

This Week's Citation Classic

Bloom B R & Bennett B. Mechanism of a reaction *in vitro* associated with delayed type hypersensitivity. *Science* **153**: 80-2, 1966.

The cell type responsible for inhibition by antigen migration *in vitro* of peritoneal exudate cells obtained from tuberculin-hypersensitive guinea pigs was studied. Exudate populations were separated into component cell types, the lymphocyte and the macrophage. Peritoneal lymphocytes from sensitive donors were the immunologically active cells in this system, the macrophages being merely indicator cells which migrate. Sensitized peritoneal lymphocyte populations upon interaction with specific antigen *in vitro*, elaborated into the medium a soluble material capable of inhibiting migration of normal exudate cells. [The *SCI*[®] indicates that this paper was cited 552 times in the period 1966-1977.]

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"It is particularly gratifying to learn that this paper has been frequently cited, because this represents the first independent research work done by my colleague, Boyce Bennett, and myself. We had each finished postdoctoral fellowships and arrived at about the same time at Albert Einstein College of Medicine. A good deal had already been learned about the mechanisms of delayed type hypersensitivity by the study of the transfer of immune cells into normal animals developed by my thesis advisor, Merrill Chase, but the problem was that once the immune cells were transferred into the recipient, it was impossible *in vivo* to analyze the biochemical mechanisms by which they mediated the passive transfer of reactivity.

"We entered into a collaboration to attempt to unravel the mechanism of delayed type hypersensitivity reaction solely by use of *in vitro* models. The principal one available at that time was the macrophage migration inhibition test originally discovered by Rich and Lewis in the 1930's, later refined by George and Vaughan and

shown to correlate with delayed type hypersensitivity reaction *in vivo* by John David.

"The question that we initially tried to approach, (and it seems inconceivable now that an answer was not known at the time) was whether both lymphocytes and macrophages possessed immunological specificity and the capability of recognizing and reacting to specific antigen. We found that purified macrophages from sensitized animals failed to react in the migration inhibition test, while the immune lymphocytes had the ability to recognize antigen. The extraordinary aspect of the results was that as few as 0.6% of lymphocytes from sensitized animals were capable of inhibiting the migration, in the presence of specific antigen, of 99.4% macrophages obtained from normal donors. This made it very unlikely that the *in vitro* reaction was mediated by a direct cell-to-cell contact, and suggested that the sensitized lymphocytes might be affecting the behavior of macrophages by secreting a soluble molecule.

"In the initial experiment in which we tested for such a soluble molecule, I recall the results were strikingly negative, although there was a peculiar wrinkling of the area of migrated macrophages. While it was tempting to dismiss this as trivial, we made one further effort to produce the *in vitro* reaction, using more concentrated supernatants of antigen-stimulated lymphocytes. The results were striking in that the supernatant of antigen-stimulated sensitive lymphocytes was able to transfer to perfectly normal peritoneal exudate cells the *in vitro* reaction hitherto seen only with sensitized cells. These results suggested for the first time that lymphocytes could communicate with other cell types by soluble non-antibody mediators. The first of these was described in this paper and termed migration inhibitory factor (MIF). Subsequently, a great number of other laboratories have both confirmed these results and added to the list over 52 such putative mediators or lymphokines.'

"It is amusing to reflect that in this work, we had between us grant funds of only \$10,000, and did all of the technical work as well as all of the glassware ourselves. And yet we felt extraordinarily fortunate to have the luxury of being able to study *in vitro* a phenomenon which had almost exclusively been regarded as one which could be studied only in the intact animal, which would have been a much more expensive proposition."