References and Notes

1. P. F. Davison and E. W. Taylor, *J. Gen. Physiol.* **43**, 801 (1960); G. G. Deffner, *Biochem. Biophys. Acta* **47**, 378 (1961).
2. E. Rojas and M. Luxoro, *Nature* **199**, 78 (1963).
3. I. Tasaki, A. Watanabe, T. Takenaka, *Proc. Natl. Acad. Sci. U.S.* **48**, 1177 (1962).
4. P. F. Baker, A. L. Hodgkin, T. I. Shaw, *J. Physiol. London* **164**, 330 (1962).
5. I. Tasaki and T. Takenaka, *Proc. Natl. Acad. Sci. U.S.* **50**, 619 (1963).
6. I. Tasaki, *J. Gen. Physiol.* **46**, 755 (1963).
7. H. G. Bungenberg de Jong, in *Colloid Science*, H. R. Kruyt, Ed. (Elsevier, New York, 1949), vol. 2.

8. We are indebted to Drs. Hodgson, Gunther, and Fischer of the University of Chile who made this joint research at the Marine Biological Station at Montemar possible.

8 July 1964

Genetic Studies on the Mixed Leukocyte Reaction

Abstract. *When leukocytes from pairs of unrelated human subjects are mixed and cultured for several days, blast-like cells appear that are capable of DNA synthesis and mitosis. This reaction can be estimated quantitatively by measuring the uptake of tritiated thymidine in the cultures. In experiments with 15 sibling pairs, the leukocytes of most individuals reacted less strongly with those of their siblings than with those of an unrelated subject.*

When leukocytes from two unrelated individuals are cultured together in the absence of phytohemagglutinin, some of the cells become transformed to immature basophilic cells capable of mitosis (*1, 2*). Only these immature cells synthesize DNA, so the intensity of the reaction between the leukocytes from a particular pair of subjects can be estimated by measuring the uptake of tritiated thymidine at the end of the culture period. The blast-like mitotic cells in cultures containing phytohemagglutinin are derived from small lymphocytes (*3*), so the immature cells in leukocyte mixtures, which are indistinguishable from those seen in phytohemagglutinin cultures, probably are also of lymphocytic origin. A similar transformation has been noted when certain antigens are added to leukocytes from single individuals (4).

These findings suggested that the mixed leukocyte reaction may be a response to foreign antigens, possibly the "individual-specific" antigens that are present in most tissues, and which are responsible for homograft rejection (*2*). Subsequently, other workers (*5*) have concluded from their data that the mixed leukocyte reaction may be helpful in the selection of homograft donors.

In our previous studies, there was no reaction in leukocyte mixtures when the two subjects were identical twins. The results with fraternal twins were variable: two pairs reacted and two pairs did not react. It was decided, therefore, to investigate mixtures of leukocytes from sibling pairs who were not twins. In this way, the effect of genetic relationship on the mixed leukocyte reaction could be studied without the results being influenced by the blood chimerism and resulting tolerance to skin grafts, which, although extremely rare, has been reported to occur between human fraternal twins (*6*).

Fifteen normal sibling pairs were studied. The culture method and quantitative estimation of the reaction by means of tritiated thymidine autoradiographs have been described previously (*1, 2*). In addition, a technique was devised for measuring the uptake of tritiated thymidine in whole cultures with a liquid scintillation counter, based on Chen's method for measuring H^3 and C^{14} in serum (*7*). In each experiment, leukocytes from the two members of the sibling pair were mixed together, and at the same time leukocytes from each member of the pair were mixed with those from a third, unrelated, individual. Unmixed control cultures were also prepared. Their H^3-thymidine uptake was always very low compared with mixtures from unrelated individuals.

The results are shown in Fig. 1. The two methods gave very similar results and correlated well, particularly within individual experiments. The mixed leukocyte reaction was diminished when the two subjects were closely related.

The experiments were performed over a period of several months, during which time minor technical changes were made to increase the sensitivity of the method. These changes contributed to the variability of the results, the liquid scintillation counts probably being affected more than the autoradiographs. Thus, it was important to compare the results within each experiment. When this was done, it was found that only five of the 30 individuals composing the 15 sibling pairs reacted more strongly with their siblings than with an unrelated subject. All of the results were analyzed by means of the *t*-test, and the mean difference between sibling-unrelated subject mixtures and the corresponding sibling-sibling mixtures was significantly greater than zero ($p < 0.01$). Whether the subjects were of the same or different sexes did not appear to make any difference.

