THE REPLACEMENT OF SERUM BY SiO_2 (AEROSIL) AND 2-MERCAPTOETHANOL OR DIETHANOLDISULFIDE IN THE IMMUNIZATION AND STIMULATION OF SPLEEN CELL CULTURES

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1. Introduction

The development of serum-free media provides many advantages for experimental work with cell cultures [1]. Here we show that in the presence of 2-mercaptoethanol (2-ME) or diethanoldisulfide (DED) SiO_2 was capable of replacing serum in the stimulation of mouse spleen cells. We have studied the effect of SiO_2 on the in vitro primary immune response [2] and on the DNA synthesis of mouse spleen cells.

2. Materials and methods

The method in [2] was used with the following modification: Cell suspensions from spleens of female 3–4-month-old BALB/c mice were prepared by gently squeezing the spleen in phosphate-buffered saline (PBS) with a spatula. The cells were washed in PBS and resuspended in Dulbecco's medium supplemented with penicillin (240 U/ml), streptomycin (240 μg/ml) HEPES (1.5×10^{-2} M), adenosine (25 μg/ml), guanosine (25 μg/ml), uridine (25 μg/ml), cytidine (25 μg/ml), essential amino acids (100 × Flow Lab.) (10 μl/ml), 2-ME (5×10^{-5} M), glutamine (1.28 mg/ml) and lysed sheep red cells [3]. The pH was adjusted to 7.2 with NaOH. The suspension of SiO_2 was prepared by mixing 4 mg Aerosil 200 (Degussa; specific surface 160 m^2/g) in 1 ml PBS. A total volume of 0.1 ml culture containing 2.5×10^6 nucleated cells were incubated in Falcon plates (no. 3040) at 37°C in an atmosphere of CO_2 (10%) and air for 4 days. Plaque forming cells (PFC) were assayed in duplicates according to [4]. Each experimental group consisted of 8 cultures which were pooled before analysis.

DNA synthesis was assessed by [^{14}C]thymidine incorporation. Cell suspensions, containing 2.5×10^6 cells in 0.1 ml were incubated for 66 h in the absence of sheep red cells. After 48h, 0.01 μCi [2-^{14}C]thymidine (56.7 mCi/mmol) was added to each culture. After 18 h the cells were filtered and washed with water in a Skatron multiple cell-culture harvester, and the radioactivity was measured in a scintillation fluid composed of omnifluor (4 g/l) and toluene. Each value shown in the figures represents the mean ± SD of 4 individual cultures. Other experimental methods were as above.

3. Results and discussion

That serum or its proteins are required in the in vitro primary immune response is generally accepted [2], however as fig.1 shows, high recoveries of PFC were obtained in the presence of SiO_2 and 2-ME despite the absence of serum and its components. Fetuin and zymosan which are capable of promoting the in vitro primary immune response in the absence of serum [3,5] inhibited the activity of SiO_2 suggesting that these materials shared a common site of action (fig.1).

It was shown in [6,7] that 2-ME has a stimulating effect on DNA synthesis in cultured cells and that this effect is strongly augmented by foetal calf serum (FCS) [6]. Fig.2 shows that 2-ME stimulated DNA synthesis under our experimental conditions and in accordance with the results in [6], FCS augmented this effect. Although SiO_2 and FCS have a similar effect, the stimulation of DNA synthesis was augmented by SiO_2 significantly more than by FCS. Thus SiO_2 was capable of replacing FCS efficiently in

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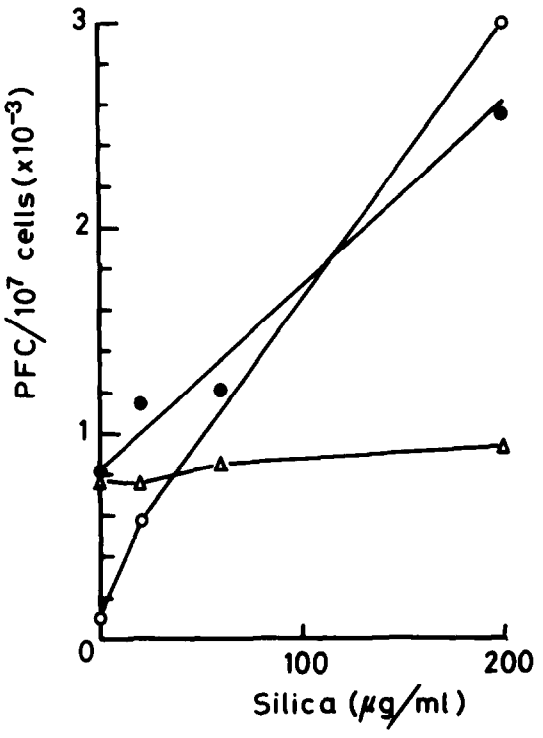

Fig.1. The effect of SiO_2 on the in vitro immune response. (•) Zymosan (5 μg/ml); (△) fetuin (400 μg/ml); (○) no further additions.

