in vivo test system. Thus, exposure to radiation may alter an indigenous quiescent virus, or its environment, giving it the ability to alter the chromosomes of blood cells which, may in turn give rise to the functionally malignant cells of leukemia.

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18. The technical assistance of Sally Antennuci and Evelyn Peters is gratefully acknowledged as is the photomicrography by Norman Rabinovitz. This work was supported in part by by grant A-84, Health Research and Services Foundation, Pittsburgh, Pennsylvania.

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

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26 November 1963

Lymphocyte Interaction: A Potential Histocompatibility Test in vitro

Abstract. *Lymphocytes from two unrelated individuals, cultured together in the same tube, undergo morphological transformation to large cells and divide. Both of these parameters may be estimated quantitatively. There is a correlation between the degree of this response and the degree of cross-reactivity of grafts from the two individuals placed on a third unrelated recipient.*

In order to evaluate potential donors for tissue transplants, it would be desirable to be able to test the compatibility of donor and recipient in vitro. Such a test might also prove useful in tissue typing. This report describes an approach to such a system in which peripheral blood lymphocytes are utilized. Genetic similarity between the donor and recipient of a transplanted tissue appears to be the major factor responsible for the success of a transplant. In skin transplantation, if the donor and recipient are genetically identical, the graft will "take" (*1*). In humans, the chance for success with kidney transplantation increases if the donor and the recipient are blood relatives. With identical twins, there is uniform immunological success.

Much work has been devoted in recent years to the problem of selecting suitable donors for tissue transplantation. In human subjects, Rapaport *et al.* (*2*, *3*) and Wilson *et al.* (*4*) have tested for histocompatibility by placing successive skin grafts from prospective donor-recipient pairs on a third unrelated individual. If A and B are the two members of the donor-recipient pair, and C is a third unrelated individual, a skin graft from A is placed on C. At a given time after the rejection of this graft, when C is sensitized to A, a skin graft from B is placed on C. If C responds to B's graft with a second-set reaction, this suggests that A and B may share transplantation (histocompatibility) antigens. Rapaport *et al.* (*2*) have been careful to indicate an alternate interpretation, relating to the contribution of hypersensitization or antigen competition to the results observed.

Table 1. The percentage of large cells (LC) and mitoses (M) in mixtures of lymphocytes cultured for 4 to 7 days.

Source of lymphocytes	After 4 days		After 7 days	
	LC	M	LC	M
X and Y	36.2	0.4	74.3	8.1
X and Z	53.1	1.4	75.7	7.6
Y and Z	32.1	0.3	52.0	3.9

This possibility is supported by the observation that the incidence of individuals exhibiting cross-reactivity appears to increase with increasing dosage of antigen, when leukocyte preparations instead of skin are used to induce sensitivity (*5*).

Brent and Medawar, using guinea pigs (*6*), and Gray and Russell, using human beings (*7*), are testing differences in immunological constitution between donor and recipient. Lymphocytes from the recipient are injected intradermally into a series of potential donors. The intensity of the delayed type-response to the lymphocytes is correlated with the rapidity of breakdown of subsequent grafts from the donors to the recipient. There is an inverse relationship between the reaction of the donor to the recipient's lymphocytes and the survival time of the donor's graft.

The immune capabilities of human peripheral blood lymphocytes in vitro have been demonstrated (*8*). These cells produce γ-globulin (*9*), presumably in the form of specific antibodies (*10*), when stimulated by antigens to which the donor of the lymphocytes is sensitized. Bain *et al.* (*11*) have shown that when human peripheral blood leukocytes from two individuals are mixed, the incorporation of thymidine into DNA is stimulated. We have also shown that in such a mixture of cells, lymphocytes are stimulated to enlarge and divide (*8*). The percentage of large cells and mitoses can be estimated quantitatively.

The lymphocytes are prepared from human peripheral blood as follows. Venous blood is mixed wtih heparin and allowed to sediment at 37°C (1 to 2 hours), and then supernatant plasma is withdrawn. This is mixed with one volume of Eagle's "minimum essential medium" (*12*) modified for suspension culture and placed in a flint-glass pre-

scription bottle. The bottle is laid on its flat side at 37°C for 1 hour, so that the polymorphonuclear leukocytes become attached to the glass. The lymphocytes are poured off and counted. The cells are washed with balanced salt solution or medium and centrifuged at 800 rev/min for 5 minutes. The culture is then diluted to a concentration of 7.5×10^5 mononuclear cells per milliliter with Eagle's medium supplemented with 20 percent fetal calf serum; 1 percent frozen glutamine (200 m*M*) is added fresh at the beginning of culture, and antibiotics are used to prevent growth of contaminants. The culture is incubated at 37°C, and the cells are harvested at the appropriate time. Before harvesting, the culture is incubated with vincaleucoblastine (*13*) for 4 hours, washed in 1 percent sodium citrate, fixed in ethanol and glacial acetic acid (3 : 1 vol/vol), air-dried on a coverslip, and stained with 0.5 percent acetic orcein; 1000 cells are counted, and the percentage of large cells and mitoses is recorded.

