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This Week's Citation Classic™

Möller G. Demonstration of mouse isoantigens at the cellular level by the fluorescent antibody technique. J. *Exp. Med.* **114**:415-34, 1961. [Institute for Tumor Biology. Karolinska Institutet Medical School, Stockholm. Sweden]

The fluorescent antibody method applied to living cells in suspension demonstrated that H-2 and non-H-2 histocompatibility antigens were localized to the cell membrane A proportion of lymphocytes, but not other cells, treated with only antiimmunoglobulm antisera exhibited staining of part of the membrane, giving rise to fluorescent crescents. This staining revealed surface bound immunoglobulin produced by the lymphocytes themselves [The *SCI*® *in*dicates that this paper has been cited in over 535 publications since 1961.]

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Citations

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"This was my second scientific publication and the result of nearly two years' frustrating work. In 1959, I started to do research in immunology and my first task was to find a suitable research project. I was reading the proceedings of a recent symposium,[1] which was introduced by Peter Medawar. The first paragraph of his paper listed a number of unsolved problems in the form of questions. This was excellent for me and I selected two questions from his list and made them into my research projects. The first was to determine the intracellular and histological localization of H-2 transplantation antigens and the second to study their phenotypic expression during ontogeny. I thought it would be easy to show the intracellular localization of the antigens by the fluorescent antibody method, which at that time was used only with tissue sections. After labelling antibodies with fluorescein — not so easy at a time when you had to start to prepare the fluoresceinisothiocyanate yourself—I applied them to tissue sections, but only obtained a diffuse nonspecific staining.

"My other Medawar-inspired project progressed better, and for various reasons I became interested in pinocytosis of cells during ontogeny. I used fluorescein labelled albumin to study pinocytosis and then observed that the great majority of cells did not take up the fluorescein and, after washing, they remained totally unstained in contrast to the tissue sections. It occurred to me that the use of living cells instead of tissue sections could solve the problem with the nonspecific staining. The first experiment with living cells worked well: all cells in the experimental group exhibited membrane staining and none in the control group. The use of living cells solved a technical problem and clearly demonstrated that H-2 antigens were membrane bound.

"However, a proportion of lymphocytes, in contrast to all other cells, stained when treated only with a fluorescent rabbit anti-mouse immunoglobulin antiserum. It was also membrane staining, but different from the ring staining seen with anti-H-2 sera. The antibodies were localized in one part of the lymphocytes only, making them look like the crescent of the moon. I showed that the stained structures were membrane bound immunoglobulin and that the immunoglobulin was produced by the lymphocytes and not passively picked up. I also found that they were absent in lymphocytes from embryos and appeared shortly after birth. This was the first demonstration of immunoglobulin receptors on the surface of B lymphocytes, although T and B cells had not yet been discovered.

"I am slightly surprised that the paper has been cited often for two reasons. First, it had little impact after its publication. It took seven years for the first confirmation of the membrane localization of H-2 antigens[2] and nine years before membrane immunoglobulin receptors were rediscovered and crescent formation renamed cap formation.[3]

"The second reason is that the fluorescent antibody method applied to living cells is now a routine method and I would not expect most immunologists to care about the original discovery. The same applies to the existence of membrane bound immunoglobulins. It is part of the common knowledge in immunology and reference to the original work done 23 years ago is not necessary."

1. **Albert F & Medawar P B,** eds. *Biological problems of grafting.* Oxford: Blackwell. 1959. 453 p.
2. **Cerottini J C & Brunner K T.** Localization of mouse isoantigens on the cell surface as revealed by immunofluorescence. *Immunology* **13**:395-405. 1967. (Cited 130 times.)
3. **Raff M C, Sternberg M & Taylor R B.** Immunoglobulin determinants on the surface of mouse lymphoid cells. *Nature* **225**:553-4. 1970. (Cited 470 times.)