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Metabolic and Structural Requirements for Concanavalin A Capping in Phagocytic Cells (40613)[1]

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The distribution of concanavalin A (Con A) receptors on the surface membrane of polymorphonuclear leukocytes is dependent upon the state of microtubule and microfilament assembly (1). Binding of fluorescein isothiocyanate–Con A (FITC–Con A) to its surface receptors induces polymerization of cytoplasmic tubulin into microtubules in polymorphonuclear leukocytes. In turn, lectin-treated polymorphonuclear leukocytes display a uniform distribution of FITC–Con A (2). Colchicine inhibits tubulin polymerization and stimulates lateral movement of Con A receptors into surface caps (2). Capping can be prevented in polymorphonuclear leukocytes by exposing the cells to cytochalasin B, a fungal metabolite known to fragment actin-microfilaments (3). In contrast to polymorphonuclear leukocytes, however, FITC–Con A capping is promoted by cytochalasin B even in the absence of colchicine in alveolar macrophages (4). The difference in capping response to cytochalasin B between polymorphonuclear leukocytes and alveolar macrophages may reflect dissimilarities in the major metabolic pathways employed by each cell to obtain energy, distinctive isoactin forms between each cell, or varied actin-microfilament topographical relationships in the cytoskeleton of the cell. In this study, we report the effect of cytochalasin B and metabolic inhibits on FITC-Con A capping guinea pig polymorphonuclear leukocytes, alveolar macrophages, and peritoneal monocytes. We also identify and compare isoactin forms from each cell type.

Materials and Methods. Polymorphonuclear leukocytes and peritoneal monocytes were obtained from male albino guinea pigs 18–72 hr after intraperitoneal injection of 12 and 1.2% sodium caseinate, respectively (5). Alveolar macrophages were harvested by bronchopulmonary lavage of guinea pigs not receiving caseinate (6). Cells were washed three times and suspended in iced Krebs–Ringer phosphate buffer, pH 7.4. Cell purity was 95% as determined by Wrights and monocyte-specific α-naphthyl esterase stains (7). Cell viability was 98–100% as determined by exclusion of 0.1% trypan blue. The capacity of lectin receptors to move into polar caps on the cell membrane was determined by observing the distribution of FITC–Con A as previously described (4). Cells were incubated 15 min at 37° with or without various drugs, stained 5 min with 50 μg FITC–Con A, and fixed in 2% formaldehyde. After pelleting, the percentage of capped cells was determined. The energy requirement for the capping response to colchicine was determined by preincubating cells for 45 min with either 1 mM potassium cyanide or 5 mM 2-deoxyglucose prior to addition of colchicine. For determination of isoactin forms, whole cell homogenates of 1×10^7 cells were prepared by homogenation in a Dounce homogenizer (8). To isolate the cellular actin, the homogenates were clarified by centrifugation at 1000g and the supernatant was made 75 mM in potassium chloride and stirred at room temperature for 1.5 hr. The solution was centrifuged at 80,000g for 3 hr (4°), and the resulting pellet was dissolved in a glass tissue grinder with a small volume of a solu-

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tion containing 0.2 m*M* adenosine triphosphate, 10 m*M* dithiothreitol, 0.2 m*M* calcium chloride, 2 m*M* Tris–HCl, pH 7.0, and was dialyzed against that buffer for 3 days. The dialysate was centrifuged at 80,000*g* for 3 hr and the supernatant was applied to DNase affinity column (9). The peak fractions from the affinity column were pooled, desalted, and dialyzed against distilled water for 18 hr. The samples were lyophilized and redissolved in 2% sodium dodecyl sulfate which was displaced with excess Triton X-100 (10). Isoelectric focusing in polyacrylamide gels containing urea and Triton X-100 was performed according to the method of O'Farrell (11), except that the gel lengths were increased to 13 cm and the focusing increased to 9000–10,000 V-hr in order to obtain better resolution of the actin forms.

