This Week's Citation Classic®

Reisner Y, Linker-Israeli M & Sharon N. Separation of mouse thymocytes into two subpopulations by the use of peanut agglutinin. *Cell. Immunol.* 25:129-34, 1976.
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The article demonstrated for the first time that a lectin from peanuts (PNA) binds selectively to the hydrocortisone-sensitive thymocytes that reside in the thymus cortex. A simple technique is described for the separation of agglutinable thymocytes from the hydrocortisone-resistant medular thymocytes, which are not agglutinated by PNA. [The *SCI*® indicates that this paper has been cited in over 425 publications.]

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During the summer of 1974 I began my study for a doctoral degree under the supervision of Nathan Sharon, the "lectin man." Sharon, together with Halina Lis and Reuben Lotan (also a graduate student at the time), was using affinity chromatography, then in its infancy, to purify lectins; he was also studying their biochemical properties.

This new field seemed to be at its peak, with many investigators employing lectins to gain further insights into the structure and organization of membranes.[1] Perhaps the most stimulating findings were those of M.M. Burger and colleagues and of M. Inbar and L. Sachs revealing that the agglutination of transformed cells by lectins is markedly enhanced compared to their normal counterparts. These early findings raised the hope that a major defect of cancer cell membranes had been identified. Like other students, I was fascinated by the new ideas and wanted to explore them further.

A major drawback in the initial agglutination studies was the lack of data on tumor cells grown *in vivo*, due to the absence of appropriate, normal controls. This problem was, and still remains, a major obstacle for such comparative studies. Nehama Haran-Ghera, a renowned tumor biologist in our institute, suggested the use of a particular thymoma that develops spontaneously in aging AKR mice and for which the normal counterpart had been defined as the hydrocortisone-resistant, mature thymocytes.[2]

In my very early study I prepared, together with Marianna Linker-Israeli from Haran-Ghera's laboratory, three cell suspensions: one from whole thymus, comprised mainly of immature thymocytes; another from medullary, hydrocortisone-resistant thymocytes, corresponding to mature cells; and a third suspension of tumor cells from the AKR thymoma. These three cell populations were then incubated with different lectins, including those from wax beans, *Lotus tetragonolobus*, and from soybeans; all were purified in our department. To my astonishment none of the tested lectins differentiated between the tumor cells and their normal counterparts.

Fortunately, peanut agglutinin had recently been purified for the first time by Lotan,[3] and he kindly gave me his first batch. As with the other lectins, peanut agglutinin did not distinguish between the thymoma cells and their normal counterparts. However, a marked difference was observed between the two normal thymocyte subpopulations. The majority of cells in the total thymocyte population were agglutinated by this lectin, whereas no agglutination was observed with hydrocortisone-resistant thymocytes, even at high lectin concentrations. This observation served as the basis of our method for the isolation of the two thymocyte subpopulations in good yield and with excellent viability. Also, it led to the extensive use of peanut agglutinin as a differentiation marker for T cells in mice and humans.

Our paper has been widely cited because it describes a simple, inexpensive method for separation of thymocytes (a "poor man's cell sorter"). This method made possible, for the first time, the purification of cortical immature thymocytes with minimal contamination by functional mature thymocytes. This goal was important for studies of the route of T-cell maturation in the thymus, an important problem in modern immunological research. In addition, the paper presented evidence that the peanut agglutinin receptor is a marker of immature cells—an idea that has been widely investigated in immunology.

Perhaps the most important outcome of our early studies has been their exciting clinical application in the field of bone-marrow transplantation. Using the same approach, but with soybean agglutinin, we were able to separate mouse and human bone-marrow stem cells from the unwanted, life-threatening T cells present in the initial bone-marrow suspension.[4,5] Since 1981 this method has helped save nearly 100 infants with severe combined immunodeficiency ("bubble children") for whom matched sibling donors were not available.[6]

1. **Sharon N & Lis H.** Lectins: cell agglutinating and sugar-specific proteins. *Science* 177:949-59, 1972. (Cited 1,240 times.) [See also: **Sharon N & Lis H.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology*. Philadelphia: ISI Press, 1986. p. 57.]
2. **Chazan R & Haran-Ghera N.** The role of thymus subpopulations in "T" leukemia development. *Cell. Immunol.* 23:356-75, 1976. (Cited 55 times.)
3. **Lotan R, Skutelsky E, Danon D & Sharon N.** The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). *J. Biol. Chem.* 250:8518-23, 1975. (Cited 395 times.)
4. **Reisner Y, Itzicovitch L, Meshorer A & Sharon N.** Hemopoietic stem cell transplantation using mouse bone-marrow and spleen cells fractionated by lectins. *Proc. Nat. Acad. Sci. USA* 75:2933-6, 1978. (Cited 120 times.)
5. **Reisner Y, Kapoor N, Kirkpatrick D, Pollack M S, Dupont B, Good R A & O'Reilly R J.** Transplantation for acute leukaemia with HLA-A and B non-identical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. *Lancet* 2:327-31, 1981. (Cited 130 times.)
6. **Reisner Y, Kapoor N, Pollack S, Friedrich W, Kirkpatrick D, Shank B, Csurny R, Pollack M S, Dupont B, Good R A & O'Reilly R J.** Use of lectins in bone marrow transplantation. (Gale R P, ed.) *Recent advances in bone marrow transplantation*. New York: Liss, 1983. p. 355-87.

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