Shape, Volume and Integrity of Reconstituted Human Red Cell Ghosts

Human red cell ghosts prepared by hypotonic lysis and reconstituted to recover cell membrane semipermeability ('resealed ghosts') have been used extensively in investigations of membrane transport phenomena. In principle this technique permits the preparation of cells with any desired intracellular solute concentrations. Some important differences in shape and volume between intact cells and ghosts have been described[1-4]. This communication investigates in more detail the volume distribution of ghosts, and their degree of resealing and membrane integrity.

Reconstituted ghosts of human red cells were prepared by a modification of the method of HOFFMAN, TOSTESON and WHITTAM[5,6], their volume distribution was obtained in detail by electronic cell counting, and their morphology and surface characteristics examined by scanning electron microscopy.

Acid-citrate-dextrose stored, washed human red cells were lysed in 3 m*M* Na_3-ATP, 3 m*M* $MgCl_2$, 1 m*M* *Tris* (pH 7.4); when counted electronically as described below, 90% of the particles in the hemolysate were below the lower limit of the size range of intact cells. The cells were reconstituted by adjusting the hemolysate to a final concentration of 140 m*M* NaCl, 10 m*M* CsCl and incubating for 30 min at 37 °C. The cells were then washed twice in an isotonic choline chloride medium[6] and resuspended to the original intact cell concentration. Recovery of ghosts, measured by electronic cell counting, was greater than 95%. Cs^+ estimation by flame photometry was used to determine the fractional resealed volume in packed ghosts.

Washed ghosts were diluted 1:10,000 in 154 m*M* NaCl, 5 m*M* *Tris* (pH 7.4) for counting with a model 'B' Coulter counter fitted with 50 μ diameter aperture tube. The instrument was calibrated to give 1 threshold unit = 1.992 μ^3, and counts were made at threshold settings of 1 unit width so as to yield a high resolution of particle volume distribution. Washed intact cells were counted in the same manner. Figure 1 is a typical volume distribution pattern of ghosts immediately after the second wash, along with the pattern for the intact cells from which these ghosts were prepared. The majority of the ghosts had volumes less than the modal volume, and the volume distribution was very diffuse, in contrast to intact cells which showed a single mode with some skewness towards higher volumes[4].

Cs^+ analysis indicated a resealed compartment typically equivalent to 40% of the total packed ghost cell volume (36% for the preparation in Figure 1). This value cannot be used to estimate the number of cells that were resealed since it was not known if there was any correlation between resealing and ghost cell volume.

Cells for scanning electron microscopy were fixed at 20 °C for 15 min in 0.5% v/v glutaraldehyde made isosmotic by addition of sodium phosphate buffer (pH 7.4). The fixed cells were washed by dilution in distilled water and after gold-palladium sputtering were examined in the Cambridge Stereoscan electron microscope.

The scanning electron microscope showed the ghosts to vary in shape, and all ghosts to possess some degree of surface irregularity. Most ghosts were of a shallow discoid shape and Figure 2 shows a typical cell with some surface detail. The membrane is pitted overall and has several relatively large indentations. The ghost membrane is clearly unlike the smooth cell membrane seen in intact erythrocytes by the scanning technique[7,8] or ghost membranes prepared by the technique of gradual osmotic lysis and examined by transmission electron microscopy[9]. It also differs from freeze-cleaved red cell ghosts[10,11] in not demonstrating any granular surface particles.

The results reported here emphazise the heterogeneous nature of reconstituted ghost preparations and the failure of the membranes of such ghosts to regain the consistency of intact cell membranes, although many studies of ion movements using these ghost preparations have confirmed that the transport system regains its functional integrity

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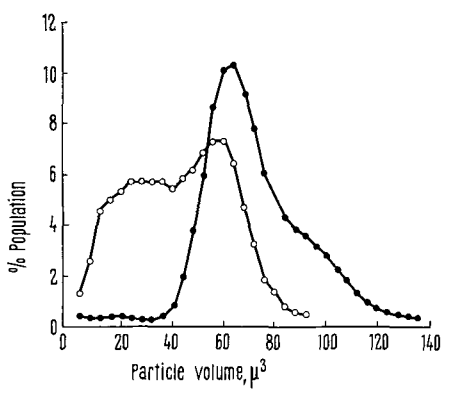

Fig. 1. Volume distribution patterns of intact red cells (●—●) and washed, reconstituted ghosts (○—○).