Results. Figure 1 shows the response of each cell type to various drugs. Treatment of cells with colchicine, a drug known to inhibit the assembly of microtubules, induced the formation of Con A caps in all cell types tested. However, the response of peritoneal monocytes to cytochalasin B as well as to cytochalasin B and colchicine was similar to that of the polymorphonuclear leukocytes. As previously reported, cytochalasin B induced Con A capping in alveolar macrophages, while, as seen in Fig. 1, cytochalasin B prevented colchicine-induced Con A capping in both the polymorphonuclear leukocytes and peritoneal monocytes. In an effort to implicate differing isoactin forms in the observed disparity of the capping response to cytochalasin B, we isoelectrophoresed homogenates from each cell type. As noted in Fig. 2, the isoelectric focusing patterns of peritoneal monocytes and alveolar macrophages were similar. In particular, two bands corresponding to β- and γ-actin were present in both cell types. Similarly, while the isoelectric focusing pattern of the polymorphonuclear leukocyte homogenate was clearly distinct from those of alveolar macrophages and peritoneal monocytes, these cells also contained both cytoplasmic actins (β and γ). As observed in other nonmuscle cells, β-actin comprises the majority of the total actin and none of the skeletal muscle actin form (α) was present in any of the three cell types.

The difference in energy metabolism among the three phagocytic cell types suggested a possible link between the varied response to cytochalasin B and the major metabolic source of energy in each cell type. Indeed, as seen in Fig. 3, the energy required for colchicine-induced capping differed among polymorphonuclear leukocytes, peritoneal monocytes, and alveolar macrophages. In both polymorphonuclear leukocytes and

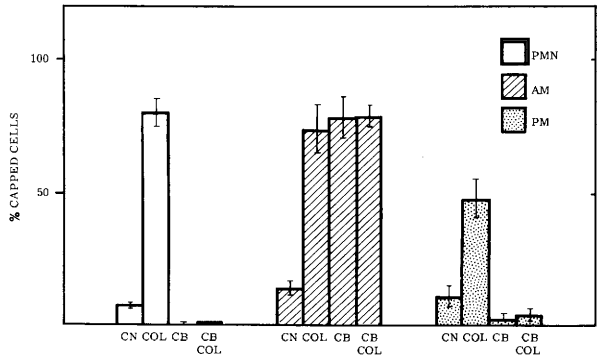

FIG. 1. Effect of colchicine and cytochalasin B on Con A capping in polymorphonuclear leukocytes (PMN), peritoneal monocytes (PM), and alveolar macrophages (AM). Polymorphonuclear leukocytes, peritoneal monocytes, and alveolar macrophages (2×10^6 cells) were preincubated at 37° in 1 ml of Krebs–Ringer phosphate buffer with no drug control (CN), 10^{-5} *M* colchicine (COL), 10 μg/ml cytochalasin B (CB), or cytochalasin B for 10 min followed by the addition of colchicine (CB, COL). After 15 min, the cells were stained with FITC–Con A (50 μg) at 37°C for 5 min, then fixed in 2% formaldehyde for 10 min at 28°. The cells were washed and then pelleted and slides were made from the cell button. The results were obtained by examining at least 200 cells per slide at random with a Zeiss fluorescence microscope (490-nm wide-band interference primary filter, 530-nm secondary filter) and classifying each cell as capped or not capped. Values represented mean ±SD of six separate experiments.

