The Journal of Immunology

More Than Just Vanilla

Pamela J. Fink

J Immunol 2008; 180:4351-4352; ;
doi: 10.4049/jimmunol.180.7.4351
http://www.jimmunol.org/content/180/7/4351

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

More Than Just Vanilla

Pamela J. Fink[1]

The year was 1969 and the immunological world mirrored the political world in its turmoil. Two camps of immunologists squared off: those who believed the thymus is a largely vestigial organ full of dying cells and those who believed this gland serves a crucial role, defined by the production of either hormones or cells, in the development and maintenance of the immune system (reviewed in Ref. 1). Those in the first camp could cite the apparently asymptomatic but dramatic postpubescent shrinking of the thymus in normal individuals and unambiguous data showing that adult thymectomy in mice minimally impacts spleen cell numbers and lifelong immunocompetence. Those in the "thymus is important" faction pointed to the findings that neonatally thymectomized mice have substantial immunological deficits as adults (2, 3) and that the effects of adult thymectomy were perhaps offset by the long lifespan of lymphocytes. This notion was reinforced by clinical observations in humans and by tracking immune function in chickens following ablation of the thymus or the bursa of Fabricius in late embryonic chicks, which suggested that two distinct lines of lymphocytes are dependent on two separate central lymphoid organs (reviewed in Ref. 4). Work conducted independently by the Miller and Mitchison groups clearly indicated that the generation of potent hapten-specific IgG Ab responses in mice required the coordinated contributions of spleen cells primed to carrier and those primed to hapten (5, 6). The function of the former cells was attributed to "antigen-specific handling," while the latter were presumed to be the Ab-secreting cells. Could these two functions elicited by priming with hapten and with carrier be performed by separate lineages of cells derived from distinct central lymphoid organs (the thymus on the one hand and the bursa or bone marrow on the other)? This fundamental question remained unanswerable as long as splenocytes were comprised of morphologically identical populations of lymphocytes. A marker to distinguish (and eventually separate) these two functionally discrete cell compartments was desperately needed. Proving once again that good things can come in small packages, that tall order was beautifully filled by a one figure, one table, three-column-long, single author paper by Martin Raff first describing Thy-1 (the theta isoantigen) as a marker of mouse T cells (7).

While working in Mitchison's division, Raff set out to test whether an antiserum recently generated by Reif and Allen in AKR mice injected with MHC-identical C3H thymocytes (8) could recognize a marker expressed by thymus-dependent lymphocytes. This antiserum appeared to identify an Ag on thymocytes from the most commonly used strains of laboratory mice. In line with the contemporaneous practice of using Greek letters to name serologically defined Ags, this marker was named θC3H (in honor of the immunizing cell type) and its allelic counterpart was named θAKR. Raff used the newly described ^{51}Cr-release assay to quantify cytolysis of CBA thymocytes, lymph node cells, and splenocytes in the presence of anti-θC3H antiserum plus hamster or guinea pig complement (7). By this simple but elegant assay, most thymocytes, over half of lymph node cells, and one-quarter to one-third of splenocytes were specifically recognized by this reagent. The distinct plateaus of cytolysis revealed that a discrete subset of θC3H$^+$ lymphocytes populates these primary and secondary lymphoid organs to a defined level. The thymus dependence of this subpopulation of lymphocytes was tested in specific pathogen-free mice treated with anti-lymphocyte serum, a reagent previously determined to deplete thymus-derived cells from the lymphoid periphery (9). Chronic treatment with anti-lymphocyte serum reduced the percentage of lymph node cells and splenocytes recognized by anti-θC3H antiserum by 80–90%. Thus, Raff had demonstrated in short order that the θ Ag serves as a marker for thymus-derived lymphocytes in the mouse. Schlesinger published similar findings at nearly the same time (10).

