CC/NUMBER 20
MAY 18, 1981

This Week's Citation Classic

Stanley E R & Metcalf D. Partial purification and some properties of **the** factor in normal **and** leukaemic **human** urine stimulating **mouse** bone marrow colony growth *in vitro. Aust. J. Exp. Biol. Med. Sci.* 47:467-83, 1969.
[Cancer Res. Unit, Walter and Eliza Hall Inst., Royal Melbourne Hosp., Victoria, Australia]

The growth factor in human urine which stimulates the formation of macrophage clones from murine bone marrow cells in agar culture was purified 200-fold. This colony stimulating factor (CSF) was active at concentrations of less than 100 ng/ml and appeared to be an acidic glycoprotein. [The ***SCI***® indicates that this paper has been cited over 150 times since 1969.]

E. Richard Stanley
Department of Microbiology
and Immunology
Albert Einstein College of Medicine
Yeshiva University
Bronx, NY 10461

April 22, 1981

"The work reported in this paper was initiated in late 1967 during the first year of my PhD with Don Metcalf at the Walter and Eliza Hall Institute in Melbourne. Don was away on sabbatical for my first nine months there. During this time, Bill Robinson, Cordon Ada, and I, with the help of Ray Bradley (the originator of the agar culture method for bone marrow colony formation), had concluded, on the basis of studies with mouse serum, that CSF was not a transforming virus, as some had thought, but probably a protein or glycoprotein. Following our discovery of CSF in human urine[1] and Don's subsequent return from sabbatical, we set about purifying it from this potentially abundant source.

"From preliminary studies with 2.0 l. batches of urine, it became clear that we were dealing with a minor urinary component and that considerable volumes of urine would be required. The resources of the entire male staff of the Institute were mobilized and urine was collected in white buckets placed by the urinals. These buckets, with their accompanying poster requesting contributions (and subsequent graffiti), became an Institute conversation piece. On Fridays, many Institute members would spend an hour or two at the local 'pub' but loyally returned to the Institute afterwards to donate. Collections on these days were almost overflowing but, to our subsequent dismay, contained subnormal CSF concentrations!

"The handling of 20-100 l. quantities of urine presented several new occupational hazards. I recall opening the door of the lab early one morning to step into three inches of water –the result of a failure in the 'automatic' system I had set up for the dialysis of large volumes.

"The division of labor was such that I was able to concentrate on the biochemistry. However, the *in vitro* agar culture assay for CSF, although sensitive, takes seven days. Progress seemed slow to me and even slower to Don. Nevertheless, during this study, we became convinced that because of its specificity, occurrence, and action at very low concentrations, we were studying a potent growth factor and that the key to understanding its role was its complete purification and radiolabeling. Despite considerable difficulties, complete purification and radiolabeling of this type of CSF was achieved.[2] We now know that human urinary CSF is a macrophage growth factor and only one subclass of the CSFs that stimulate granulocyte and/or macrophage production.[3]

"I think this publication has been highly cited because it was the first detailed description of the characteristics of a white blood cell growth factor. It indicated that complete purification was feasible and emphasized its possible physiological role as a humoral regulator *of* hemopoiesis.

"I am pleased to see a thoroughly Australian paper become a *Citation Classic*. I hope this can continue in spite of the low priority given to the funding of basic research by the Australian government."

1. **Robinson W A, Stanley E R & Metcalf** D. Stimulation of bone marrow colony growth *in vitro* by human urine. *Blood* 33:396-9, 1969.
2. **Stanley E R & Heard P M.** Factors regulating macrophage production and growth. Purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. J. *Biol. Chem.* 252:4305-12, 1977.
3. **Stanley** E R. Colony stimulating factor (CSF) radioimmunoassay: detection of a CSF subclass stimulating macrophage production. *Proc. Nat. Acad. Sci. US* 76:2969-73. 1979.