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Positively charged polymer polylysine-induced cell adhesion molecule redistribution in K562 cells

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We have recently demonstrated that if human K562 erythroleukemic cells, which normally grow in suspension, are grown in polylysine-coated culture flasks for 48 h, they adhere to these flasks and grow in an anchorage-dependent like manner. Important changes in both membrane conductivity (ionic transport across the cell membrane) and membrane permittivity (static distribution of charges across the cell membrane) were also observed, indicating perturbations in membrane lipids, proteins and polysaccharides. In order to better understand the changes occurring in K562 cells exposed to polylysine and because of the important role played by cell adhesion molecules (CAMs) in cell/cell and cell/substratum interactions, and in cellular adaptation to the surrounding environment, the possible redistribution of these molecules after exposure to polylysine were investigated. In particular, the CD54 (ICAM-1), CD58 (LFA-3) and $\alpha_V\beta_3$ (vitronectin receptor) molecules were investigated at different times of growth both quantitatively and qualitatively utilizing flow cytometry and immunofluorescence microscopy, respectively. The data indicate that there were no significant quantitative variations in the CAMs examined at all the times tested. In addition, no qualitative changes were observed at 48 h (as well as 24 h) of exposure. However, shorter treatment times (30 min, 1 and 2 h) did induce important CAM reorganization. The results seem to demonstrate that this cycle of CAM redistribution may, in part, be responsible for cellular adaptation to the new growth environment of K562 cells and for the variations in membrane electrical properties observed.

1. Introduction

One of the fundamental questions in biomaterials research is the effects that surface charge may have on cell structure and function and, consequently, in determining the biocompatibility of a specific material. Surface charge is particularly important, especially when considering that the cell membrane, also endowed with its own characteristic charge distribution, comes into direct contact with the biomaterial. Although much knowledge has been gained regarding biomaterials surfaces and cell membrane dynamics, the effects that cell/biomaterial surface charge interaction may have on cell structure and function as well as on the biocompatibility of the biomaterial itself, still need much further clarification.

Two major types of phenomena, both of which involve various types of forces (i.e. electrostatic and electrodynamic), may de distinguished to explain cell adhesion: specific interactions where ligand/receptors, enzyme/substrates, etc., are involved and allow very precise recognition and binding, and non-specific ones which do not depend upon complex charge specificity. Polylysine is a basic polyamino acid with a net positive charge. Because cells do not normally have specific receptors for this polyamino acid, it is believed that polylysine enhances adhesion by means of electrostatic interaction between anionic sites of plasma membrane components and cationic sites on its surface [1].

We have recently demonstrated that, if human K562 erythroleukemic cells, which normally grow in suspension, adhere and grow for 48 h in polylysine-coated culture flasks, important changes in both membrane conductivity (ionic transport across the cell membrane) and membrane permittivity (static distribution of charges across the cell membrane) are observed [2]. These changes in membrane electrical parameters were hypothesized to be a consequence of the new growth conditions induced by the polylysine polymer which result in a perturbation of the K562 cell membrane through reorganization of membrane lipids, proteins and polysaccharides.

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In order to attempt to understand the mechanisms which are at the basis of this membrane reorganization, the possible variations occurring in three important cell adhesion molecules (CAMs) after 48 h growth were investigated. In particular, CD54 (ICAM-1), CD58 (LFA-3) and $\alpha_V\beta_3$ (the vitronectin receptor) were investigated quantitatively and qualitatively by flow cytometry and immunofluorescence microscopy, respectively. It should be recalled that specific cell adhesion events do take place in cells through CAMs and that many of these proteins are present on the K562 cell membrane. In addition, it should also be emphasized that these molecules have charges associated with them and that these may interact with the positive charge of polylysine and thus bring about changes in the membrane of these cells. The data indicate that there were no significant quantitative variations in the CAMs examined at all the times tested. In addition, no qualitative changes were observed at 48 h (as well as 24 h) exposure. However, shorter treatment times (30 min, 1 and 2 h) did induce important CAM reorganization. Taken together, the results seem to indicate that the non-specific interaction between K562 cells and polylysine induces strong perturbations in the K562 cell membrane and these changes may be responsible for the variations in membrane conductivity and membrane permittivity observed at 48 h.

