THE PRELIMINARY STUDY OF HUMAN BLOOD MONOCYTES ISOLATION USING PERCOLL DENSITY GRADIENT

Chuenchitra T[1], Japatanakul N[2], Kiatakekasit T[2], Junwongkaew J[3], Urnarom A[3], Sukwit S[1] and Sirisopana N[1]

1 Armed Forces Research Institute of Medical Sciences, Bangkok 10400, Thailand, 2 Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand, 3 Triam Udom Suksa School, Bangkok 10330, Thailand

Abstract

Monocytes and macrophages are widely used in immunological research especially for the study of intracellular pathogens. Several methods for monocyte isolation from peripheral blood are adherence after Ficoll-Hypaque purification of peripheral blood mononuclear cells (PBMC) and positive and negative immuneselection. However, monocyte isolation method based on density gradient centrifugation is still attractive alternative because it is convenient, simple and cheap. To study and establish the method of human blood monocyte isolation by using percoll density gradient for an alternative method in our laboratory at AFRIMS, we used a two step procedure with single gradients in each step for monocyte isolation from whole blood. First a Ficoll-Hypaque gradient (density=1.070 g/ml) was used for separation of PBMC and then a slight hyperosmolar percoll gradient (density=1.064 g/ml). Percoll was prepared mixing 1:1 (v/v) isosmotic Percoll with PBS/Citrate. The gradient centrifugation was done at room temperature, 2000 rpm, 30 min. PBMC was counted and viability was estimated by trypan blue dry exclusion. Percentage of monocytes after the Percoll gradient was determined by CD14+ staining and FACS analysis. In our preliminary study showed the variable yield of monocytes (about 40-70%) and lower than other studies (90%). It is probable due to few samples and this technique need to be developed and improve more steps such as centrifugation step and Percoll solution preparation to get the more yield of monocytes and high viability. However, Percoll density gradient procedure can be done with usual reagents and equipment of average laboratory. Thus, this procedure can be used as an alternative method although it requires more skill and time consumimg.

34th Phramongkutklao Hospital Annual Scientific Meeting, Bangkok, Thailand, 23-25 November, 2006 (Poster)