant into 1 to 2 ml of this medium and incubated at 25°C in a 16-mm tube shaken with a wrist-action shaker, growth is very pro-