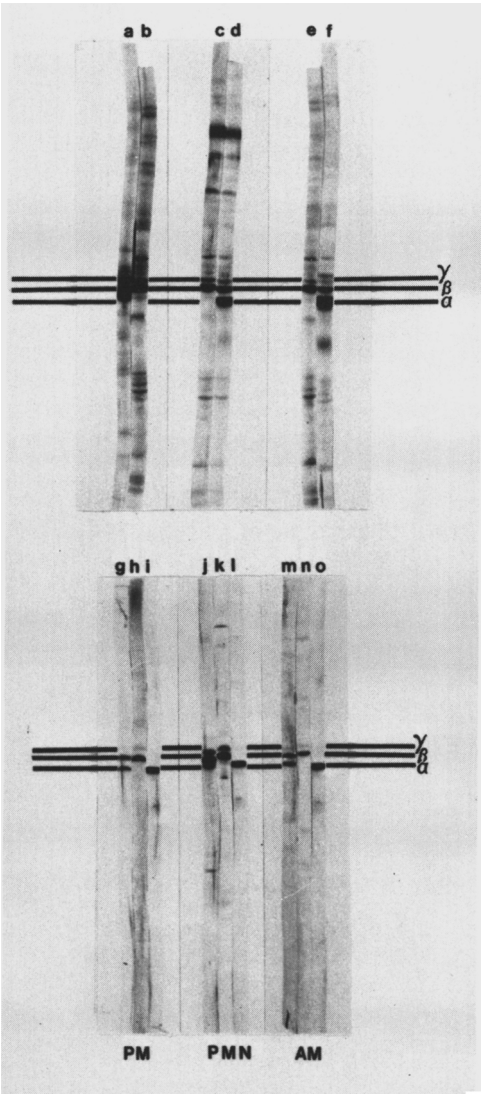

FIG. 2. Isoelectric focusing patterns of total homogenates and DNase purified actins from PMN, PM, and AM. In gels b, c, and e, 30 μg of a SDS-solubilized homogenate from each of the cells indicated was applied to polyacrylamide gels (length 13 cm) containing urea and Triton X-100 with focusing carried out 9000–10,000 V-hr. In gels a, d, and f, 10 μg of murine skeletal muscle actin (α-actin) was mixed as an internal standard with the various homogenates. Gels h, k, and n contain DNase purified actin (5–7 μg) from each cell type as indicated, while 3 μg of murine skeletal muscle actin was included in gels g, j, and m. Gels i, l, and o each contain 3 μg of skeletal muscle actin alone.

peritoneal monocytes, colchicine-induced capping was significantly attenuated by preincubation with 2-deoxyglucose, a glycolytic inhibitor, while cyanide, an inhibitor of oxidative phosphorylation, had only minimal effect on these two cells which are both dependent upon glycolysis for their major source of energy. In contrast, cyanide completely abolished colchicine-induced capping in the alveolar macrophage, a cell dependent upon oxidative phosphorylation for energy, while 2-deoxyglucose had a much less dramatic response in these cells.

Discussion. The major findings in this study were that the primary metabolic pathway employed for energy production by each cell line correlated with the response of the respective phagocytic cell to cytochalasin B-induced capping. On the other hand, there was no difference observed in the isoactin forms seen in homogenates from each cell type. Thus, by these criteria, the results suggest that the observed differences in capping properties among the cells were not due to qualitative variation among the actin forms; rather the capping response in the alveolar macrophage may depend upon a unique topographical relationship of microfilaments to the membrane (4) which may be amenable to perturbation by altering the metabolism of the phagocytic cells as has been seen in virally infected or transformed cells (12, 13). The biological basis of capping in cells of various types is not known, but differences in this parameter such as those shown here may ultimately be related to cell differentiation and metabolism.

Summary. Colchicine induced Con A capping in polymorphonuclear leukocytes, peritoneal monocytes, and alveolar macrophages, which was prevented in the polymorphonuclear leukocyte and peritoneal monocyte by cytochalasin B. Isoelectrophoresis of whole cell homogenates and purified cytoplasmic actin revealed β- and γ-actin to be present in similar amounts in all three cell types. Alveolar macrophages failed to cap in the presence of 1 m*M* potassium chloride whereas polymorphonuclear leukocytes and peritoneal monocytes maintained the capping response. In contrast, alveolar macrophages continued to cap during treatment with 5 m*M* 2-deoxyglucose abeit at lower levels than control; whereas in polymorphonuclear leukocytes and peritoneal monocytes, capping was substantially attenuated. These studies demonstrate the differing metabolic requirements

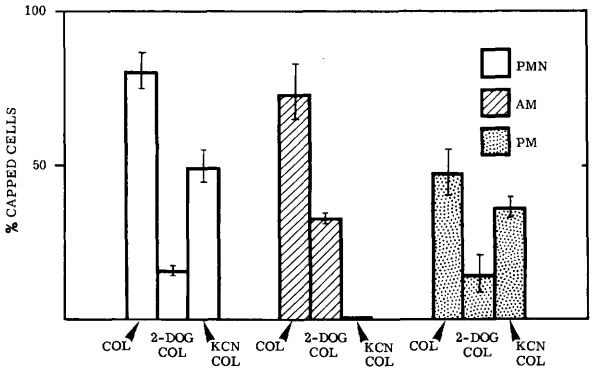

FIG. 3. Effect of metabolic inhibitors on colchicine-induced Con A capping in PMN, PM, and AM. Cells were preincubated for 45 min in either 1 m*M* potassium cyanide (KCN) or 5 m*M* 2-deoxyglucose (2-DOG) before addition of 10^{-5} *M* colchicine. After 15 min, cells were treated as in Fig. 1. All values represented mean ± SD of three separate experiments.

for capping in phagocytic cells and suggest that different topographical relationships of actin-microfilaments to the plasma membrane might exist in the alveolar macrophages compared to the phagocytic cells dependent on anaerobic metabolism.

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