2. Materials and methods

2.1. Cells

K562 cells (a human erythroleukemic cell line which normally grows in suspension) were grown in RPMI 1640 supplemented with 10% foetal calf serum, non-essential amino acids, 100 IU ml^{-1} penicillin and 100 $\mu g\,ml^{-1}$ streptomycin and incubated at 37 °C in a 5% CO_2 atmosphere. Aliquots of the same batch of frozen cells were thawed and used for all experiments at about the same number of passages (between 15 and 18 passages) in order to make certain that no major variations in the cell populations had occurred between each trial.

2.2. Polylysine exposure

For control samples, cells were counted using a Coulter Counter (Coulter Electronics Ltd, Luton, UK) and seeded in Petri dishes at a concentration of 10^6 cells in 2 ml. For polylysine exposure, glass coverslips were washed and sonicated in ethanol, air dried, sterilized by autoclave, placed in sterile Petri dishes and coated overnight at room temperature with sterile 0.1 mg ml^{-1} poly-L-lysine (hydrobromide, lyophilized form; Sigma, St Louis, MO) dissolved in deionized water (pH 7.4). Coating was performed 24 h before use. Cells to be exposed to polylysine were counted by a Coulter Counter, seeded in the Petri dishes containing the polylysine-coated coverslips at a concentration of 10^6 cells in 2 ml and maintained for 30 min, 1, 2, 24 and 48 h at 37 °C in a 5% CO_2 atmosphere. It should be pointed out that within 10 min, K562 cells adhere to the coverslips and that these cells continue to grow in an anchorage dependent-like manner on these coverslips throughout the entire time period of exposure.

2.3. Flow cytometry

For flow cytometric determination of CD54 (ICAM-1), CD58 (LFA-3) and $\alpha_V\beta_3$ (vitronectin receptor) (Chemicon International, Inc., Temecula, CA), the cells were incubated for 30 min at 4 °C with monoclonal antibodies directed against these cell surface molecules. After washing with ice-cold phosphate-buffered saline (PBS) containing 10 mM NaN_3, 0.5% bovine serum albumin (BSA; Sigma) and 0.002% EDTA, cells were incubated for 30 min at 4 °C with F(ab′) fragment of goat anti-mouse IgG fluorescein-conjugate (Sigma) used at a working dilution of 1:50. After washing, cells were immediately analyzed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) equipped with a 15 mW, 488 nm, air-cooled argon ion laser. In order to discriminate between live and dead cells, propidium iodide (PI) was added to the cell suspension immediately before cytofluorimetric analysis. Fluorescence emissions were collected after passage through 530 and 570 nm band-pass filters for fluorescein or PI signals, respectively. Data were collected and analyzed on a Hewlett-Packard model 310 computer interfaced with the FACSan. Fluorescence data were collected on a four-decade log scale. For negative controls, cells were incubated with the second antibody only.

2.4. Immunofluorescence labeling

For cell surface labeling of CD54 (ICAM-1), CD58 (LFA-3) and $\alpha_V\beta_3$ (vitronectin receptor), K562 control cells were collected from the Petri dishes, centrifuged (1000*g* for 5 min) and the cells incubated for 40 min at 4 °C with 20 µl of a 1:50 dilution of each of the monoclonal antibodies. After washing with ice-cold PBS supplemented with 0.5% BSA, cells were incubated with 20 µl of a 1:100 dilution of anti-mouse IgG fluorescein-labeled antibody at 4 °C for 40 min. Samples were then resuspended in PBS containing 3.7% paraformaldehyde at 4 °C for 15 min. After adhesion on polylysine-coated coverslips for 10 min, samples were mounted with glycerol/PBS (1:1). Cells exposed to polylysine by direct growth on polylysine-coated coverslips for 30 min, 1, 2, 24 and 48 h were washed with ice-cold PBS and the coverslips with the adhered cells were directly incubated with the primary antibody and treated as described above. All samples were analyzed with a Nikon Microphot fluorescence microscope.

