Culturing of BHK-21 cells in a medium, containing adult bovine serum after treatment with hydrophilic silicon particles

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Summary. The lipid concentration in adult bovine serum (ABS) is $7.93 \pm 1.29 \text{ mM}$ and in fetal bovine serum (FBS) $2.90 \pm 0.39 \text{ mM}$. A high lipid concentration in the medium reduces the tested cell culture parameters. When a culture medium for BHK-21 cells, containing FBS, has the same lipid concentration as a medium containing the same volume fraction of ABS, the cell density and protein synthesis after 7 days, are reduced by about 15%. However, the cell density and the protein synthesis, in this medium with FBS and additional lipids, are still about twice as high as in a medium with ABS. The hydrophilic silicon particles Aerosyl have a specific affinity for lipids. Incubation in suspension of ABS with 2.5 g Aerosyl 300/liter serum, before use in a culture medium, yields a maximal increase of the cell density of BHK-21 cells and protein synthesis. However, after this incubation, the lipid concentration in ABS is still $2.40 \pm 0.71$ times higher than in FBS. Some other materials (gels) remove lipids from ABS, but do not yield an increase of the cell density and protein synthesis, comparable with ABS treated with Aerosyl 300. These observations indicate that, besides lipids, Aerosyl 300 also removes other components from serum. Presumably some of these removed components inhibit cells in culture and others are essential for cells in culture. This is supported also by the observed maximal stimulation of cell in culture in a medium with FBS, treated with 2.5 g Aerosyl/liter serum.

Key words: Aerosyl, BHK-21 cells, Cell culture medium, Fetal bovine serum, Lipids, Serum

1. Introduction

Serum contains many components that stimulate proliferation and protein synthesis by mammalian cell cultures [3]. Serum can be used in the medium up to a volume fraction of about 20%, yielding an almost linear relation with the stimulation of a cell culture. Higher volume fractions of serum often lead to toxicity [22]. Among many other factors, lipids are also essential components in serum for maximal cell proliferation and protein synthesis. The main lipids in serum are cholesterol and fatty acids. Except for non-esterified fatty acids, the lipids are complexed in blood with apolipoproteins, mainly by hydrophobic interaction, yielding lipoproteins. The non-esterified fatty acids (NEFA) are complexed with albumin, by means of a combined hydrophobic and electrostatic interaction. The plasma lipoproteins are classified on the basis of their flotation density [10]. The fatty acids are for a considerable part esterified as triacylglycerol (TGC) and phospholipids (PL) [13]. Very low density lipoprotein (VLDL) contains the highest content of TGC; high density lipoprotein (HDL) contains the highest content of PL. The most abundant lipoproteins in blood, low density lipoprotein (LDL) [13] and HDL [15], contain the highest content of esterified and non-esterified cholesterol (respectively -CH and ne-CH).

All lipids can be synthesized by the cells de novo from polyunsaturated fatty acids [24]. However, addition of low concentrations of cholesterol and fatty acids (respectively complexed with apolipoprotein and albumin), yields a strong stimulation of the proliferation by some cell cultures [8]. In fetal bovine serum (FBS), the concentration of cholesterol varies between 500 and 1500 µM and NEFA between 150 and 350 µM, TGC between 650 and 950 µM, PL between 200 and 550 µM and NEFA between 150 and 350 µM [4, 14, 20, 27, 28]. The protein concentration in FBS varies between 30 and 50 g/liter. After birth, the concentration of the lipids and proteins in serum rapidly increases with the age of the donors [12, 15, 27, 28].

Various reports describe the inhibition of cell cultures by high concentrations of lipids in the medium. The viability of cells is reduced by lipidlike extracts from blood plasma [11]. FBS with a high concentration of protein, cholesterol, and phospholipids causes a lower plating efficiency of various cells [27]. A lower NEFA content in FBS is correlated with an enhanced cell culturing [4]. The plating efficiency is reduced by the addition of LDL to a medium containing serum [29]. Addition of more than 100 mM NEFA, in combination with albumin, inhibits cells cultures [6].

