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CHROMATOGRAPHIC BEHAVIOUR OF MOUSE SPLEEN CELLS ON GLASS BEAD COLUMNS

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SUMMARY

The chromatographic behaviour of murine spleen cells on glass bead columns was investigated at 25° and 37°. Adhered cells were eluted by phosphate-balanced solutions containing increasing concentrations (1.5–4 m*M*) of the disodium salt of ethylenediaminetetraacetic acid and 20% of calf serum. Chromatography at 25° led to greater elution of the cells. The elution profiles of mouse spleen cells showed fewer peaks than rat bone marrow cells. The number and differential counts of cells in the five main fractions were determined.

INTRODUCTION

The fractionation of cell populations by chromatographic methods is still only at the very beginning of development, although several reports have been published on this problem. This lack of published information makes investigations in this field promising[1–8].

In a previous paper[9] we achieved a certain chromatographic fractionation of rat bone marrow cells on glass bead columns at 37°. Cell fractions were eluted stepwise by basal Eagles' medium (BEM) containing increasing concentrations of the disodium salt of ethylenediaminetetraacetic acid (EDTA).

In the present work, we extended this work and investigated the chromatographic behaviour of another, more simple, type of cell population from mouse spleen. The method of adherence chromatography on glass beads was again used and different experimental conditions (temperature and composition of media) were tested.

MATERIALS AND METHODS

Solutions

The phosphate-balanced solution (PBS) used for washing the beads consisted of 8 g of NaCl, 0.2 g of KCl, 2.89 g of $Na_2HPO_4 \cdot 12H_2O$, 0.2 g of KH_2PO_4, 100 000

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I.U. of penicillin, 62.5 mg of streptomycin, and distilled water to 1000 ml. For the chromatographic elution of cells, we used a PBSC solution prepared by the addition of one part of calf serum (Sevac, Prague, Czechoslovakia) to four parts of PBS.

Standard EDTA solutions were prepared by adding EDTA to PBSC to give final concentrations of 1.5, 2, 3 and 4 m*M* of EDTA. All solutions were adjusted to pH 7.2 with 0.1 *N* sodium hydroxide.

Mouse spleen cells were obtained from the spleens of a group of four H mice (male) aged 2–3 months and freshly killed by cervical dislocation. The spleens were washed with sterile saline (0.9% NaCl) and then teased with forceps in PBSC solution. The crude cell suspension was filtered through a fine-mesh polyamide sieve, centrifuged for 5 min at 600 *g*, re-suspended in PBSC and centrifuged again. The cells were then suspended in the same medium to give a final concentration of $75 \cdot 10^6$/ml nucleated cells; the number of erythrocytes was about $45 \cdot 10^6$/ml. The cells were immediately used for chromatography.

Chromatography

A slurry of 18 ml of glass beads[9] (0.2 mm diameter, batch No. 16, Jablonec Glass Works, n.p., Czechoslovakia), pre-treated separately with 65% nitric acid at 95° for 90 min and rinsed repeatedly with distilled water[9], was placed in a jacketed chromatographic column (30 × 1.3 cm) connected to a Hoeppler ultrathermostat.

The pre-treatment of the glass beads and the columns before adherence chromatography is already reported[9]. However, for equilibration of the column we used the more practical and standard PBS and PBSC instead of saline and BEM solutions. A 1-ml volume of cell suspension containing $75 \cdot 10^6$ cells was applied and soaked in the column, then PBSC was applied in two 1-ml portions. After standing for 30 min at the given temperature, the cells were eluted with 40 ml of PBSC (in 3-ml portions). The flow-rate of 0.5 $ml \cdot cm^{-1} \cdot min^{-1}$ after three fractions was increased to 1 $ml \cdot cm^{-1} \cdot min^{-1}$ and was maintained at this level until the end of the chromatography. The elution by PBSC was followed by the elution of PBSC containing 1.5, 2, 3 and 4 m*M* EDTA. The methods of application and turbidity measurement of the fractions have been described earlier[9]. After turbidity measurement, the eluates were combined in five fractions, which corresponded approximately to the different EDTA concentrations. A portion of these five fractions (after the removal of EDTA by double centrifugation and double washing with PBSC) was used for the determination of colony-forming cells. The results of these biological experiments will be published elsewhere.[11]

The combined fractions were also used for the determination of the number of cells and their morphological evaluation. A mixture of one drop of the concentrated cell suspension from each fraction and one drop of calf serum was used on a hematological smear; after overnight drying in air, the smear was stained by the May–Grünwald and Giemsa–Romanowski techniques. Differential counts were made by counting 200 cells in each smear. The viability of cells was tested by trypan blue exclusion.

