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Invited Review

Linkage between cell membrane proteins and actin-based cytoskeleton: the cytoskeletal-driven cellular functions

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Summary. Asymmetric organization of the plasma membrane and cytosolic organelles is fundamental for a variety of cells, including bacteria, yeast and eukaryotic cells (Nelson, 1992). The degree into which cells polarize is characterized by their ability to create and maintain morphologically and biochemically distinct plasma membrane domains. The generation and maintenance of polarized distribution of membrane components (proteins and lipids) is thus critical to the ability of cells to perform complex activities such as cell-to-cell interactions, vectorial transport and secretion, cellular immunity, development and morphogenesis. Modification of cellular polarity may potentially lead to abnormal cellular activities and various pathological disorders (Molitoris, 1991; Carone et al., 1994; Chen et al., 1995). Our review shows the complex interplay between membrane proteins and the cytoskeletal network in determining the "polarized phenotype" in the cell. We provide evidence that membrane/cytoskeleton interaction is the key to regulation of the vast majority of cellular functions.

Key words: Cytoskeleton, Membrane, Skeleton, Polarization, RM, Membrane proteins

Introduction

Although cells differ in function and architecture, most cell types polarize either during the acquisition of a final phenotype (e.g. epithelial cells) or in a transient manner during the development of a specific function (e.g. leukocytes). Of course the prototype of polarized membrane domains are the apical and basolateral surfaces of simple epithelial cells. However, the helper T lymphocytes that interact with antigen-presenting cells rapidly polarize their cytoskeletal components toward the antigen-presenting cells (Stowers et al., 199.5).

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The interactions between plasma membrane and cytoskeleton play an essential role in various cellular functions (Luna and Hitt, 1992; Kusumi and Sako, 1996; Assoian and Zhu, 1997; Tsukita et al., 1997). Among the proteins that have been suggested to link cytoskeleton to the plasma membrane are members of the ezrin family (ezrin, radixin, moesin and merlin). The ERM proteins (ezrin, radixin and moesin) are found in the microvilli, filopodia, membrane ruffles and cell-to-cell contact sites where they colocalize with actin (Berryman etal., 1997; Tsukita et al., 1997). Human ezrin, radixin and moesin are 73-81% identical to each other, and their activities are particularly interchangeable (Tsukita et al., 1997). These three closely-related –80 kDa proteins have been shown to function as molecular links that connect some cell-surface transmembrane proteins (Table 1) to the actin cytoskeleton, in a variety of cells (Tsukita et al., 1997). Furthermore, an association with the actin cytoskeleton has been shown for other membrane proteins that are the key for the development of a proper immune response (e.g. the ζ chain of T cell receptor, TCR) (Caplan et al., 1995).

In this review we present some morpho-functional evidence suggesting that the cytoskeletal-driven polarization of cellular functions is a common mechanism involving both naturally polarized cells (epithelial cells) and cells that continuously modify their polarization during the development of specific functions (immune cells).

Discussion

The aim of our review is to trigger a discussion about the possibility that the actin cytoskeleton is the main driver of cellular functions. A great deal of evidence suggests that the interactions between the plasma membrane and the actin cytoskeleton are involved in many diverse functions of eukaryotic cells, including development and control of cell morphology and polarity, adhesion of a cell to extracellular matrix and cell-to-cell adhesion, membrane stability, and membrane domain organization (Luna and Hitt, 1992).

Table 1. Membrane proteins association with the actin-based cytoskeleton.

MEMBRANE PROTEIN	CELLULAR SOURCE	CYTOSKELETAL PROTEINS	REFERENCE
ICA-1	cos-1	ezrin	Heiska et al., 1998
ICAM-2	cos-1	ezrin	Heiska et al., 1998
CD44	BHK	ezrin, radixin	Tsukita et al., 1994
ICAM-3	pbl	moesin	Serrador et al., 1997
CD43	pbl	moesin, ezrin	Serrador et al., 1998
TCR ζ chain	pbl	cytoskeleton	Pardi et al., 1992
LFA-1	pbl	cytoskeleton	Pardi et al., 1992
CD2	pbl	tubulin	Offringa and Bierer, 1993
CD45	mouse T-lymphoma cells	fodrin, spectrin	Lokeshwar and Bourguignon, 1992
Na^+-K^+ ATPase	epithelial cells	actin cytoskeleton (band 4.1 family)	Arpin et al., 1994