Less than four months (including two major holidays) later, Raff coauthored a paper demonstrating that surface Ig can be detected by autoradiography and immunofluorescence on a subset of peripheral lymphocytes (11) and that the surface Ig-positive and θ-positive lymphocytes represented nonoverlapping subsets (12). The longed for markers of bone marrow-derived and thymus-derived lymphocytes now had been realized. Raff also crafted the last piece of the puzzle by performing a reprise of the earlier Mitchison experiment adoptively transferring spleen cells from carrier-primed mice into lightly irradiated recipients that were then primed with hapten-conjugated carrier and assayed for hapten-specific serum Abs 10 days later. Treatment of carrier-primed splenocytes with anti-θ plus complement before transfer greatly diminished their ability to augment the recipient's Ab response (13). Thus, the surface marker for thymus-derived lymphocytes also identifies functionally distinct cells, tagging lymphocytes that help Ig-producing cells do their job. The era of "thymus-marrow synergism" (13) was born. Not all immunologists were immediate fans of this work, and one noted scholar disparaged the notion of functionally and phenotypically separate populations of lymphocytes, saying at the time that " 'b' and 't' are the first and last letters of 'bull- - - -.' " This immunologist will of course remain nameless.

[1] Address correspondence and reprint requests to Dr. Pamela J. Fink, University of Washington, Department of Immunology, I-607H Health Sciences Center, Campus Box 357650, Seattle, WA 98195. E-mail address: pfink@u.washington.edu

The notion that Abs can recognize antigenic markers of discrete cell lineages performing distinct functions is so fundamental to the way that immunologists design experiments now that it is difficult to imagine a time when this was not so. The Ab reagents have improved, moving from complex antisera to mAbs, some of the first of which were specific for Thy-1.1 and Thy-1.2, the current nomenclature for θAKR and θC3H, respectively. The cytolysis assay largely has been replaced by flow cytometry and magnetic bead separation, and Abs are still used routinely as a means to enrich and track defined subpopulations of cells. It is even more gratifying that this seminal discovery was presented in such a succinct and simple paper, with none of the grandstanding and self-aggrandizement we have come to expect in papers published today. Perhaps even more astounding, despite mentoring Raff, Mitchison declined authorship on this work, preferring instead to see the spotlight on this young scientist.

References

1. Miller, J. F. A. P. 2006. Vestigial no more. *Nat. Immunol.* 7: 3–5.
2. Miller, J. F. A. P. 1961. Immunological function of the thymus. *Lancet* 2: 748–749.
3. Arnason, B. G., B. D. Jankovic, and B. H. Waksman. 1962. Effect of thymectomy on "delayed" hypersensitive reactions. *Nature* 194: 99–100.
4. Good, R. A. 2002. Cellular immunology in a historical perspective. *Immunol. Rev.* 185: 136–158.
5. Miller, J. F. A. P., and G. F. Mitchell. 1968. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J. Exp. Med.* 128: 801–820.
6. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. I. Measurement of the effect with transferred cells and objections to the local environment hypothesis. *Eur. J. Immunol.* 1: 10–17.
7. Raff, M. C. 1969. Theta isoantigen as a marker of thymus-derived lymphocytes in mice. *Nature* 224: 378–379.
8. Reif, A. E., and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exp. Med.* 120: 413–433.
9. Levey, R. H., and P. B. Medawar. 1966. Nature and mode of action of antilymphocytic antiserum. *Proc. Natl. Acad. Sci. USA* 56: 1130–1137.
10. Schlesinger, M., and I. Yron. 1969. Antigenic changes in lymph-node cells after administration of antiserum to thymus cells. *Science* 164: 1412–1413.
11. Raff, M. C., M. Sternberg, and R. B. Taylor. 1970. Immunoglobulin determinants on the surface of mouse lymphoid cells. *Nature* 225: 553–554.
12. Raff, M. C. 1970. Two distinct populations of peripheral lymphocytes in mice distinguishable by immunofluorescence. *Immunology* 19: 637–650.
13. Raff, M. C. 1970. Role of thymus-derived lymphocytes in the secondary humoral immune response in mice. *Nature* 226: 1257–1258.