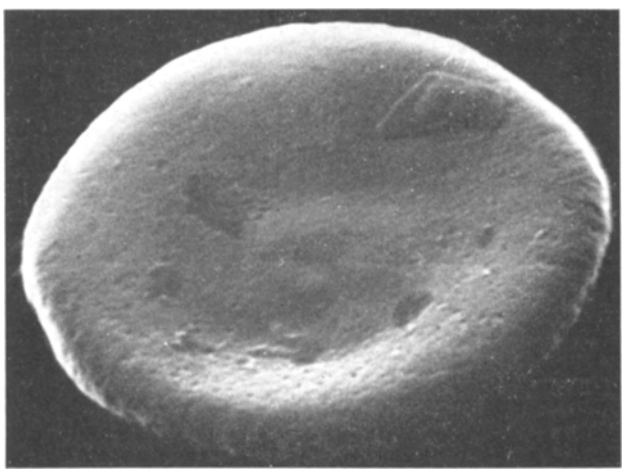

Fig. 2. Scanning electron micrograph of a typical reconstituted ghost (× 13,200).

in at least some of the cells. Although the cells gave a fractional resealed volume of only 40%, no obvious differences in morphology could be observed to separate the cells into 2 populations, and all cells showed some surface irregularities[12].

Resumen. El contenido de cationes en una preparación de eritrocitos resellados, usado como criterio de resellado, dió un volumen parcial de 40%. La morfologia de la superficie de dicha preparación ha sido investigada mediante el microscopio electrónico de barrido. Todas las células presentaban irregularidades en su superficie. La distribución del volumen de células reselladas medida en el contador electrónico de células es diferente de la de celulas intactas, pero las diferencias morfológicas observadas no eran suficientes para distinguir entre células reselladas y permeables.

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[12] We thank Cambridge Scientific Instruments, Ltd., for scanning electron microscope facilities.

Suppressed Multiplication of Pulmonary Tubercle Bacilli in Bacillary Trypsin Extract-Immunized Mice

Previous evidence that bacillus-free, water-soluble extracts of trypsin-digested tubercle bacilli will immunize mice and guinea-pigs specifically against experimental tuberculosis[1-3]. has been indirect. Herein we present direct confirmation of this.

Materials and methods. Preparation of our immunogen has been detailed previously[1,4]. CF-1 female mice used in groups of 30 were injected s.c. on days 0 and 7 with 0.1 ml volumes of water-in-oil emulsion[5], containing either no antigen (nonimmune control group) or containing 0.25 mg quantities of trypsin extract (test group) or acetone-killed bacilli (immune control group; cf. ref. [5]. On day 35 each mouse was infected i.v. with 0.2 ml of a second subculture on Middlebrook 7H9 medium of human-type tubercle bacilli, maintained in stock culture on Kirschner medium. Lungs were removed from 3 mice of each group at various intervals after infection, homogenized aseptically in 10 ml of 7H9 medium, and plated by the drop technique[6] in serial 10-fold dilutions in duplicate on Middlebrook 7H10 medium. Colonies were counted after 4 weeks' incubation at 37 °C and results are reported here in mean total culturable tubercle bacilli per set of lungs.

Results. Our data are summarized in the Figure. Challenge infections for these 3 experiments read 45 (on the Klett-Sumerson colorimeter), 12, and 45, respectively. H37Rv tubercle bacilli were used for the first 2 experiments and Erdman for the third, constituting severe, moderate, and very severe challenges, respectively.

As seen, immunization with either trypsin extract or acetone-killed bacilli significantly suppressed pulmonary tubercle bacillus multiplication. This bacteriostatic immunity was more obvious against heavy than against moderate challenge, and it did not appear until after the 12th day of infection.

Discussion. These experiments indicate that the lungs of trypsin extract-immunized mice suppress bacillary multiplication just as has been reported for the lungs of BCG-immunized mice[7,8], including even the puzzling but well-confirmed[7], commonly described delay in the appearance of bacteriostasis. Reasons for this 12-day delay are unclear but may relate to the use of Tween-80 in challenge bacillus culture medium. It is absent when Tween is not used[9-11] and thus may not be associated with time required for multiplication of challenge bacilli to a postulated level sufficient to evoke local immunity[12]. The delay also is avoided when Tween-cultured bacilli are used for challenge if Tween-grown immunizing bacilli are delivered directly to mouse lungs by aerosol or i.v. injection[13]. Perhaps Tween-associated tubercle bacilli have an immunogenic specificity of their own. This could explain why Ribi et al.[14] found that in contrast to Ribi's 'oil disruption product' and to BCG, Youmans' 'particulate fraction'[15] immunized mice poorly against either i.v. or airborne challenge; because in these experiments both immunizing and challenging bacilli and the oil disruption product were at one time or another in their use exposed to Tween, whereas the particulate fraction was not.

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