3. Results

In order to evaluate the possible quantitative variations in cell adhesion molecules of K562 cells induced by a 48 h exposure to polylysine, flow cytometric analyses were conducted. Specifically, CD54, CD58 and $\alpha_V\beta_3$ were analyzed. As can be seen in Fig. 1a, no important differences were apparent in the quantity of these molecules in treated cells with respect to controls

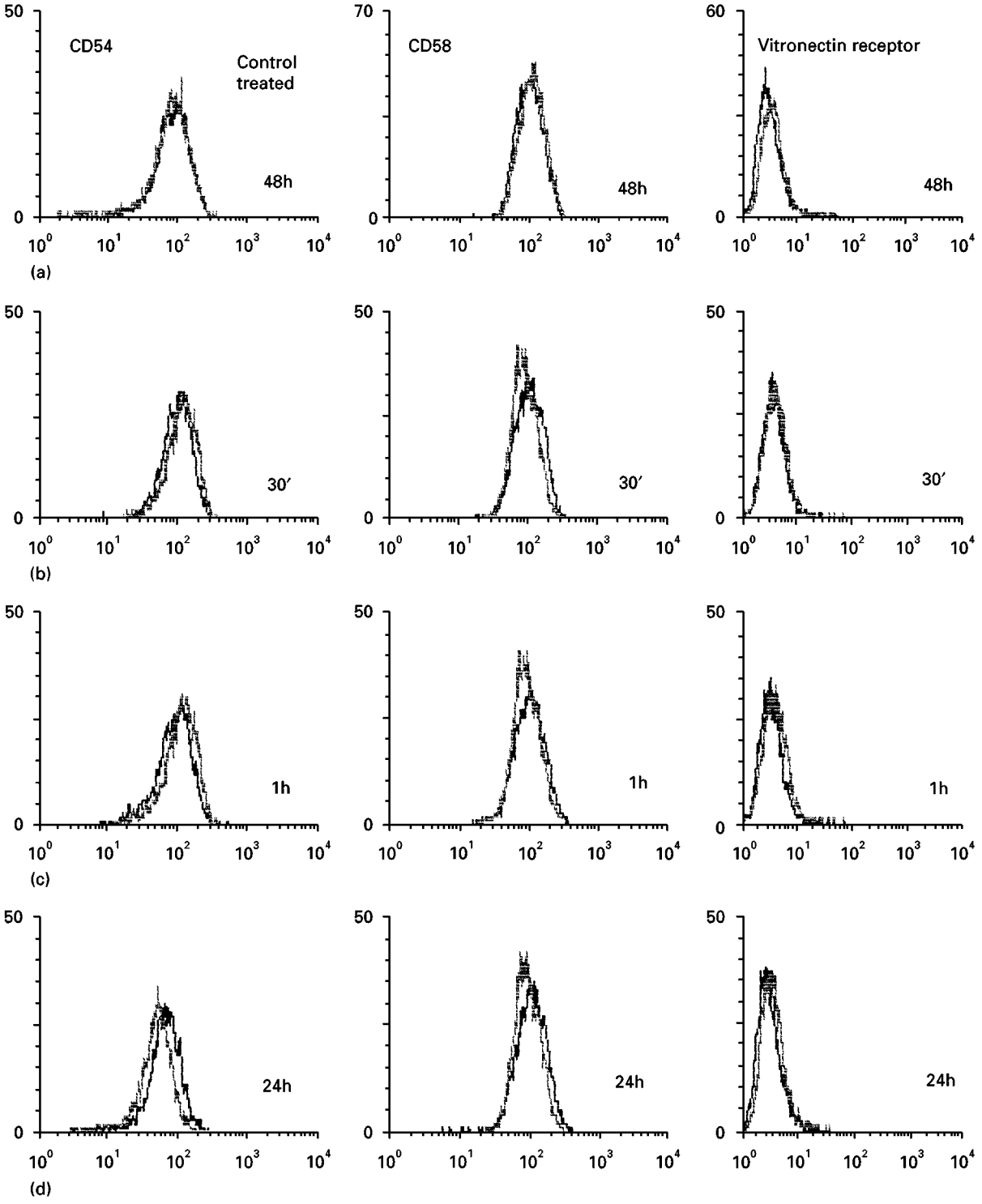

Figure 1 Flow cytometric quantitative analyses of CD54 (ICAM-1), CD58 (LFA-3) and $\alpha_V\beta_3$ (vitronectin receptor) cell adhesion molecules on control K562 cells and of these cells examined after (a) 48 h, (b) 30 min, (c) 1 h and (d) 24 h of adhesive growth on polylysine. The abscissa indicates the fluorescence intensity, and the ordinate the cell number. As can be seen, there is little variation in the expression of all three of these molecules at all the times tested.

at this time. In addition, in order to better examine the changes occurring at shorter exposure times, the quantity of the above CAMs were also evaluated at 30 min, 1, 2 and 24 h. As is evident in Fig. 1b–d respectively, no variations in CD54, CD58 and $\alpha_V\beta_3$ were observed. The 2 h time points are not shown because the results are nearly the same as those obtained at 1 h.

Although no variations in the amount of CAM expression were noted with flow cytometry, polylysine may, nonetheless, alter the distribution of these molecules on the K562 cell surface. In order to examine this eventual redistribution of CAMs induced by polylysine, immunofluorescence microscopy was conducted on control and treated erythroleukemic cells at the same exposure times as described above. As can be seen in Fig. 2, there is little rearrangement of CD54 in cells grown for 48 h on polylysine-coated culture flasks. In fact, although control cells (Fig. 2a) appear well-separated from each other while treated cells