RESULTS AND DISCUSSION

In order to confirm the specific effect of EDTA on the elution of adhered cells,

we made two parallel runs with the same cell suspension on two columns. Cells from the first column were eluted as usual with PBSC containing an increasing concentration of EDTA while cells from the second column were eluted with PBSC in the absence of EDTA. This solution was applied in volumes equal to those of the solutions containing EDTA.

Fig. 1 shows the elution patterns of both chromatographic runs. The elution curves are markedly different. The two highest peaks obtained with PBSC containing 1.5 m*M* and 3 m*M* EDTA were not eluted by PBSC in the absence of EDTA. The numbers of cells eluted during these two separations were also different. In the absence of EDTA, only about 25% of the applied cells was eluted, while PBSC containing EDTA eluted about 60% of the applied cells. It is evident that the stepwise loosening of cells adhering to the glass is significantly dependent on the EDTA concentration and not on non-specific mechanical stirring, etc.

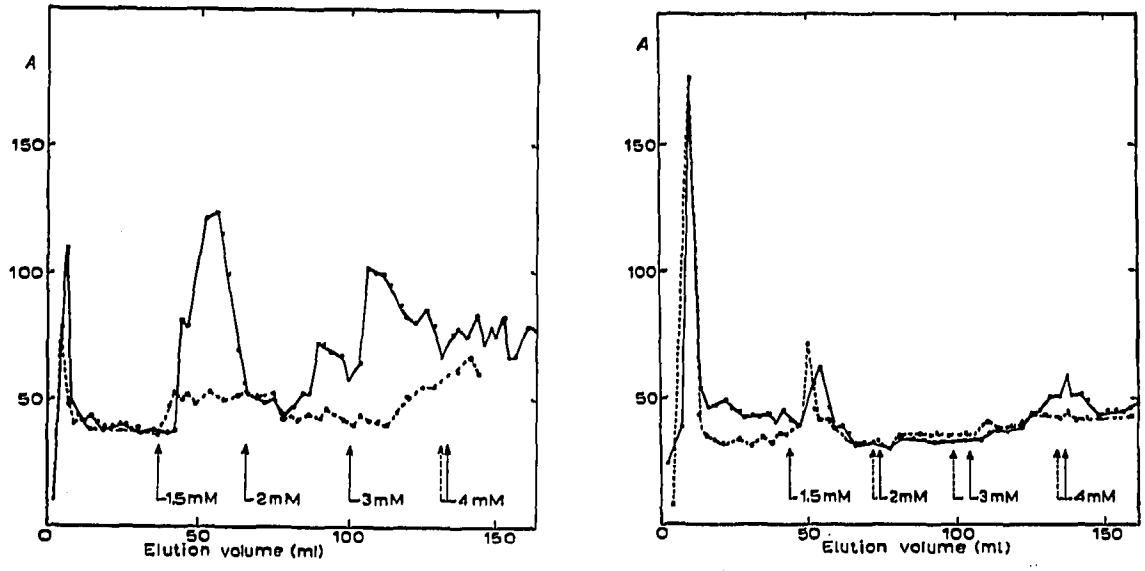

Fig. 1. Adherence chromatography of mouse spleen cells at 25°. Comparison of two elution curves obtained by two parallel runs of the same cell suspension on two columns. One column was eluted with PBSC (broken line), the other with PBSC containing increasing concentrations of EDTA (solid line). Both solutions were applied in equal volumes. The applications and concentrations (m*M*) of EDTA are indicated by arrows.

Fig. 2. Adherence chromatography of mouse spleen cells at 25°. Elution with PBSC and PBSC containing increasing concentrations of EDTA. The applications and concentrations (m*M*) of EDTA are indicated by arrows.

The chromatographic behaviour of mouse spleen cells was followed at two different temperatures. The suspension of cells from mouse spleen was separated on glass beads at 25°; typical elution curves are shown in Fig. 2. These curves were obtained by parallel runs of identical cell suspensions on two identical columns. At the beginning of the elution with PBSC a high peak was always formed that contained approximately 50% of the total recovered cells, and about 30% of the cells in

this fraction were erythrocytes. The second peak, formed after the addition of PBSC containing 1.5 m*M* EDTA, contained about 20% of the total recovered cells. In this and in all of the following fractions, most of the cells were small lymphocytes. The number of cells increased slightly after the elution with PBSC containing 2 or 3 m*M* EDTA, while the addition of 4 m*M* EDTA appeared to be without any substantial effect on the number of eluted cells. The final peak consisted mainly of granulocytes and monocytes, *i.e.*, cells that adhered easily to the glass surface. In general, after chromatography 60% of the total number of applied mouse spleen cells were recovered.