Five of the sibling pairs, when compared with unmixed controls, showed no reaction that could be detected either by autoradiographs or by liquid scintillation counting. Some of these pairs were studied twice, and no reaction was seen on either occasion. Similarly, other sibling pairs consistently showed a positive reaction. All of these individuals

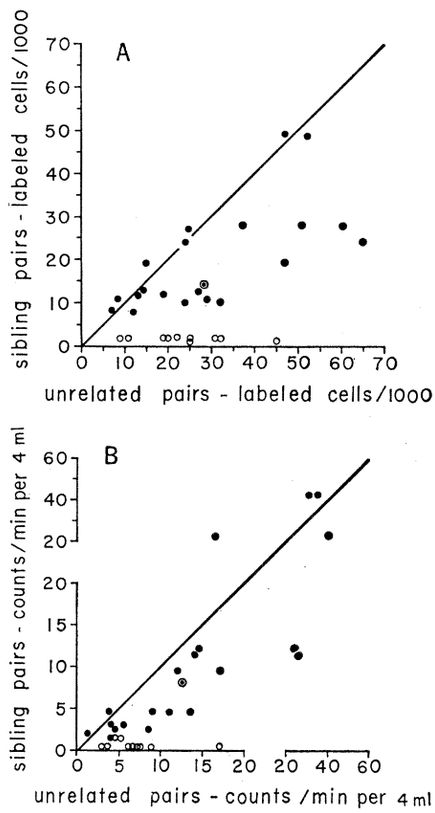

Fig. 1. Tritiated thymidine uptake in leukocyte mixtures. (*A*) Autoradiographs. (*B*) Liquid scintillation counts. For each point, the ordinate is the reaction between an individual and his sibling, and the abscissa is the reaction between the same individual and an unrelated subject. Each sibling pair is thus represented by two points on the graph. Symbols: closed circles, experiments where sibling pair showed positive reaction; open circles, experiments where sibling pair showed no detectable reaction; open circle with center point, mean value of mixtures from all sibling pairs versus mean value of mixtures from each sibling and a third, unrelated individual. The diagonal line shows where the points would lie if sibling pairs and unrelated pairs reacted equally.

reacted with unrelated subjects, as have all 100 pairs of unrelated normal subjects studied so far. These results with siblings and our limited data on fraternal twins suggest that siblings and fraternal twins do not differ in their behavior in the mixed leukocyte reaction.

These results are compatible with the laws of inheritance: siblings have, on the average, 50 percent of their chromosomes in common, so that they are more similar to one another than they are to nonrelated members of their species. However, the number of chromosomes that they have in common can theoretically vary from 0 to 100 percent, and an appreciable number of sibling pairs will lie towards one or the other of the two extreme ends of this scale. On the other hand, a child always inherits 50 percent of his chromosomes from his mother and 50 percent from his father.

Thus, in a situation such as the selection of a donor for a kidney homograft, one of the recipient's brothers or sisters might be much more compatible than either parent, while another sibling would be relatively incompatible (*8*). If the mixed leukocyte reaction proves to be effective in predicting histocompatibility, it may be particularly useful as an indicator of compatibility between siblings. The pairs which react most strongly may be those whose genetic similarity lies towards the "completely dissimilar" end of the scale, and those who show no reaction may be placed closer to the "completely identical" extreme.

BARBARA BAIN*
LOUIS LOWENSTEIN
Division of Hematology,
Royal Victoria Hospital,
Montreal, Canada

References and Notes

1. B. Bain, M. Vas, L. Lowenstein, *Federation Proc.* **22**, 428, abstract (1963).
2. ———, *Blood* **23**, 108 (1964).
3. W. H. Marshall and K. B. Roberts, *Quart. J. Exptl. Physiol.* **48**, 146 (1963); W. H. Marshall, K. B. Roberts, F. Wanless, M. R. Young, *J. Physiol.* **170**, 54P, abstract (1964).
4. G. Pearmain, R. R. Lycette, P. H. Fitzgerald, *Lancet* **1963-I**, 637 (1963); M. W. Elves, S. Roath, M. C. G. Israëls, *ibid.*, p. 806; R. R. Lycette and G. E. Pearmain, *ibid.* **1963-II**, 386 (1963).
5. F. Bach and K. Hirschhorn, *Science* **143**, 813 (1964); A. L. Rubin, K. H. Stenzel, K. Hirschhorn, F. Bach, *ibid.*, p. 815.
6. M. F. A. Woodruff, M. Fox, K. A. Buckton, P. A. Jacobs, *Lancet* **1962-I**, 192 (1962).
7. P. S. Chen, *Proc. Soc. Exptl. Biol. Med.* **98**, 546 (1958).
8. D. R. Newth, *Plastic Reconstruc. Surg.* **27**, 452 (1961).