During the last years the number of membrane proteins that have been shown to directly link to the actin cytoskeleton in human lymphoid and epithelial cells are impressively growing (Table 1). To date, the linkage between the actin cytoskeleton and the adhesion receptors (e.g. intercellular adhesion molecules, ICAMs) is largely the most studied (Tsukita et al., 1994; Heiska et al., 1998; Serrador et al., 1998; Yonemura et al., 1998). Actually this is very intriguing in that when non-polarized cell are induced to polarize through the formation of an uropod-like structure (following actin polimerization), the actin-based cytokeleton (Fig. 1a) and ICAM-1 (Fig. 1b) localize in the uropod region. This is a phenomenon common to a variety of cells, including macrophages (Fig. 1c) and human primary T cells following mitogen stimulation (Fig. 1d). Moreover, the acquisition of a uropod-driven polarized shape may be invariably induced by both adhesion on plastic or glass substrate and cytokine stimulation (Fais et al., 1994). Notably, uropods and cell-to-cell adhesion sites form and disappear continuously in cells observed in culture, suggesting that the concentration of the cytoskeleton-associated membrane molecules may change over time. In fact, substances inihibiting actin polymerization, such as cytochalasin D, can disrupt both actin microfilament organization and various intra-cellular signaling (e.g. integrin-mediated signaling via tyrosine kinase) (Clarke and Brugge, 1995; Maniotis et al., 1997). Examination of integrin-mediated adhesion sites has revealed a variety of cytoskeletal and signaling molecules (Clarke and Brugge, 1995; Yamada and Geiger, 1997). These large adhesion and signaling complexes have been found both in integrin-matrix interactions and at sites of cell-to-cell adhesion (Clarke and Brugge, 1995; Yamada and Geiger, 1997). The cytoskeleton-driven formation of polarized large aggregates of membrane proteins, signal transduction molecules and actin-cytoskeletal protein may be a highly conserved mechanism, common to all cells and species, that provides high local concentration of receptors, enzymes, substrates and structural molecules in polarized membrane sites, which facilitate activation of intracellular signaling and cellular functions, with a *minimum* of protein neosynthesis. Therefore, the uropod shape of circulating cells, such as lymphocytes and monocytes, does not simply represent a specialized structure with important function for motility and adherence (Del Pozo et al., 1996). It is notewhorthy that polarization of ICAM-1 on human monocytes (Fig. 2a) leads to monocyte-to-monocyte fusion as a final differentiation state (Fais et al., 1997). Of interest is that multinucleated giant cells (MGC), derived from monocyte fusion, lose both the α-actinin (Fig. 2c) and the actin (Fig. 2d) polarization observed in single cells, becoming unpolarized cells uncapable of recruiting new cells for fusion (Fais et al., 1997). In fact, the ability to fuse forming MGC is peculiar to monocytes, being differentiated macrophages unable to form MGC (Fais and Pallone, 1995; Fais et al., 1996, 1997; Most et al., 1997). Another important example of cellular polarization, not directly related to adherence, motility or transendothelial migration, is the HIV-1-induced polarization during cytoskeletal-driven unidirectional budding (Fais et al., 1995b). In fact, acutely HIV-1-infected human lymphoid cells show a highly polarized staining for viral proteins both in single cells (Fig. 3a) and, contrarily, to the monocyte-derived MGC in syncytia (Fig. 3b) (Fais et al., 1997). In these structures, ICAM-1 and HIV-1 co-localize both in single cells and syncytial uropods (Fais et al., 1997). Of interest is that very recently an association between the actin-based cytoskeleton and some HIV-1 proteins has been shown (Bukrinskaya et al., 1998). Notably, the phenomenon of the uropod-driven unidirectional budding is common to yeasts and bacteria, as well (Drubin and Nelson, 1996; Aroeti et al., 1998). Moreover, a crucial role of membrane-to-cytoskeleton linkage has been postulated, and partially demonstrated, both in yeasts and bacteria (Nelson, 1992; Chant, 1994). These data strongly suggest that the cytoskeleton-driven cellular polarization is an ubiquitary phenomenon, but the consequences of polarization are highly cell specific (Fais et al., 1997).

Monocytes

Monocytes continuosly change their shape during culture observation (Fig. 4). Notably, monocyte differentiation may be strongly changed during adherence to glass or plastic, and above all with the addition of specific or non-specific cytokines (Fais et al.,

1997). The shape that monocytes acquire during differentiation is strictly related to diverse functions: (i) flat unpolarized shape (Fig. 4a), scavenging and/or phagocytosis; (ii) spindle shape (Fig. 4b), endothelial-like function; (iii) interdigitating/dendritic shape (Fig. 4c), antigen processing and presentation; and (iv) MGC (Fig. 4d), granulomatous inflammatory reaction. Moreover, monocytes (showing a phagocytic behaviour) may reach an impressive size which is 10 to 50 folds larger with respect to the surrounding homotypic cells (Fig. 4e). Of interest is that, independently from the cell shape, a monocyte may temporarily form uropods with a polarized distribution of cytoskeleton-associated membrane proteins. These features strongly suggest that