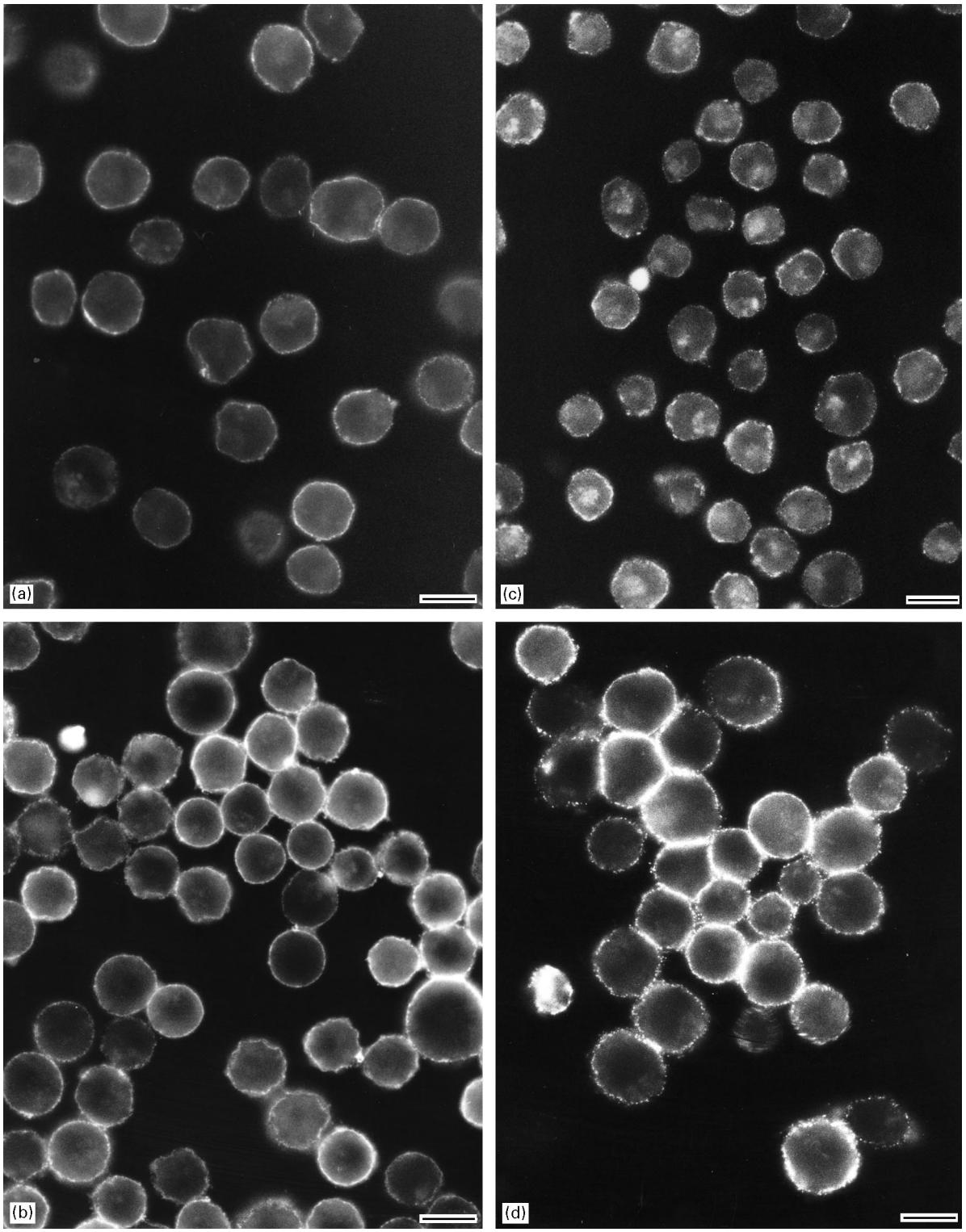

Figure 2 Immunofluorescence microscopy of control K562 cells and of these cells examined after 48 h adhesive growth on polylysine. Cells were labeled with antibodies directed against CD54 (a, b) and CD58 (c, d) cell adhesion molecules. In control cells (a, c) as well as in treated cells (b, d), both CD54 and CD58 molecules appear uniformly distributed on the cell surface, indicating that growth on polylysine of these cells does not induce any important variations in the distribution of these CAMs. The bars represent 10 μm.

(Fig. 2b) grow in clusters, there appears to be little redistribution of this molecule after 48 h growth. In particular, CD54 molecules appear as luminous points uniformly distributed on the cell surface (ring appearance) in both controls and treated cells. Similar results were also observed in the distribution of CD58 because this molecule also appears uniformly distributed in both controls (Fig. 2c) and in K562 cells grown for 48 h on polylysine (Fig. 2d). Uniform distribution was also observed at 24 h polylysine exposure (data not shown). Thus, it appears that 48 h growth of these cells on polylysine does not induce important variations in the distribution of these molecules. It should be noted that in these figures the fluorescence intensity appears greater in treated samples than in controls. However, this is due to the clustered growth of treated cells which are close to each other and, consequently, the light intensity is due to enforced and not to a real

