

# This Week's Citation Classic®

Ledbetter J A & Herzenberg L A. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63-90, 1979.

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Monoclonal rat antibodies that recognize antigens expressed on mouse lymphocytes were produced and characterized. The antigens included several previously known through work with polyclonal antisera, such as T200, Thy-1, Lyt-1, Lyt-3, ThB, and others identified for the first time with the antibodies described here. [The SC7® indicates that this paper has been cited in over 660 publications, making it the most-cited paper for this journal.]

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When I arrived at Stanford University in January 1978 to start a postdoctoral fellowship with Len Herzenberg, he had just returned from a sabbatical in César Milstein's lab. Herzenberg was there during some of the earliest work on construction of monoclonal antibody (MAb)-producing hybridoma cells and arrived back at Stanford with a flask of NS-1 cells, a good fusion partner for immunized mouse or rat cells. I was, therefore, in a fortunate position and was able to begin fusions of NS-1 with spleen cells from rats that had been immunized with mouse lymphocytes.

A key factor in the successful isolation and characterization of the rat anti-mouse monoclonals was the high level of technology available in the lab. For example, all of the screening to detect interesting antibodies was done using immunofluorescence and flow cytometry. The fluorescence-activated cell sorter (FACS) instrumentation was primarily developed in Herzenberg's lab, which made it among the best facilities available. The use of flow cytometry for screening MAbs is the method I still use successfully today.

Another factor that contributed to the impact of this work was the biochemical characterization of the glycoprotein antigens on two-dimensional charge-versus-size gels. Thus, even though the anti-Lyt-1 and anti-Lyt-2 MAbs detected non-polymorphic epitopes on glycoproteins known to be polymorphic and previously studied using alloantisera against the polymorphic epitopes, the biochemical analysis allowed us to demonstrate that the MAbs recognized these same molecules. This result also showed that xenogeneic immunization, that is, immunization from one species to another, would allow detection of potentially important antigens even if they were not polymorphic.

Finally, and probably most importantly, this paper is highly cited because the antibodies and the hybridoma cell lines themselves were made available to the scientific community. This has ensured their continued use in many laboratories. Several of these cell lines are still available through the American Type Culture Collection, and I regularly send out other cells in response to individual requests.

How is this work connected to work being done today? The importance of studying lymphocyte differentiation antigens lies in the discovery and understanding of their function, and an MAb against an antigen can provide a way to approach this problem. One relevant example is the anti-Lyt-1 antibody and the anti-CD5 (Tp67) antibody against the human homologue of Lyt-1.<sup>1</sup> The anti-Lyt-1 MAb (53-7) described here was first used to show that Lyt-1 may function to transmit a signal to T-cells that stimulates T-cell growth.<sup>2</sup> Anti-Lyt-1 stimulates production of Interleukin-2 by T-cells,<sup>3</sup> a function that is shared by anti-CD5 MAbs to the human Lyt-1 counterpart.<sup>4</sup> The receptor function in the mouse and human appears to involve regulation of cytoplasmic calcium levels.<sup>3,5</sup> Although the natural ligand for Lyt-1 has not yet been identified, injection of anti-Lyt-1 into tumor-bearing mice suggested that this system may be useful for *in vivo* immunopotentialization of the immune system.<sup>6</sup>

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3. Stanton T, Stevens T L, Ledbetter J A & Wofsy D. Anti-Lyt-1 antibody induces Interleukin 2 release from T cells. *J. Immunology* 136:1734-7, 1986.
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