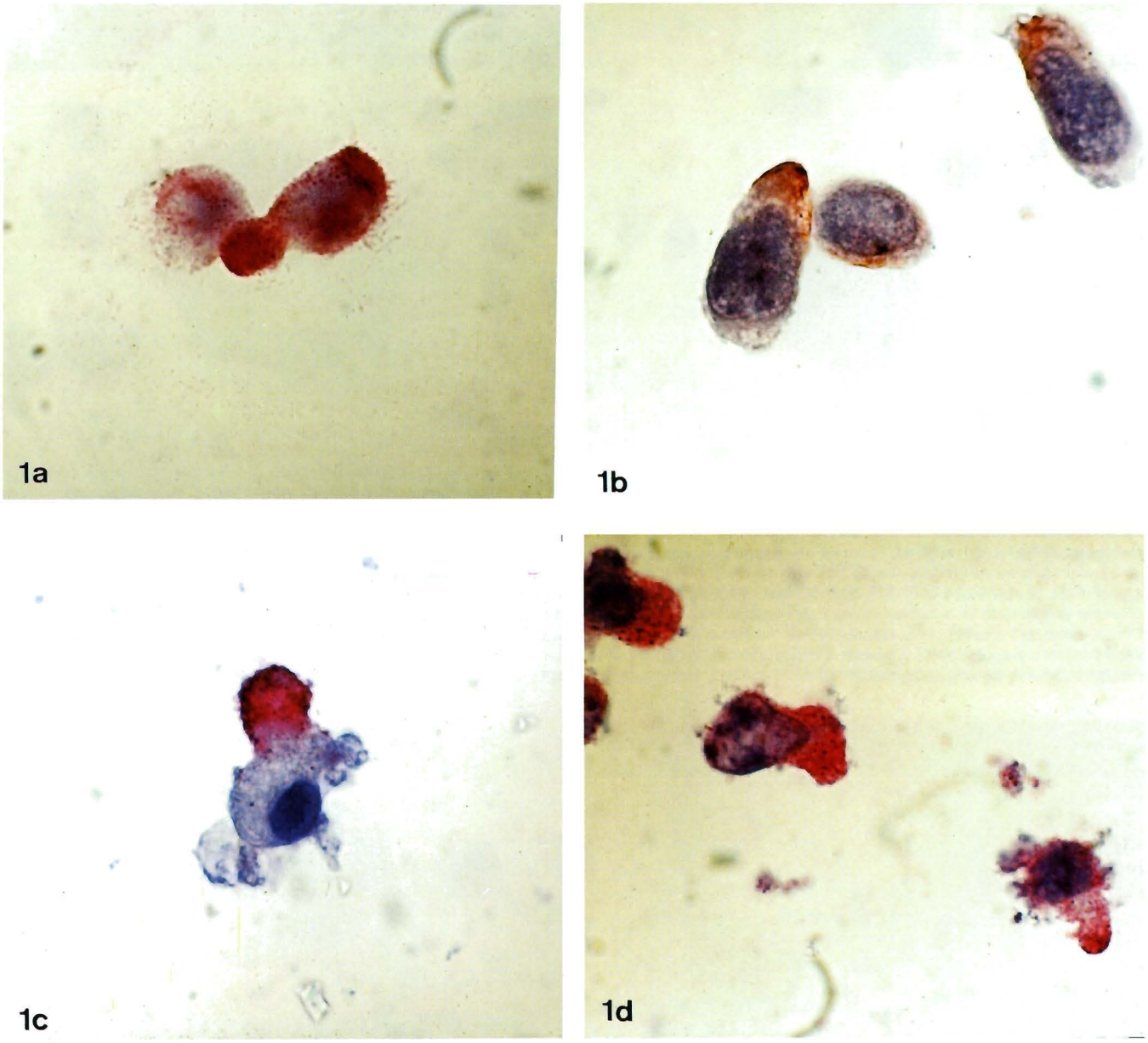


Fig. 1. Polarization through uropod formation in lymphocytes and monocytes. **a.** Polarization of actin cytoskeleton on the uropod of a human lymphoblastoid cell in chamber slide preparation. Anti-actin mAb, Peroxidase-anti-peroxidase (PAP) method, DAB as chromogen and Mayer's haematoxylin counterstaining. **b.** ICAM-1 polarization on the uropod of a human lymphoblastoid cell in chamber slide preparation. Anti-ICAM-1 mAb, Peroxidase-anti-peroxidase (PAP) method, DAB as chromogen and Mayer's haematoxylin counterstaining. **c.** ICAM-1 polarization on the uropod of a human primary monocyte following interferon-γ (IFN-γ) stimulation in chamber slide preparation. Anti-ICAM-1 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. **D.** ICAM-1 polarization on the uropod of human primary T cells following mitogen stimulation in chamber slide preparation. Anti-ICAM-1 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. x 1,000