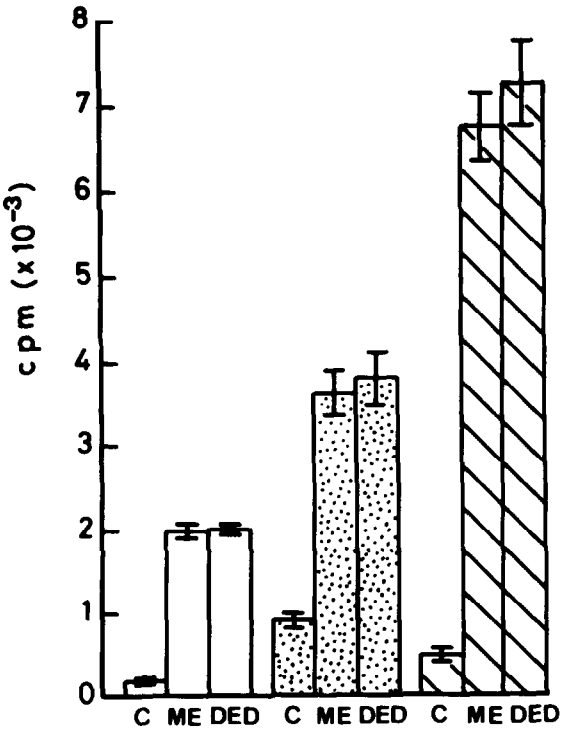

Fig.2. The effect of SiO_2 on [^{14}C]thymidine incorporation by mouse spleen cells. Suspensions contained 2.5 × 10^6 cells/0.1 ml: dashed columns = SiO_2 (200 μg/ml); dotted columns = foetal calf serum (5%); empty columns = no further additions; C = controls; 2-ME was 5 × 10^{-5} M; DED was 5 × 10^{-5} M.

the system described here. It may be of interest that under our experimental conditions the oxidation product of 2-ME, DED had the same effect on DNA synthesis as 2-ME [8]. In experiments with the in vitro primary immune response (not shown), DED also had a similar effect as 2-ME.

It is an advantage to use SiO_2 instead of serum as it is easily removable from the medium and therefore facilitates the isolation of the specific control factors liberated from the cells during in vitro immune response. From the theoretical point of view, it is of interest that SiO_2 can be used instead of a complex serum system. The most plausible assumption about the role of SiO_2 may be that it interacts with the cells. A support for this possibility was obtained in experiments in which the effect of SiO_2 on different densities of cells was compared (fig.3). These experiments showed that the optimally active concentration of SiO_2 was dependent on the cell density.

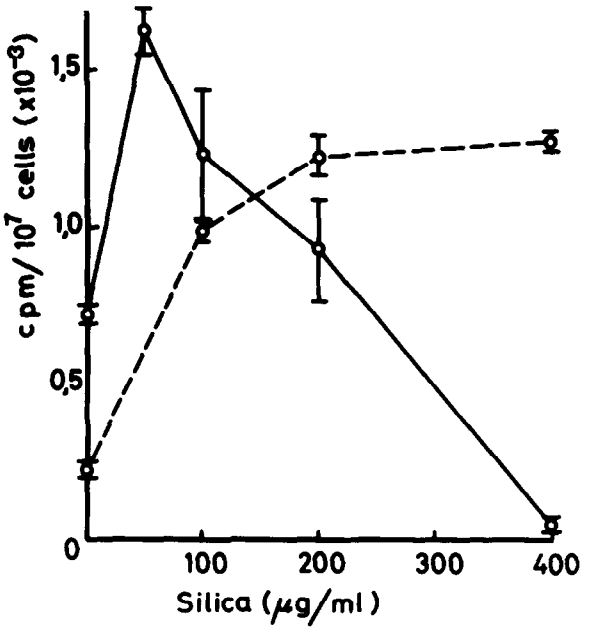

Fig.3. Influence of cell density on the [^{14}C]thymidine incorporation by mouse spleen cells in the presence of 2-ME cell densities of 5 × 10^6/ml and 25 × 10^6/ml were used.

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