

# This Week's Citation Classic

Mosier D E. A requirement for two cell types for antibody formation in vitro. *Science* 158:1573-5, 1967. [Department of Pathology, University of Chicago, IL]

Mouse spleen cells were separated into two populations by allowing one fraction to adhere to plastic culture dishes. Both the adherent and nonadherent fractions were required to support an *in vitro* primary antibody response to sheep erythrocytes. The adherent fraction consisted mainly of macrophages and the nonadherent fraction mainly of small lymphocytes. Only the adherent fraction had to be directly exposed to sheep erythrocytes to induce an antibody response. It was concluded that two cell types must interact to induce antibody formation and that the macrophage-rich population functioned to process or present antigen to the lymphocytic precursors of antibody-forming cells. [The *SCI*<sup>®</sup> indicates that this paper has been cited in over 565 publications since 1967.]

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"In the spring of 1966, I had just finished my first year of medical school and I was overcome with the ennui that only a year of gross anatomy taken among a herd of over-achieving med students can produce. I took a leave of absence and sought refuge in the laboratory. I had become interested in immunology while working with Felix Haurowitz as an undergraduate at Indiana, and I was both intrigued and perplexed by the claims of Fishman and Adler<sup>1</sup> that 'immune RNA' from macrophages could stimulate antibody formation by naive lymphocytes. While considerable evidence suggested that macrophages bound antigen and that lymphocytes were the precursors of antibody-forming cells, that macrophage messenger RNA could encode specific immunoglobulin

molecules or even that functional messenger RNA could be transferred from one cell to the next was viewed with considerable skepticism by molecular biologists.

"I began working on an *in vitro* model of macrophage-lymphocyte interaction using the (then) recently described method of Mishell and Dutton<sup>2</sup> for obtaining anti-sheep erythrocyte antibody responses from cultured spleen cells. This work was initiated with the help of Frank Fitch and Don Rowley in the department of pathology and I soon found myself a graduate student in that department. Rabinowitz<sup>3</sup> had just published a method for adhering macrophages to glass-bead columns, but I couldn't get enough cells that didn't stick to these columns to do my experiments. Not only that, but my unfractionated spleen cell cultures were giving meager antibody responses. I conferred with Fitch who made two key suggestions—first, that I go to San Diego to learn from Mishell and Dutton how to set up cultures correctly (buy the right fetal calf serum), and second, that I try just letting macrophages adhere to the standard culture dishes I was using. Thereafter, the experiments worked well and I was able to characterize both plastic adherent and nonadherent populations and show that they both were required for the immune response *in vitro*.

"We observed subsequently that both T and B lymphocytes were included in the nonadherent lymphocytes and that both were required for immunocompetence *in vitro*.<sup>4,5</sup> By this time, almost three years later, I was sufficiently rejuvenated to return to medical school, which I finished with as great dispatch as possible.

"This paper probably has been quoted frequently because it combines a direct demonstration of what was already widely suspected, that macrophages 'process' antigen for lymphocytes, and it introduced a simple method for cell separation. Subsequent improvements in lymphoid cell separation by adherence techniques have been made, most notably by Ly and Mishell."<sup>6</sup>

1. Fishman M & Adler F L. Antibody formation initiated in vitro. II. Antibody synthesis in X-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen. *J. Exp. Med.* 117:595-602, 1963.
2. Mishell R I & Dutton R W. Immunization of normal mouse spleen cell suspensions in vitro. *Science* 153:1004-5, 1966.
3. Rabinowitz Y. Separation of lymphocytes, polymorphonuclear leukocytes, and monocytes on glass columns, including tissue culture observations. *Blood* 23:811-28, 1964.
4. Mosier D E & Coppelson L W. A three-cell interaction required for the induction of the primary immune response in vitro. *Proc. Nat. Acad. Sci. US* 61:542-7, 1968.
5. Mosier D E, Fitch F W, Rowley D A & Davles A J S. The cellular deficit in thymectomized mice. *Nature* 225:276-7, 1970.
6. Ly I & Mishell R I. Separation of mouse spleen cells by passage through columns of Sephadex G-10. *J. Immunol. Method.* 5:239-47, 1974.