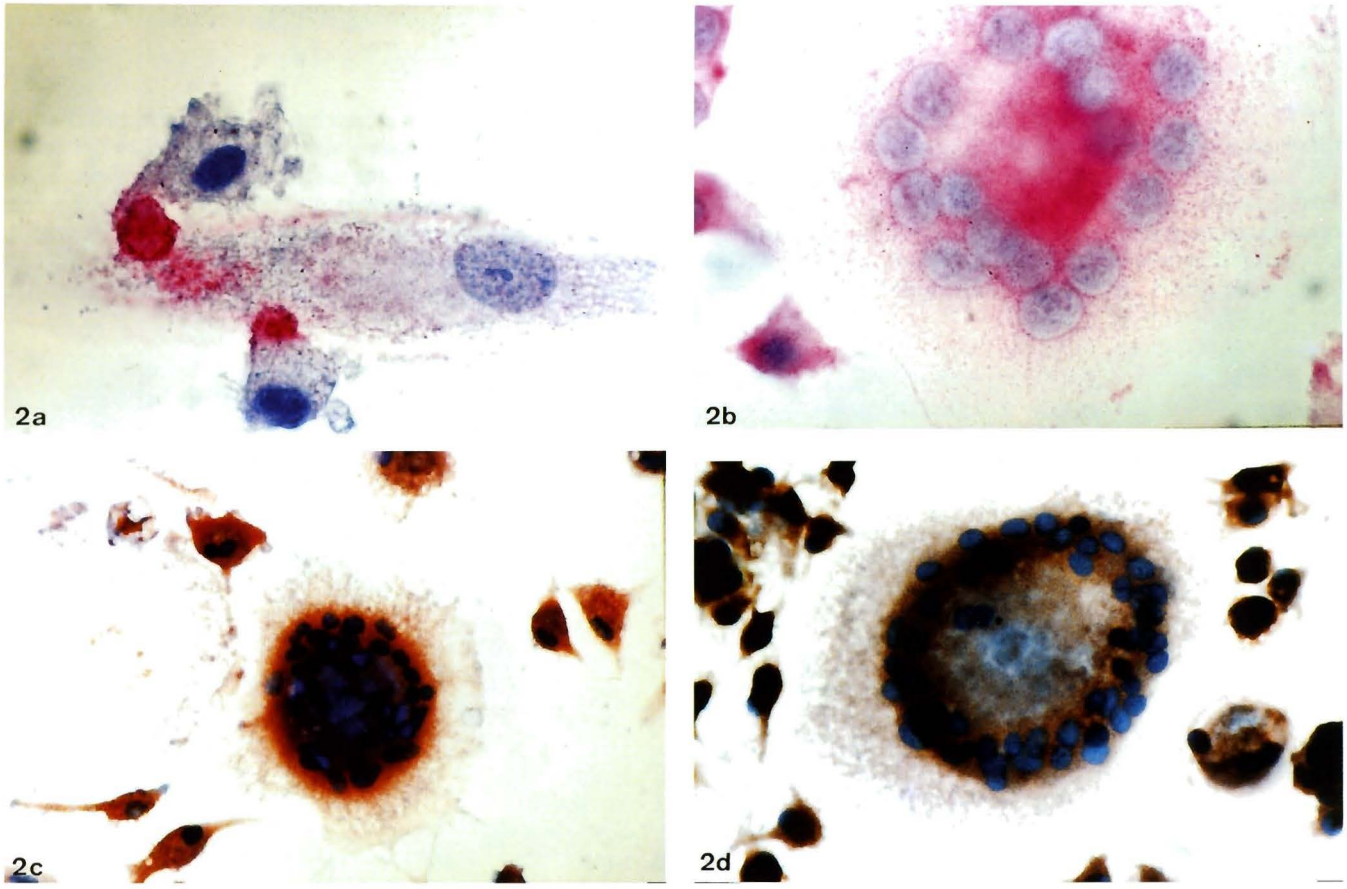


Fig. 2. Role of cytoskeletal-driven polarization in multinucleated giant cell (MGC) formation from human primary monocytes. **a.** Immunocytochemistry in slide-chamber preparation of human primary monocytes after IFN-γ treatment showing a small cluster of monocytes. ICAM-1 is clearly polarized on uropods of two monocytes at the level of cell-to-cell contact with a giant macrophage. Anti-ICAM-1 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. **b.** Immunocytochemistry in slide-chamber preparation of a human primary monocyte-derived MGC with a centrally localized immunostain for ICAM-1. Anti-ICAM-1 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. **c.** Immunocytochemistry in slide-chamber preparation of a human primary monocyte-derived MGC with a centrally localized immunostain for α-actinin. Anti-α-actinin mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstainin. **d.** Immunocytochemistry in slide-chamber preparation of a human primary monocyte-derived MGC with a centrally localized immunostain for actin. Anti-actin mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. x 1,000

