

Raff M C. Two distinct populations of peripheral lymphocytes in mice distinguishable by immunofluorescence. *Immunology* 19:637-50, 1970.
[National Institute for Medical Research. Mill Hill, London. England]

In immunofluorescence studies using anti- θ (now called Thy-1) and anti-immunoglobulin (Ig) antibodies on cell suspensions prepared from mouse peripheral lymphoid tissues, thymus-dependent T lymphocytes were shown to be Thy-1⁺ and Ig⁻, while thymus-independent B lymphocytes were shown to be Ig⁺ and Thy-1⁻. [The SC® indicates that this paper has been cited in over 570 publications since 1970.]

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"In 1968, I went to the National Institute for Medical Research at Mill Hill, London, to work on the immune system with Avron Mitchison. I had just completed my training in clinical neurology in Boston, and this was my first real taste of science. It was an exciting time in cellular immunology: it was becoming clear that there were two classes of lymphocytes—now called T and B cells—and several laboratories, including Mitchison's, were gathering evidence that T and B cells collaborated with each other in making antibody responses. Since the two types of lymphocytes looked the same and were always found together in lymphoid tissues, methods were badly needed for distinguishing and separating them. Mitchison pointed me toward the θ (Thy-1) antigen as a possible marker for T cells.

"Reif and Allen had discovered Thy-1 in 1964 and showed that it was on the surface of mouse thymus lymphocytes by killing these cells with anti-Thy-1 antibodies and complement.^{1,2} I³ (and, independently, Schlesinger and Yron⁴) used a similar approach to show that T cells, but not B cells, in peripheral tissues were also Thy-1⁺. In order to

visualize Thy-1 on T cells, I turned to indirect immunofluorescence, using fluorescent anti-Ig antibodies to detect the binding of anti-Thy-1 antibodies. The method worked beautifully but turned up an unexpected result: in control experiments where the anti-Thy-1 antibodies were omitted, the fluorescent anti-Ig labelled a substantial proportion of lymphocytes on its own. Roger Taylor and Michel Sternberg, working across the hall from me, independently found the same thing using radiolabelled anti-Ig antibodies, and we published our observations together in *Nature* in 1970.⁵ These findings were exciting because they provided strong support for an important corollary of the clonal selection hypothesis—that lymphocytes have antibodies on their surfaces that function as receptors for antigen. On the other hand, they raised the question of why most lymphocytes were Ig⁻. Interestingly, in 1961, Möller had observed that fluorescent anti-Ig antibodies labelled a small number of lymphocytes but, since the concept of antibody-like receptors on lymphocytes was not at the forefront of immunological thinking, the implications were missed.^{6,7}

"In the paper published in *immunology* in 1970, I showed that the Ig⁺ lymphocytes were B cells whereas the Ig⁻ lymphocytes were T cells. The paper has been widely cited, I suspect, because it was the first direct demonstration that B cells but not T cells have detectable Ig on their surfaces. Since the publication of this paper, the presence of surface Ig has been the defining characteristic of B cells. Although not often cited in this regard, the paper also raised the possibility for the first time that T cell receptors for antigen may not be classical antibody molecules. This began a prolonged and heated controversy concerning the nature of T cell receptors, which has only been resolved recently with the demonstration that these receptors are homologous to, but distinct from, Ig molecules (reviewed in reference 8).

"It is ironic that both the *Nature* and *Immunology* papers on cell-surface Ig were originally rejected by *Science* and by the *Journal of Experimental Medicine*, respectively, because they were not considered sufficiently important."

1. Reif A E & Allen J M V. The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exp. Med.* 120:413-33. 1964.
2. Reif A E. Citation Classic. Commentary on *J. Exp. Med.* 120:413-33. 1964. *Current Contents/Life Sciences* 26(5): 17, 31 January 1983.
3. Raff M C. Theta isoantigen as a marker of thymus-derived lymphocytes in mice. *Nature* 224:378-9. 1969. (Cited 415 times.)
4. Schlesinger M & Yron I. Antigenic changes in lymph node cells after administration of antisera to thymus cells. *Science* 164:1412-14, 1969. (Cited 80 times.)
5. 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.)
6. Möller G. Demonstration of mouse isoantigens at the cellular level by the fluorescent antibody technique. *J. Exp. Med.* 114:415-34, 1961.
7. Citation Classic. Commentary on *J. Exp. Med.* 114:415-34. 1961. *Current Contents I./e Sciences* 27(27):20. 2 July 1984.
8. Williams A F. The T-lymphocyte antigen receptor—elusive no more. *Nature* 308: 108-9. 1984.