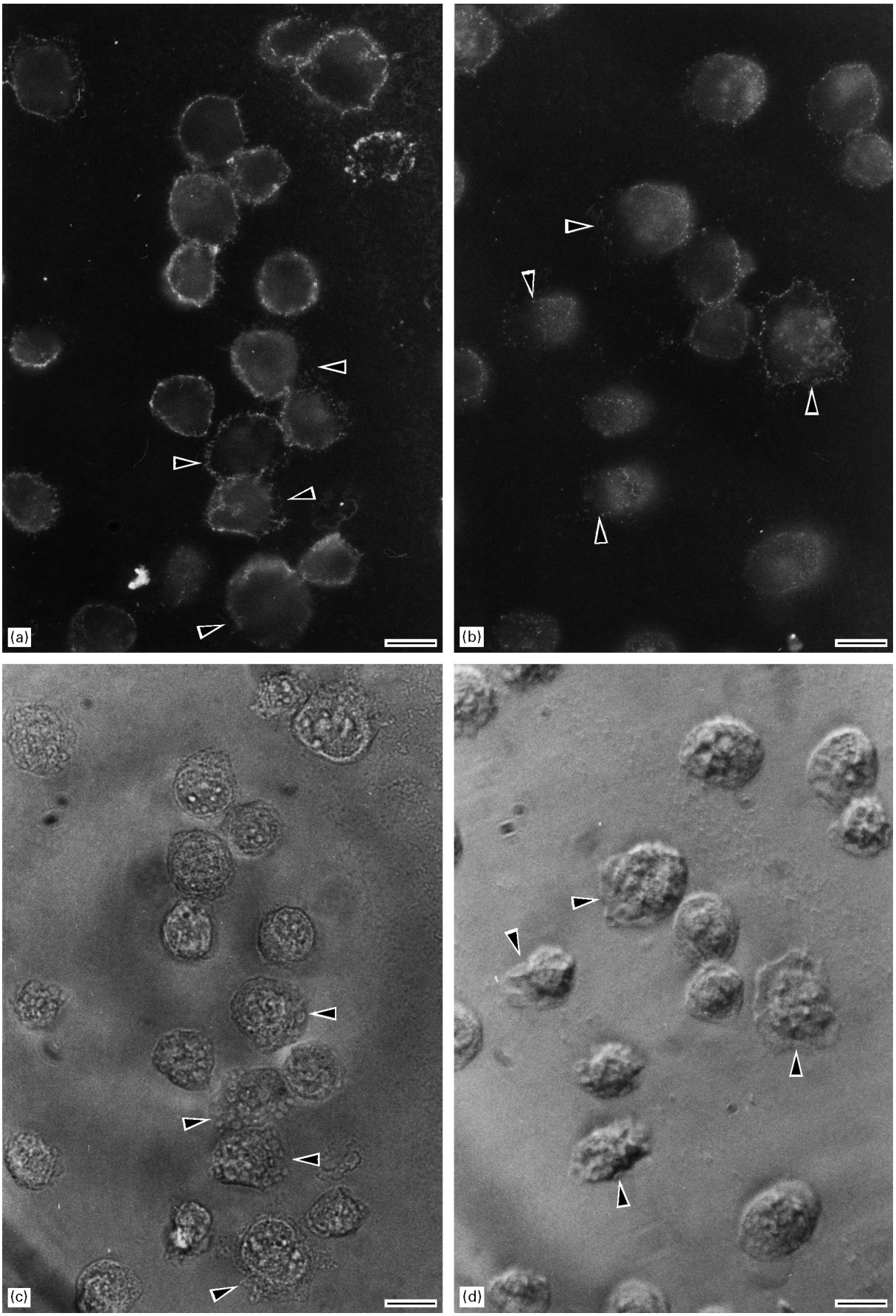

Figure 3 Immunofluorescence and light field microscopy of K562 cells grown for 30 min on polylysine. Cells were labeled with antibodies directed against (a) CD54 and (b) CD58. As is evident from the micrographs, the uniform distribution of these molecules observed at 48 h is no longer present, but rather the organization of these CAMs appears highly disrupted. In addition, as is pointed out by the arrows in the corresponding light field micrographs for (c) CD54 and (d) CD58, the variations in CAM reorganization seem to accompany alterations in cell morphology. The bars represent 10 μm.

increment in the expression of these molecules as was measured with flow cytometry.

