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This Week's Citation Classic

Jerne N K & Noriln A A. Plaque formation in agar by single antibody-Firoducing cells. *Science* 140:405, 1963.
[Dept. Microbiology, Univ. Pittsburgh Medical School, Pittsburgh, PA]

Distinct plaques, each of **which is** due **to the release of hemolysin by a single antibody-forming cell, are revealed by complement after incubation, in an agar layer, of a mixture of sheep red cells and lymphoid cells from a rabbit immunized with sheep red cells. [The *SCI*® indicates that this paper has been cited over 1,805 times since 1963.]**

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"Nineteen hundred and sixty-two was my sixth year in the World Health Organization, Geneva. Sitting there at my desk, I tried to devise a research plan for the fall of that year when I would join the department of microbiology in the School of Medicine of the University of Pittsburgh. At that time, I had become convinced that single lymphocytes responding to antigen will secrete about a thousand identical antibody molecules per second, and that they will continue to do so when suspended in a suitable medium. From my earlier research career, I had quite some experience with the assay of bacteriophage by plaque formation in semi-solid agar. In March 1962, I made the following entry in my diary: 'Antibody molecules diffusing from a single cell into agar containing red cells and complement should lyse a sufficient number of surrounding red cells to produce a visible plaque.'

"In Pittsburgh, in October that year, Albert Nordin (now at the National Institutes of Health), who had just obtained his PhD, became my research associate, working in the laboratory room next to my chairman's office. We adopted the classical phage plating technique in petri dishes. Nordin was extremely diligent and intelligent at the bench, and persisted in trying out minor modifications in the face of early failures. When I returned from my Christmas vacation in January 1963, he showed me a plate with hundreds of tiny plaques that, after staining the red cell background with benzidine, looked like stars in a cloudless sky. We spent a few weeks making spectacular photographs, and then, on February 2, sent our paper to *Science.*

"The importance of being able to count the number of cells secreting a specific antibody was immediately clear to everybody to whom we showed these pictures. These included Macfarlane Burnet, Max Delbrück, Peter Medawar, and Hilary Koprowski. The editors of *Science* proposed to place one of our pictures on the front page, but changed their mind at the last minute before publishing our paper in the April 26, 1963, issue.

"Claudia Henry and Hiroshi Fuji then joined our efforts. Henry improved the technique by adding DEAE-dextran to the agar,[1] and Fuji devised an'indirect'plaquing technique for visualizing cells secreting immunoglobulins of the IgG-classes. Unfortunately, this improvement was first published by others.[2,3] The adoption of this experimental technique in all immunological laboratories has resulted in the high citation frequency of our original paper. The term 'plaque forming cell' (PFC) has become a standard term in immune response assays. An extensive review article[4] on the methodology and theory of plaque formation by antibody secreting lymphocytes, including certain technical modifications introduced by others, appeared in 1974.

"Our paper must be one of the shortest to become a *Citation Classic.* In fact, it was shorter than the present commentary!"

1. **Jernc N K, Nordin A A & Henry C.** The agar plaque technique for recognizing antibody-producing cells. (Amos B & Koprowski H, eds.) *Celt-bound antibodies.* Philadelphia: Wistar Institute Press. 1963. p. 109-25.
2. **Sterzl J & Riha I.** Detection of cells producing 7S antibodies by the plaque technique. *Nature* **208**:858, 1965.
3. **Dresser D W & Wortis H H**. Use of antiglobulin serum to detect cells producing antibody with low haemolytic efficiency. *Nature* **208**:859. 1965.
4. **Jerne N K, Henry C, Nordin A A, Fuji H, Koros A M C & Lefkovits I.** Plaque forming cells: methodology and theory. *Transplant. Rev.* **18**:130-91, 1974.