Many reports describe the need for lipids [17] or lipoproteins [16] in serum-free culture media for mammalian cells. LDL stimulates the proliferation of
various cell cultures at a concentration below 100 mg/liter medium (about 150 μM lipid) and is toxic above this concentration (not always [33]). HDLs strongly stimulate cell cultures and are not toxic up to 1 g/liter medium (about 1 mM lipid) [16]. Up to 20 μM linoleic acid in combination with 1 g/liter albumin stimulates the proliferation of various cells in a serum-free medium. Addition of NEFAs without albumin is toxic for cells [2]. Cholesterol stimulates cells in a serum-free medium up to a concentration of 25 μM [33]. Phospholipids stimulate cells up to a concentration of 5 μM [18]. Addition to a medium of delipidated FBS yields a strong reduction of the proliferation of fibroblasts. The growth can be restored by the addition of 0.2 μM linoleic acid. Reduction of the growth is observed above 0.5 μM linoleic acid [25]. More than 90% cholesterol may be removed from FBS without affecting the cell culture [26].

For the removal of lipids from serum, the lipoproteins can be removed by means of ultracentrifugation [16] or by extraction of the lipids from serum by means of organic solvents [7, 30]. The former method is time consuming; the latter method may denature proteins. Adsorption by hydrophilic silicon particles from a hydrophobic solution [19] and by hydrophilic particles with hydrophobic aliphatic ligands from a hydrophilic solution are common techniques for the separation of lipids from a complex solution. In comparison with other silica particles, hydrophilic Aerosyl yields the highest adsorption of lipids per fixed mass of the particles [21]. By incubation in suspension of 20 g Aerosyl 380 with 1 liter serum for 2 h, about 98% cholesterol and TGC and about 22% NEFAs are removed [1]. Under these conditions, more than 70% of the β-lipoproteins (VLDL and LDL) and 60% of the other β-globulins are removed (except transferrin) [23, 31]. Aerosyl also effectively removes mycoplasma from serum [21].

In this paper a method is described for the removal of lipidlike components from adult bovine serum (ABS) by means of incubation with a low concentration of Aerosyl 300. The aim is to achieve a maximal increase of the proliferation and protein synthesis by BHK-21 cells in a medium containing ABS. In comparison with a medium with FBS, a medium with ABS contains a higher lipid concentration and yields a reduced proliferation and protein synthesis. A reduction of the lipid concentration in a medium containing ABS may enhance the culturing of cells.

2. Materials

A. Equipment
1. H1 Flow cytometer
2. Wallac 1410 Liquid Scintillation Counter

B. Disposables (sterile)
1. Cell culture flasks (TCPS), 25 cm², No. 3025

2. Cell culture flasks (TCPS), 225 cm², No. 3000
3. Cell culture cluster dishes (TCPS), 6 × 9.5 cm², No. 3406
4. Stripettes (PS): 2 ml, No. 4021; 5 ml, No. 4051; 10 ml, No. 4101 and 25 ml, No. 4251
5. Biofreeze vials (PP), Nos. 2028, 2128 and 2228
6. Bottles (PS): 250 ml, No. 8390; 1000 ml, No. 8396
7. Centrifuge tubes (PP) 15 ml, No. 3216
8. Microtitration plates (PS) 96 wells (for the determination of lipids), No. 3796
9. Reaction vials (PP) 1.5 ml, No. 616201
10. Tubes (PP) 50 ml, No. 22726
11. Acrodisc PF, 0.8/0.2 μm, 2.8 cm², No. 4187
12. Serum Acrodisc, 0.2 μm, 7.5 cm², No. 4525
13. Counting vials, Pony vial H/I

C. Media and chemicals

1. Serum
   a) Serum, used in all cases:
      (1) Adult bovine serum (ABS), No. S-0018
   b) Fetal bovine serum (FBS), No. S111a

2. Tissue culture medium and reagents
   a) Minimum essential medium eagle (modified) with Earle’s salts (EMEM), containing 20 mM HEPES (N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid], No. 12-104
   b) EMEM, containing 2 g/liter sodium bicarbonate, No. 12-104
   c) L-Glutamin (200 mM), No. 16-801
   d) Tryptose phosphate broth, No. 16-821
   e) Penicillin (5 × 10⁶ IU/liter) and streptomycin (5 g/liter), No. 16-700
   f) Amphotericin B (0.25 g/liter), No. 16-723
   g) Trypsin (0.05%, rrdv) and EDTA (ethylendiaminetetra-acetic acid, 0.02%, m/v) in a salt solution, No. 16-891
   h) Lipids, solution, cholesterol rich, from ABS

3. Chemicals
   a) DMSO (dimethyl sulfoxide), No. 16743
   b) TCA (trichloroacetic acid, 40%), No. 811
   c) NaOH (sodium hydroxide, 1 M), No. 9956
   d) [4,5-3H]-Leucin, specific activity: 120–190 Ci mmol/liter, 5 Ci/liter, No. TRK
   e) Aerosyl 300