Chromatographic fractionation of mouse spleen cells at 37° was generally similar to that at 25°. Fig. 3 shows typical elution curves obtained at 37°. The elution with PBSC in the absence of EDTA again loosened 50% of all of the eluted cells, mainly small and large lymphocytes corresponding to the second peak. However, in contrast to the fractionation at 25°, PBSC containing 4 m*M* EDTA at 37° eluted a separate fraction with a small number of cells, predominantly polymorphonuclear leucocytes.

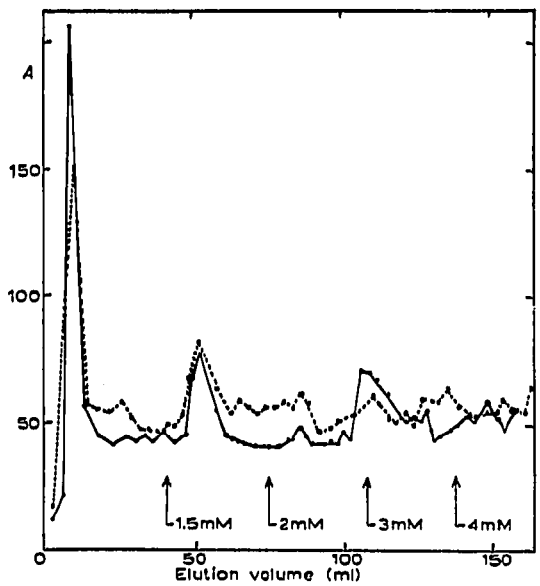

Fig. 3. Adherence chromatography of mouse spleen cells at 37°. Elution of the same cell suspension on two columns with PBSC and PBSC containing increasing concentrations of EDTA. The applications and concentrations (m*M*) of EDTA are indicated by arrows.

The total number of cells eluted at 25° was approximately 10% higher than that at 37°, which is in agreement with other work[10].

The eluates corresponding approximately to the given EDTA concentrations were combined into five fractions, and the number and differential counts of cells in those fractions were determined. Table I shows the relative number of cells eluted at different EDTA concentrations and also the cell types in individual fractions. The viability of the cells did not show any significant changes.

The eluates of mouse spleen cells chromatographed with PBSC containing an

TABLE I

NUMBER OF CELLS IN ELUTED FRACTIONS

Fraction	*Percentage of cells ± mean square deviation*	*Mean differential cell count*			
		Erythrocytes	*Small lymphocytes*	*Large lymphocytes*	*Other cells*
I (No EDTA)	26.2 ± 3.28	46.8	41.9	5.25	16
II (1.5 m*M* EDTA)	16.3 ± 2.05	4.6	53.7	9.9	33.8
III (2 m*M* EDTA)	8.4 ± 1.06	7.4	62.1	8.4	22
IV (3 m*M* EDTA)	6.0 ± 0.61	4.5	61.4	6.5	27.8
V (4 m*M* EDTA)	3.9 ± 0.21	3.6	48.8	6.7	40.9
Retained in column	39.2				

increasing concentration of EDTA gave the elution curves shown in Fig. 2. However, the reproducibility of the elution curves in detail is not perfect (see the deviation in Table I), owing to the fact that the system under study consists of a complicated mixture of populations of living cells of different types and ages, a complicated medium, complicated phase systems, etc. The present experimental technique is obviously not yet adequate to deal with all of the major parameters. Better reproducibility was obtained only in parallel chromatographic runs on columns of identical dimensions and when the same cell suspension was used (see Fig. 2). A satisfactory mutual comparison of various elution patterns was possible only between the two first peaks, obtained by elution with PBSC and PBSC containing 1.5 m*M* EDTA.

The absorbance during the turbidity measurements on different cell fractions was not always satisfactorily related to the real cell count, although in separate determinations with one type of cells there was a linear relationship between turbidity and cell concentration. This discrepancy could be explained partly by the different optical densities of different cell types and partly by the possibility that the turbidity is increased by non-cell light-scattering particles.

The results of the morphological evaluation show that during chromatography on glass beads, mouse spleen cells, as rat bone marrow cells[9], were fractionated not only according to their morphological type but also according to their individual ability to adhere to glass at different EDTA concentrations. The separation of erythrocytes and their concentration into the first peak was most succesful. Erythrocytes were almost absent in the second and further fractions. Cells that were classified as small lymphocytes were present in all fractions.

In general, the elution profiles of mouse spleen cells have fewer peaks in comparison with the chromatographic behaviour of rat bone marrow cells[9]. This fact seems to reflect certain qualitative differences between both types of complicated cell populations. However, it is now necessary to develop and use more specific biological and biochemical tests in order to be able to distinguish the different functional properties of cells in different fractions. Until this is achieved, it is difficult to judge whether or not a given chromatographic fractionation is effective. Furthermore, it is also probable that the use of other carriers or other solutions could give better results. Experiments on this aspect are being carried out and the results will be published later.

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