Cells from each individual are incubated either for 7 days with nothing added, to check for the spontaneous production of large cells or mitoses (normally less than 5 percent large cells and less than 0.1 percent mitoses), or for 72 hours with phytohemagglutinin (PHA) (*8*) to check the ability of the cells to respond (normally over 90 percent large cells and 1 to 5 percent mitoses).

Cells from several pairs of individuals who showed normal reactions to PHA and normal control values when nothing was added were mixed in equal quantities to establish a time curve of stimulation. To check whether extracts would also cause stimulation after repeated freezing and thawing, cells from one individual were freeze-thawed five times and then added to whole cells of the other individual. This was done in both directions, and in each case, the freeze-thawed extract represented a number of cells equal to the number of whole cells present. However, the extract was added in very small volume in order to maintain the same whole-cell concentration. The graph in Fig. 1 shows the time curve of the whole-cell mixture of one pair of individuals.

Maximum stimulation in mixtures of whole cells appears after approximately 7 to 8 days of incubation. Results from extracts after 4 and 6 days show similar

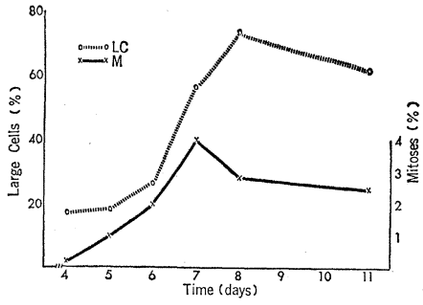

Fig. 1. The percentage of large cells (*LC*) and mitoses (*M*) in mixtures of lymphocytes after 4 to 11 days in culture.

stimulation in both directions, with the percentage of large cells comparable to the percentage of large cells in whole-cell mixtures which have been incubated the same length of time.

We have been able to study three individuals, X, Y, and Z, whose immunological cross-reactivity was determined previously (*14*). This was done by sensitizing a series of recipients with either white cells or white-cell extracts from one of the three donors, followed by a skin transplant from another of the three donors. Rapaport *et al.* (*15*) were able to show that of these three donors, Y and Z cross-reacted most, indicating that they probably share the most major histocompatibility antigens.

We cultured lymphocytes from X, Y, and Z in all combinations of two. Slides were prepared on the 4th and 7th days of incubation, and the percentage of large cells and mitoses was noted.

As shown in Table 1, Y and Z showed a significantly lower percentage of large cells ($p < .001$) and mitoses ($p < .001$) after 7 days in culture than either X and Z or X and Y. After 4 days, X and Y also showed quite a low degree of stimulation. The significance of a low response before the peak of stimulation (7 to 8 days) will need further evaluation. The results obtained with mixtures of cells at the peak of stimulation agree with those obtained by Rapaport *et al.* (*14*), in that they appear to be correlated with the high degree of cross-reactivity. In view of the reservations relating to the interpretation of the results in vitro (*2*), the correlation between our results and skin transplant cross-reactivity must be futher evaluated.

Because so little is known about human histocompatibility antigens, the question arises whether "compatibility" of tissue transplanted from A to B necessarily means that tissue transplanted from B to A will also be "compatible." From the results of our experiments, in which extracts were added one to another in both directions, it would appear that in general, cross-compatibility may exist, since we have not observed the extract from A stimulating B's cells to a very different degree than the reverse. However, the possibility exists that a mutation may change a major histocompatibility antigen to a minor one, or vice versa, or that an amorph at that site could be produced.

As large numbers of intra- and inter-family studies are done in this manner, a genetic analysis might not only give some understanding of the complexity of the histocompatibility genes in man, but might also make it possible to type and set up a panel of white blood-cell donors similar to the panels of red blood-cell donors now in existence. This could then be used for typing a recipient and all potential donors.

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16. Work supported by grants from the U.S. Public Health Service (HD00542), American Heart Association (K.H., established investigator), New York City Health Research Council (U-1030), and the Association for the Aid of Crippled Children.

28 January 1964