In order to exclude any possible early rearrangement of CAMs induced by polylysine on the K562 cell membrane, the distribution of CD54 and CD58 adhesion molecules were also examined at 30 min, 1 and 2 h by fluorescence microscopy and the corresponding cellular morphology studied by light field microscopy (Fig. 3). Surprisingly, these molecules were completely redistributed at all three shorter exposure times with respect to both controls and to cells exposed for longer times. In fact, the uniform distribution of these molecules on the cell surface, which appears as a luminous ring, is no longer present (Fig. 3a and b), but rather these CAMs appear to accompany morphological variations occurring in the cells themselves. This latter point is particularly well stressed by observation of the corresponding light field micrographs (Fig. 3c and d) where these morphological variations are pointed out by arrows. Because similar results were obtained at all three exposure times, only data observed after 30 min treatment with polylysine are shown.

Although the fluorescence intensity of the vitronectin receptor was too weak to photograph and, consequently, is not reported, it also showed a similar pattern of rearrangement exclusively at shorter exposure times (30 min, 1 and 2 h) while at longer exposure times (24 and 48 h) no such reorganization took place.

4. Discussion

The data presented in this report indicate that when K562 cells adhere to polylysine, CAMs undergo important reorganization at short exposure times, but that these changes in CAM distribution disappear at longer treatment times when important alterations in membrane electrical parameters persist. This cycle of temporal events seems to be necessary for the cellular adaptation of K562 cells to their new growth environment seen at longer times. It should be recalled that CAMs modulate a great number of cell/cell and cell/substratum events by binding to specific receptors. These ligand/receptor interactions are involved in cell signalling [3–5] and, ultimately, in cell survival. Adhesion of cells to polylysine is a non-specific charge interaction because specific receptors for this polyamino acid are not normally present on cells. Thus, it would appear that even though the positive charge of polylysine is not acting on the CAMs examined in a direct manner, it is, nonetheless, varying their distribution at short exposure times. This altered distribution may signify that non-specific adhesion of K562 cells on to polylysine is somehow mimicking the adhesive events which take place between CAMs and their specific receptors such that signals for adaptation and survival are received by the cell. If this were not the case, the "forced" adhesion of K562 cells on to polylysine would have led to cell death and not to cell survival and proliferation.

Additional support for this hypothesis is given by the return of CAM distribution in adapted cells to that observed in controls. In fact, this return may indicate that once the CAMs have served as "signalling molecules" for cell survival, there is no further need for their redistribution and, consequently, their original organization is restored. However, it should also be recalled that this return of the CAMs to their original state is not accompanied by a similar reversal of membrane electrical properties to control values at 48 h. Thus, because CAMs do not seem to be directly responsible for these long-lived variations, other possible molecules must be considered. Of these, especially with regard to membrane permittivity, membrane lipids should be considered quite seriously because of the important role played by these molecules in the maintenance of cell structure. If, in fact, CAM redistribution is necessary for cell signalling, membrane lipids may be one of the targets of these signals and may be directly responsible for the structural reorganization of the cell membrane and, therefore, for the alterations in membrane permittivity. In fact, preliminary data obtained by our group using proton nuclear magnetic resonance spectroscopy has demonstrated that not only the distribution of membrane lipids may be the cause of these electrical changes, but that lipid synthesis itself may also be involved (paper in preparation).

Other cellular structures such as the cell cytoskeleton, which together with the cell membrane is primarily responsible for the structural integrity of the cell, may also be targets of the CAM molecules. Consequently, the involvement of the cytoskeletal network in the growth and adaptation of K562 cells on to polylysine is also being investigated. Experiments are also underway in our laboratory in order to test the importance of CAM redistribution in the survival of K562 cells grown on polylysine by utilizing specific antibodies which are able to block the CAMs.

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