Technical methods

Cell suspension culture for studying development of macrophages

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Although morphometric analysis is gaining an increasing place in clinical pathology,¹ no attention has so far been given to the use of this technique in relation to disorders of the maturation of mononuclear phagocytes. One of the reasons for this is the tendency of macrophages to adhere to foreign surfaces, thus losing the random orientation necessary for morphometry. To overcome this problem the following method was devised to study the development of mononuclear phagocytes in suspension culture.

Material and methods

Venous blood (40 ml) was collected and defibrinated with polystyrene granules under sterile conditions. An equal volume of 3% dextran 150 in 0.9% saline was added, and aliquots of suspension were carefully layered over twice their volume of lymphocyte separation medium (Flow Laboratories) and centrifuged for 30 minutes at 400 g at a temperature between 18°C and 20°C. The layer of mononuclear cells at the interface of the plasma and separation medium was aspirated and transferred to a centrifuge tube. It was washed twice with three times its volume of Hanks's balanced salt solution (Flow Laboratories) and centrifuged at 100 g for 10 minutes. The cells were then resuspended in minimum essential medium Eagle for suspension cultures (Flow Laboratories) containing 2 g sodium bicarbonate/l. Ten millilitres of 200 mM L-glutamine, 10 mg gentamicin/l, and 10% by volume of autologous serum inactivated by heat were added to give a concentration of between 1 and 2×10^6 cells/ml. Aliquots of cell suspension (2-5 ml) were transferred to sterile 13 ml polypropylene tubes (Sarstedt) and gassed with 5% carbon dioxide in air. The tubes were continuously rotated (to prevent adherence) at six cycles/minute at 37°C, the medium being changed every two days.

At the end of the culture period cell viability was assessed using trypan blue exclusion. The cells were

then washed with sterile isotonic saline, pelleted into beam capsules, fixed with 1.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for one hour, and washed with 7% sucrose in 0.1M cacodylate buffer. The pellet was removed from the tube, cut into 1 mm³ blocks, washed with buffer, and postfixed in 2% unbuffered osmium tetroxide at 4°C for one hour. The specimens were then further processed for electron microscopy by standard methods.²

Results

Specimens examined on days 0, 1, 2, 4, and 6 showed greater than 90% cell viability. A mixed population of mononuclear phagocytes and lymphocytes was present. During the early intervals the mononuclear phagocytes had the morphological appearance of monocytes, while in the later intervals cells with the characteristic features of macrophages (Figure) were predominant. Lymphocytes were readily distinguishable from mononuclear phagocytes and showed no ultrastructural evidence of transformation. There was no evidence of loss of mononuclear phagocytes during culture. A test suspension initially containing 2.3×10^6 monocytes was estimated at days 1, 4, and 6 to contain 2.4, 2.9, and 2.8×10^6 macrophages, respectively. Subsequent microscopic examination of the walls of the containers showed no adherent cells.

Typical macrophage in culture (six days) from normal subject, illustrating suitability for morphometric analysis. Nuclear profiles, mitochondria, Golgi apparatus, phagosomes, and lysosomal granules can be clearly identified. Original magnification $\times 8600$.

Discussion

The preparations obtained by this method present the cells in random orientation, so that the profiles seen on electron microscopy fulfil the first criterion for morphometric analysis; quantitative studies can therefore be made of morphological changes that take place during the maturation of monocytes into macrophages. Reports have indicated that mononuclear phagocyte maturation is disordered in patients with malignant disease,³ and a morphometric analysis is required to define ultrastructural changes. Our method could be adapted for study of the specific effects of soluble factors, and, therefore, the presence of lymphocytes in the culture would need to be considered. Cytochemical and functional assays could also be carried out on the cultured macrophages, lymphocytes being removed if necessary by density gradient centrifugation.

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References

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Donor screening for antibody to hepatitis B surface antigen

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The acute shortage of hepatitis B surface antigen (HBsAg) immunoglobulin has prompted an investigation into the most practical methods of screening blood donors for the presence of antibody suitable

for harvesting. Several methods were explored in a preliminary study of 1203 random donor sera.

Agarose diffusion¹ is inexpensive (about 1 p per test) and easy to perform but for our purposes slow, not quantitative, and too insensitive (minimum level detected: about 20 IU/cm³). Reverse radioimmunoassay² requires overnight incubation if consistent results are to be achieved, and although it is sensitive (minimum about 1 IU/cm³), it is too expensive for large scale screening (23p per test). A commercial haemagglutination test (Serodia) is sensitive (minimum <1.0 IU/cm³) and easy to perform, but, at over 30p per test, it too is uneconomical.

Miniaturised haemagglutination techniques in Terasaki plates have been well documented^{3,4} and have been in use in this centre for some years. A further modification has now been evolved that is simple to perform, gives easily read results within 30 minutes, is inexpensive at 3p per test, like the original commercial method, and can also detect <1.0 IU/cm³ and be used for measurement.

Material and methods

The Serodia commercial passive haemagglutination kit was used (Diamed Diagnostics, Bootle, Merseyside), which uses fixed chicken cells coated with purified inactivated HBsAg. The freeze dried cells were reconstituted in 0.5 cm³ kit buffer as recommended by the manufacturer, then diluted with a further 0.5 cm³ kit buffer.

Test sera were diluted 1/40 in phosphate buffered saline in a World Health Organisation Tendix plate (Appleton-Woods, Birmingham, England) using a Compupet (General Diagnostics, Eastleigh, Hants, England), and then 5 µl of each diluted serum was transferred to a well of a Terasaki plate.

Diluted test cells (5 µl) were added to each well using a Hamilton multidispenser (Pierce and Warriner, Chester, Cheshire). The plates were centrifuged for 60 seconds at 200 g in a Beckman TJ-6 centrifuge. They were then placed at room temperature on a light box angled at 45° and left for 10 minutes before reading.

A positive control (23 IU/cm³ obtained from Blood Products Laboratory, Elstree, Herts, England) was titrated with test and control cells in the range 1/40–1/20480, and a 1/10 negative control was put up without titration.

A positive test is characterised by a clear disc of cells at the bottom of the well, a negative test by a discrete crescent. Differentiation was very clear. After the preliminary study a further 4165 random sera were screened by this method alone.