To the Editor:

Ziegler-Heitbrock et al. recently reported the tumor necrosis factor-α, interleukin-1 (IL-1), and IL-6-expressing activities of regular CD14+ and a novel subset of CD14+CD16+ small monocytes. In their studies, they have used previously frozen and stored mononuclear cells (MCNs) for further fractionation of monocyte subpopulations and analyses of cytokine expression by the isolated subsets. Because they did not indicate the reason for using cryopreserved cells over fresh cells, I presume that they used frozen cells on the assumption that freezing has very little or no effect on the expression of cytokines by these subsets.

However, it must be pointed out that even under the controlled conditions of freezing, the functional activities of both T-lymphocyte and monocyte subsets are significantly affected by freezing.* We have previously shown that the supernatants collected from frozen, lipopolysaccharide-stimulated, unfractionated, and monocyte-enriched populations of MNCs contained significantly larger quantities of IL-1 than those from fresh cells.3 We attributed the observed increase to its enhanced production by a subset of monocytes because of functional inactivation of the other subset of cryosensitive-suppressor monocytes. In addition, freezing also has been shown to enhance the IL-2–producing abilities of human MNCs.4 Besides, frozen MNCs from both healthy subjects and from patients with lung cancer have been shown to generate a significantly larger number of IFN-producing cells than fresh cells.5

It is apparent from these studies that the differential expression of cytokines by the frozen monocyte subsets described by Ziegler-Heitbrock et al. may or may not be comparable to those of fresh cells. This important factor should be taken into consideration before arriving at any definite conclusions from their studies. It would have been more informative if they had also used fresh cells in their study.

M. VENKATARAMAN
Department of Medicine
Mount Sinai Hospital Medical Center
Chicago, IL

REFERENCES


RESPONSE

In his letter, Venkataraman addresses the important issue of a possible influence of cryopreservation on leukocyte properties. We have always used a mild procedure of cryopreservation, which includes a rapid freezing step at −22°C for 1 minute starting at −7°C sample temperature, a strategy that prevents reheating and damage of the sample during crystallization. We thaw samples at 37°C. We have controlled for monocyte phenotypes, as determined by monoclonal antibodies (unpublished observation), and for monocyte function, i.e., the tumor necrosis factor (TNF)- mediated cytotoxicity,1 and we found no detrimental effect of our procedure. The average 1.5- to 3-fold higher values for IL-1 observed by Venkataraman may, in fact, be unique to their strategy, which includes a constant freezing rate of −1°C/min. down to −30°C and a rapid thawing at 45°C.2 Because IL-1 lacks a signal...
peptide for secretion, it is conceivable that this largely cell-associated cytokine is released in higher amounts after mild damage.

With our strategy and the controls mentioned, we are convinced that cryopreservation does not account for the differences observed in monocyte subsets. Still, purification of large numbers of minor subsets, such as the CD14⁺/CD16⁺ cells, is a time-consuming procedure, involving elutriation and cell sorting, and extending over 2 days. This manipulation may differentially inhibit or activate cells. We are currently analyzing this question by polymerase chain reaction analysis of TNF mRNA in small numbers of cells that can be isolated within a few hours.

H.W.L. ZIEGLER-HEITBROCK
Institute for Immunology
University of Munich
Munich, Germany

REFERENCES
