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This Week's Citation Classic

Hirschhorn K, Bach F, Kolodny R L, Firschein I L & Hashem N. Immune response and mitosis of human peripheral blood lymphocytes in vitro. *Science* **142**:1185-7, 1963.
[Department of Medicine, New York University School of Medicine, NY]

This paper described the initial experiments demonstrating the usefulness of peripheral blood lymphocyte cultures for immunologic studies. It demonstrated lymphocyte responses on exposure to antigens for which the donor was sensitized, as well as the mixed lymphocyte response found with co-cultivation of cells from unrelated individuals. [The *SCI*® indicates that this paper has been cited in over 555 publications since 1963.]

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"In 1959, we and others began using phytohemagglutinin (PHA) for the purpose of obtaining chromosome preparations from peripheral blood cultures. We noted, in addition to dividing cells, that many of the PHA stimulated cells resembled the graft versus host cells described by Gowans[1] in their morphology and staining properties, and we also showed that these cells were lymphocytes. It seemed possible to us that the then current dogma that peripheral blood lymphocytes are end cells might be wrong. We therefore began a series of experiments using cells from individuals who were tuberculin positive, had been vaccinated against diphtheria and pertussis, or had been shown to be sensitive to penicillin. Culture of such cells in the presence of the appropriate antigen resulted in the appearance of the same enlarged cells and mitoses seen with PHA, although in smaller numbers, while no such response was observed in cells from nonsensitized individuals. We interpreted these findings to indicate that peripheral blood lymphocytes were in fact not end cells but demonstrated immunologic memory.

"In a study of the effect of fibroblasts and their extracts on the lymphocytes of patients with eczema, we had noted that there was a small and variable amount of lymphocyte stimulation upon exposure to fibroblasts from unrelated individuals. We therefore postulated that peripheral blood lymphocytes may demonstrate a response to histocompatibility differences and proceeded to co-cultivate peripheral blood lymphocytes from unrelated individuals. We again found enlarged cells and mitoses and concluded that such mixed lymphocyte cultures represented an *in vitro* model of graft rejection.

"Thousands of papers have appeared since that time which have confirmed, utilized, and expanded upon these two fundamental principles. (See, for example, reference 2.) Such peripheral blood lymphocyte cultures have become standard techniques for the study of many aspects of normal immunology, as well as various immunologic abnormalities, in man as well as many other species. The mixed lymphocyte response has been shown to be the best correlate of donor-recipient compatibility in relation to organ transplantation, and has been found to be determined by an allelic series of surface antigens of the D and DR specificity.

"There was great excitement in our laboratory in those early days of the establishment of the field of cellular immunology. Of the many people working in my laboratory in the early-1960s, for short or long periods, several were coauthors on this paper. Fritz Bach, a postdoctoral fellow at the time, has gone on to carry the mixed lymphocyte response to a fine art and has become a leading immunobiologist, currently at the University of Minnesota. Roselyn Kolodny, a medical student on an elective, is a pediatrician in Boston. Lester Firschein, also a postdoctoral fellow, is a geneticist and anthropologist at the City University of New York, and Nemat Hashem, a visiting scientist from Egypt, is now a leading human geneticist in that country and has continued to work on lymphocyte cultures.

"I am personally convinced that the work was done and that it succeeded because, as geneticists, we naively pursued an observation in another discipline, immunology, without the full realization that our results would question established dogma. In the years since then, I have consistently encouraged students and fellows not to fear a fresh viewpoint and to use their techniques in other fields. I believe that this paper has been frequently cited because the various preliminary findings reported attracted many proper immunologists to use a simple technique of cell culture for the study of numerous immunologic phenomena."

1. **Gowans J L.** The fate of parental strain small lymphocytes in F_1 hybrid rats. *Ann. NY Acad. Sci.* **99**:432-55, 1962.
2. **Hume D A & Weidemann M J.** *Mitogenic lymphocyte transformation.* Amsterdam: Elsevier/North-Holland Biochemical Press, 1980. 251 p.