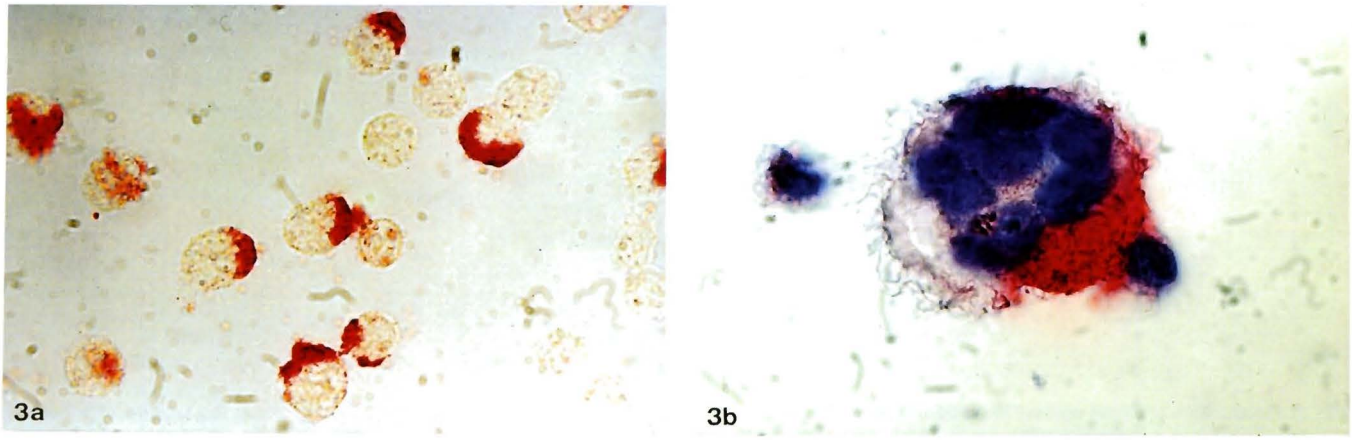


Fig. 3. Syncytia formation in HIV-1-infected human lymphoblastoid cells. **a.** Immunocytochemistry in slide-chamber preparation of human lymphoblastoid cells infected with HIV-1. Infected cells clearly show polarization of HIV-1 on uropods or at the level of cell-to-cell contact sites. Anti-p24 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen, without counterstaining. **b.** Immunocytochemistry in slide-chamber preparation of syncytia derived from human lymphoblastoid cells infected with HIV-1. Syncytium clearly shows polarization of HIV-1 on a giant uropod in recruiting a new cell. Anti-p24 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen, Mayer's haematoxylin counterstaining. x 1,000

cytoskeleton drives both the functional differentiation of monocytes and a time-dependent activation of specific functions, through the polarization of the specific membrane proteins in the forming uropods. Very recently, we have shown that the multidrug resistance protein-1 (MDR-1), that normally localizes in a perinuclear area in monocytes, becomes polarized on a cell uropod after interferon-γ (IFN-γ) treatment, colocalizing with ezrin on the same uropod (Puddu et al., 1999). These data suggest that the integrity of cytoskeleton is crucial in regulating the activity of this important protein. It is therefore conceivable that monocyte functions strictly depend on the actin cytoskeleton activity.

