ELSEVIER

Cryopreserved Hepatocytes Are Poor Stimulators of In Vitro Cytotoxicity

F.M. Karrer, B. Bilir, R. Gill, A. Ostrowska, M. Stegall, M. Wachs, and I. Kam

HEPATOCYTE transplantation has been used to correct inborn errors of metabolism and to treat fulminant hepatic failure in animal models.[1,2] The use of allogeneic or xenogeneic cells has resulted in limited survival due to immunologic destruction.[3,4] Strategies then require immunosuppression of the recipient or reduction/elimination of the immunogenicity of the graft.[5] To investigate the effect of various modifications of the hepatocyte (HPC) preparation on the ability of these cells to stimulate an in vitro cytotoxic response, the following experiments were undertaken.

MATERIALS AND METHODS

Animals

BALB/c ByJ (H-2^d) and CBA (H-2^k) male mice were used as the source of target and responder cells, respectively.

Hepatocyte Preparation

HPC were isolated by modified in situ collagenase perfusion.[6] Three different HPC preparations were investigated;

1. crude HPC (filtered and washed only)
2. HPC purified by centrifuge separation using 70% Percoll; and
3. HPC processed as in #2 then cryopreserved using a computerized controlled-rate freezing apparatus. Viability and yield were determined by cell count after trypan blue exclusion. The purity of the preparations was determined by cytospin of 10^5 cells followed by CD45 staining.

Mixed Hepatocyte-Lymphocyte Cultures and Cytotoxic Assay

Two million responder lymphocytes were co-cultured with either irradiated splenocyte controls or one of the HPC preparations. In each instance, 3.2×10^4 viable cells were used in culture. After 5 days in culture, 100 μL (1/20th of the culture) was plated in quadruplicate and serially diluted for a 4-hour chromium release assay. Chromium-labeled P815 cells (H-2^d) (1×10^4) were used as targets. The cytotoxic activity was calculated and expressed as a percent of specific lysis.

RESULTS

Hepatocyte preparations

HPC yields after collagenase dissociation were from 5 to 10×10^6 HPC/gm. HPC viability was 75% for the crude preparation, 98% after Percoll centrifugation and 65% for the cryopreserved cells. Contamination by CD45 + cells was 9.3%, 0.7%, and 0.5% respectively.

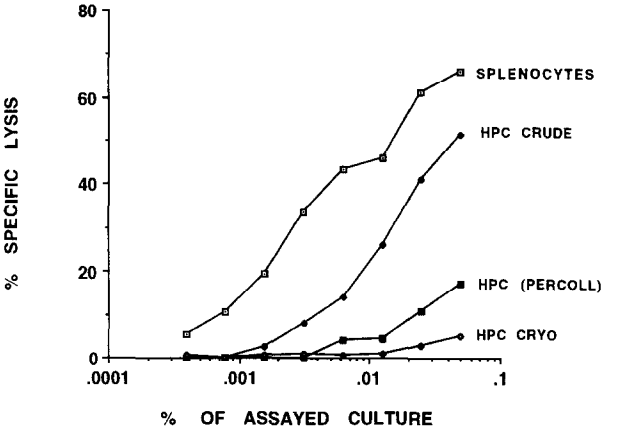

Fig 1. Cytotoxic assays of different cell preparations in mixed lymphocyte culture.

Cytotoxic Assay

The results of the cytotoxic assays for each of the cell preparations is shown in Fig 1. Splenocytes and crude HPC demonstrated significant stimulation of a cytotoxic response, whereas Percoll HPC were relatively poor stimulators. The least stimulatory preparation was cryopreserved HPC.

DISCUSSION

Survival and function of transplanted syngeneic hepatocytes has been shown in several animal systems. However, when allogeneic hepatocytes are used, cells are rejected. Addition of immunosuppression has still only achieved limited success. Thus, not only is immunosuppression insufficient but it

From the University of Colorado School of Medicine, and Barbara Davis Institute, Denver, Colorado, USA.

Supported, in part, by Hepatobiliary Research Center—Pilot Feasibility Grant, NIH Grant No P30 DK34914.

Address reprint requests to Dr F.M. Karrer, The Children's Health Center, 1950 Ogden Street, B323, Denver, CO 80218.

0041-1345/97/$17.00
PII S0041-1345(96)00453-8

also negates one of the advantages of cell transplantation. Since HPC do not express class II antigens under normal circumstances, another strategy would be to modify or "purify" the HPC preparation to remove contaminating cells that might initiate the immune response. By separating the cells using Percoll, a significant reduction in in vitro cytotoxicity was shown. Further reduction in the cytotoxic response was seen after cryopreservation of the hepatocytes. Possibly this reduced immunogenicity is due to fewer contaminating (CD45+) cells in the preparation. CD45+ cells, such as Kuppfer cells, lymphocytes, leukocytes not only express MHC Class II antigens but are also able to provide the 2nd signal necessary for initiation of the immune response. In conclusion, purified cryopreserved hepatocytes are poor initiators of in vitro cytotoxicity.

REFERENCES

1. Gupta S. Chowdury JR: Hepatology 15:156, 1992
2. Demetriou A, Whiting J, Feldman D, et al: Science 233:1190, 1986
3. Makowka L, Falk RE, Rotstein LE et al: Surgery 88:244, 1980
4. Coburn CS, Makowka L, Falk JA et al: Transplant Proc 19:1002, 1987
5. Lafferty KJ, Prowse SJ, Simeonovic CJ: Ann Rev Immuno 1:143, 1983
6. Seglen PO: Methods Cell Biol 13:29, 1976