* J. B. Collip fellow in medical research, McGill University.

10 July 1964

Antigenic Behavior of Moloney Lymphomas: Independence of Virus Release and Immunosensitivity

Abstract. *Mouse lymphomas induced by Moloney virus were compared with regard to their ability to elicit humoral antibodies against Moloney cells, to sensitize against Moloney isografts, and to respond to established isograft resistance. The first two properties were parallel, while the third was independent. The former, but not the latter, is attributed to the release of infectious virus.*

Lymphomas induced by the Moloney virus are capable of inducing specific resistance against the transplantation of other Moloney lymphomas to genetically compatible, isologous hosts (*1, 2*). Humoral antibodies reacting with Moloney lymphoma cells can be detected in the serum of resistant animals by the cytotoxic or the indirect fluorescent antibody test (*2*). The resistance can be induced by at least three methods: by inoculating homografts from Moloney lymphomas that fail to grow or regress after temporary growth, by isografting subthreshold numbers of Moloney lymphoma cells, or by inoculating homogenates containing Moloney virus (*2*). All lymphomas tested released virus even after serial passage. A single dose of irradiated tumor cells, incapable of multiplication but competent to release virus, induces formation of antibody to the cells, the antibody lasting throughout most of the lifetime of the recipient animal (*3*). In contrast, treatment of the x-irradiated cells with hydroxylamine caused complete inactivation of its capacity to induce antibody formation.

While all Moloney lymphomas tested induced a specific immunological response against themselves and other Moloney lymphomas as judged by formation of antibody and by resistance to transplantation, the degree of susceptibility to the rejection response of sensitized hosts varied considerably. A number of lymphomas were not transplantable to isologous recipients at all, even if large numbers of cells were inoculated, unless the recipients were irradiated; with large cell numbers others were transplantable to irradiated and nonirradiated hosts alike, while small inoculums often failed to take. The threshold dose in untreated recipients could be diminished by several orders of magnitude by total body irradiation of 400 roentgens. Lymphomas of the latter transferable type were highly susceptible to the rejection response of sensitized hosts. They did not grow progressively even when large numbers of cells were grafted and the hosts were irradiated prior to challenge. On the other hand, certain lymphomas were characterized by a much lower threshold dose, by only small differences in the takes of small inoculums in irradiated (compared to unirradiated) hosts, and by only a minor increase of the threshold cell number or only a slight prolongation of the latency period upon inoculation into specifically presensitized hosts.

Thus, while all tested tumors released virus, they showed very great variations with regard to their sensitivity to the virus-induced rejection response (VIR). From results with the mouse antibody-production (MAP) test for quantitative assay of the virus (*3*), it appeared that the same number of irradiated tumor cells from different lines released different, but for each line fairly constant, amounts of virus, as judged by the antibody titers 35 days after inoculation. The question arose whether differences in the sensitivity to virus-induced rejection are related to the degree of virus release. An experimental study of this question may define the mechanism of the rejection response.

Since the Moloney agent is an RNA virus that matures by budding from the cell membrane, antiviral antibodies may combine with it on the surface of releasing cells, with complement binding and cell lysis as a consequence. If this were the case, a correlation is expected between virus release and sensitivity to virus-induced rejection in different tumors. Alternatively, the two phenomena may be independent, as in the polyoma system (*4*). Sensitivity to such rejection would then be determined by new cellular antigens, and would appear in virus-induced neoplastic cells and not be related to virus release as such. It is also possible to postulate a dualistic scheme where virus maturation and release occur in cells *not* primarily responsible for tumor proliferation. Dividing neoplastic cells would be characterized by a more "moderate" interaction of virus and cell, where genetic informa-