Lymphocytes

All the above reported findings may suggest that

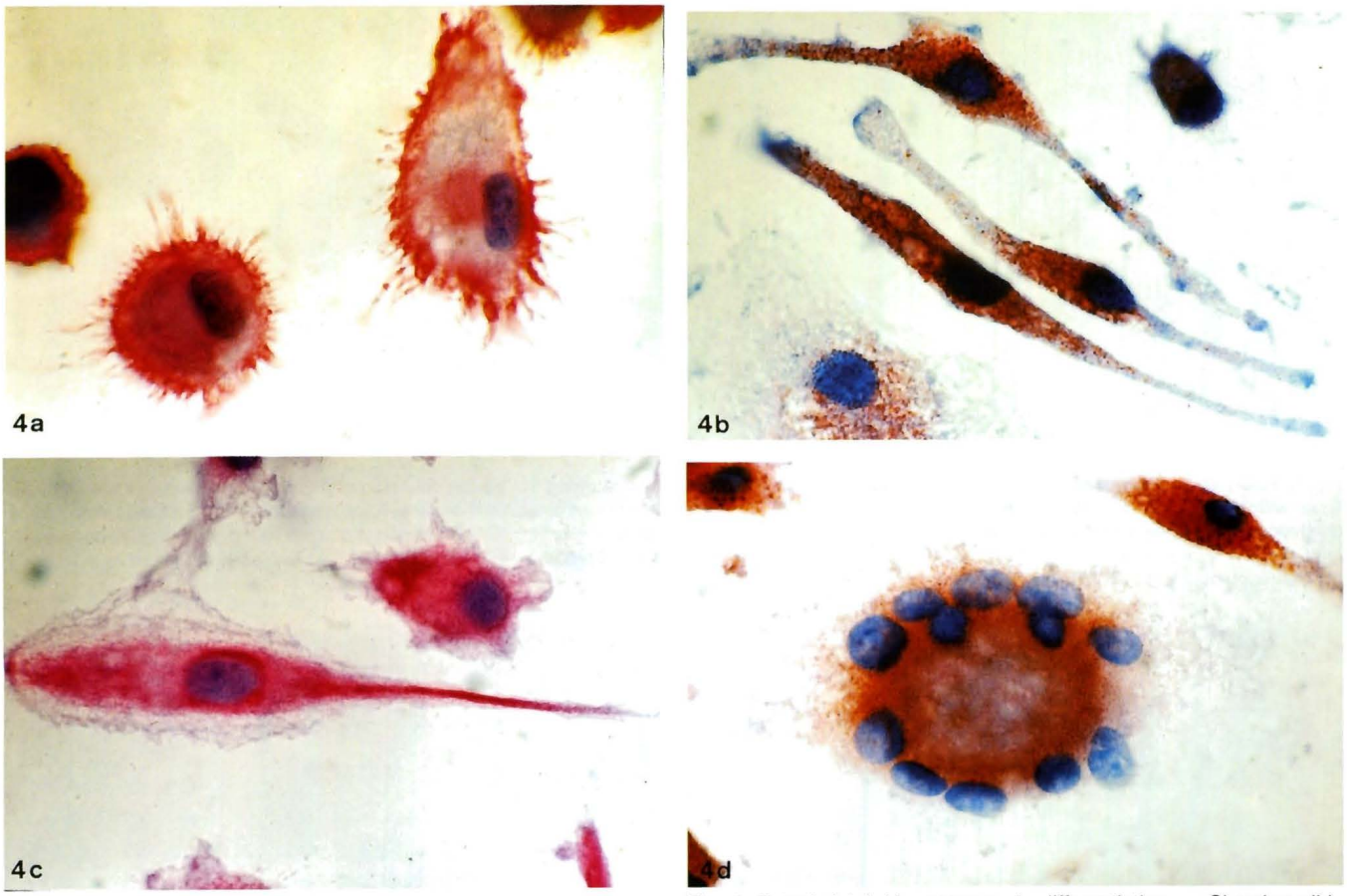


Fig. 4. Cytoskeletal-driven monocyte differentiation. **a.** Chamber slide preparation of human primary macrophages with a flat unpolarized morphology. Anti-α-actinin mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. **b.** Chamber slide preparation of human primary macrophages with a spindle morphology. Anti-actin mAb, Peroxidase-anti-peroxidase (PAP) method, DAB as chromogen and Mayer's haematoxylin counterstaining. **c.** Chamber slide preparation of human primary macrophages with a dendritic/interdigitating morphology. Anti-actin mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. **d.** Chamber slide preparation of human primary macrophage-derived MGC. Anti-actin mAb, Peroxidase-anti-peroxidase (PAP) method, AEC as chromogen and Mayer's haematoxylin counterstaining. **e.** Chamber slide preparation showing the phagocytic behaviour of a macrophage with a size that appears 10-15 folds larger than that of the surrounding cells. Anti-α-actinin mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. x 1,000

cytoskeletal components serve as a matrix, which regulates the efficiency of interactions between cell-surface-expressed transmembrane receptors and various catalytic and noncatalytic molecules of the signal transduction cascade. Some evidence strongly suggests that the membrane-cytoskeleton interactions are impressively active in lymphocytes, as well. It is well known that immune activation induces morphological changes in lymphocytes, such as switching from a round to a pear-like polarized shape (Fig. 5a) (Del Pozo et al., 1996). It is straightforward that the cell polarization is a direct consequence of the formation of uropods. The development of these cytoplasmic projections in lymphocytes involves the cell as a whole (Fig. 5a), while in monocytes only 1/3 of the cell may be involved (Fig. 5b). This means that, particularly in lymphocytes, when the cell is induced to assume a cytoskeleton-driven uropoidal shape the majority of membrane proteins, and

