

This Week's Citation Classic

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Wybran J, Chantler S & Fudenberg H H. Isolation of normal T cells in chronic lymphatic leukaemia. *Lancet* 1:126-9, 1973.
[Sect. Haematology and Immunology, Dept. Medicine, Univ. California Sch. Medicine, San Francisco, CA]

This paper presented data showing that T cells isolated, by rosetting with sheep red blood cells, from the blood of patients with chronic lymphatic leukemia normally react to mitogens. This paper introduced two new techniques: a rosette technique to identify human T cells and a technique to isolate T cells from the peripheral blood. These T cell populations were rather pure and functional. [The *SCI*[®] indicates that this paper has been cited over 325 times since 1973.]

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"The work was done while I was a fellow in the laboratory of H. Hugh Fudenberg, at the University of California, San Francisco. At that time, Chantler was also spending one year in the department doing immunofluorescence on B cells. In fact, this paper was probably one of the first practical implications of the rosette system which we had previously described as a marker for human T lymphocytes.^{1,2}

"The idea for this paper came rather obviously when I realized that rosettes (T lymphocytes surrounded by sheep red blood cells) should be heavier than nonrosettes. Thus, by first rosetting human lymphocytes with sheep red cells (a newly introduced terminology in human immunology) and layering them carefully over Ficoll-Hypaque, these rosettes should be recovered as T cells at the bottom whereas the nonrosetted cells (non T cells) would remain on top.

"The *Lancet* paper was the first application of this idea since we described the isolation of normally reactive T cells from the blood of patients with chronic lymphatic leukemia of B cell type. Other authors have since also approached this specific

problem and using similar or different techniques have shown that T cells can be normal or slightly abnormal in B chronic lymphatic leukemia. This issue, although fundamental, will not be discussed here.

"The data presented in the *Lancet* article were very easily obtained. In fact, the experimental work lasted only two weeks. The writing was rapid and the article was accepted in two weeks by *Lancet*, even though it is probably, for its English style, my worst paper.

"I take the present opportunity to thank Fudenberg who always backed my efforts to prove that rosettes were a marker for human T cells.

"The concept was met with much scepticism by the immunological community and it took 18 months to have these fundamental observations published in the *Journal of Clinical Investigation*.²

"The reasons why this *Lancet* paper is so frequently quoted appear to be multiple. Indeed, this article contains two new techniques: the first, a fast (one hour) rosette technique to identify all human T cells, and the second, a simple procedure for isolating T cells. These techniques allowed the identification or isolation of T cells in disease and in health so that it was easily possible to study their functions. Furthermore, since the rosette techniques are simple, they very quickly became a much used tool for any investigator interested in human immunology. They do not require sophisticated equipment (a centrifuge, an incubator, and a microscope are sufficient). Therefore, I strongly suspect that this simple technique helped in the growing practical interest in clinical immunology by nonimmunologists. Not only have these techniques become powerful tools in understanding various immunological phenomena in medicine but appear to contribute to other new areas like immunopharmacology or more recently the identification of enkephalin receptors on human T lymphocytes.³

"The appearance of more sophisticated tools like the monoclonal antibodies against human T cells will probably take the place for identifying T cells, but I believe that the rosette technique will still remain much used in view of its simplicity, its low cost, and its requirement for only basic equipment."

1. Wybran J & Fudenberg H H. Rosette formation, a test for cellular immunity. *Trans. Assn. Amer. Physicians* 84:239-46, 1971.
2. Wybran J, Carr M C & Fudenberg H H. The human rosette forming cell as a marker of a population of thymus-derived cells. *J. Clin. Invest.* 51:2837-43, 1972.
3. Wybran J, Appelboom T, Famaey J P & Govaerts A. Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human blood T lymphocytes. *J. Immunology* 123:1068-70, 1979.