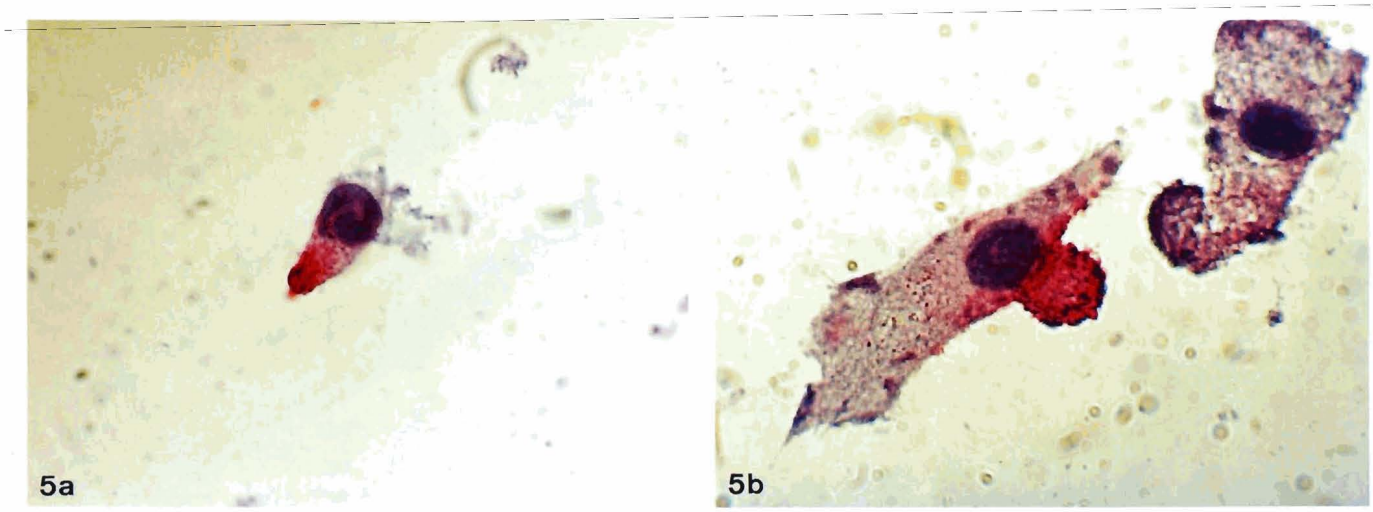


Fig. 5. Polarization of lymphocytes and monocytes. **a.** Chamber slide preparation showing the typical pear-shape of mitogen-stimulated human primary T cell with polarization of ICAM-3 on the cell uropod. Anti-ICAM-3 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. **b.** Chamber slide preparation showing ICAM-3 polarization on a monocyte uropod that involves only 1/3 of the cell. Anti-ICAM-3 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. x 1,000

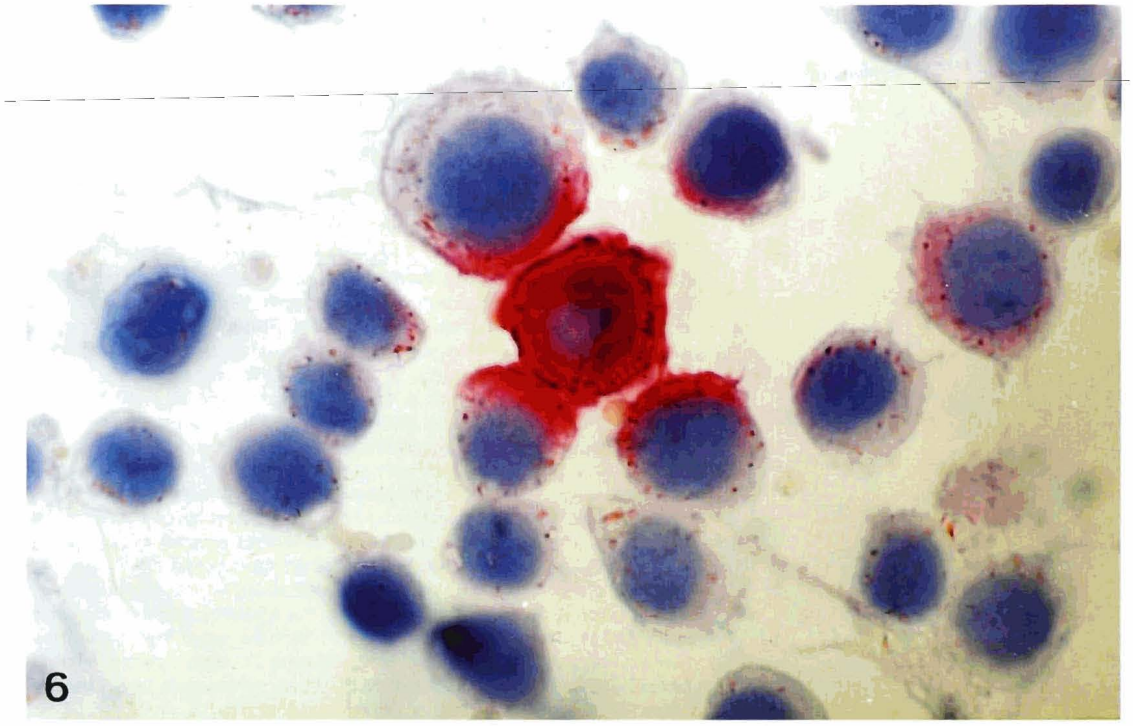
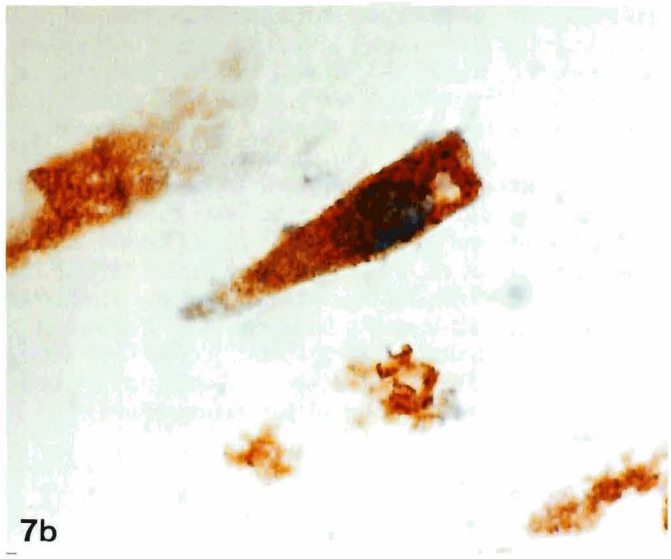


Fig. 6. HIV-1 cell-to-cell infection. Chamber slide preparation showing a mechanism of HIV-1 cell-to-cell infection through polarization of virus at the level of cell-to-cell contact sites. Anti-p24 mAb, Alkaline Phosphatase-anti-alkaline phosphatase (APAAP) method, fast red as chromogen and Mayer's haematoxylin counterstaining. x 1,000

as a consequence the majority of cellular functions, are recruited in the uropod. Polarization of helper T cells toward antigen-presenting cells (APC), as mediated by the interaction of the T-cell receptor (TCR) with antigen bound to proteins encoded by the major histocompatibility complex, is a clear example of cell polarity that is rapidly induced in the direction of an external signal (Geiger et al., 1982; Kupfer et al., 1986, 1994). Interaction between helper T cells and an APC is the key to the regulation of the immune response. Upon contact between an APC and a helper T cell, a tight interface forms between the two cells, and the T cell polarizes its cytoskeleton toward the APC (Geiger et al., 1982; Kupfer et al., 1986, 1994). It has been shown that in T cells, the mammalian Ras-related GTPase CDC42 (homologous of yeast CDC42, a protein involved in budding polarity) can regulate the polarization of both actin and microtubules toward APC (Stowers et al., 1995). Notably, this is a demonstration for a conserved CDC42 function between yeast and T cells, suggesting that this and many other GTPase may be a general regulator of cytoskeletal polarity in a variety of cell types. Furthermore, it has been shown that the cell-surface-expressed ζ chain of TCR is anchored to the cytoskeleton in resting T cells (Caplan et al., 1995), and that the TCR, upon engagement, initiates a cascade of biochemical events that lead to receptor association with cytoskeleton (Rozdzial et al., 1998) and culminate in the rearrangement and reorientation of the cytoskeleton (Omann et al., 1987), production of lymphokines, and cellular proliferation and differentiation. It has also been shown that antigen-(Ag-) and Ia-specific helper T cells form 1:1 cell couples with Ag-pulsed hybridoma B cells expressing the specific Ia determinants (Kupfer et al.,



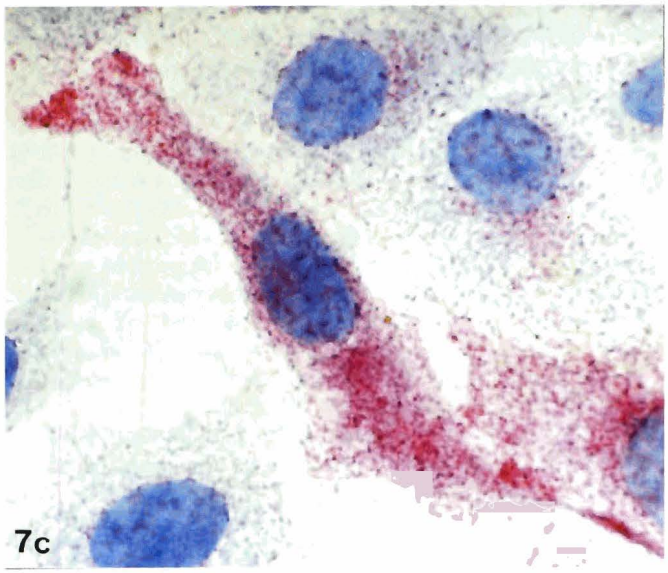


Fig. 7. The epithelial shape. a. Chamber slide preparation of a human lymphoblastoid cell showing an epithelial-like shape. Anti-bcl2 mAb, Peroxidase-anti-peroxidase (PAP) method, DAB as chromogen and Mayer's haematoxylin counterstaining. b. Chamber slide preparation of a human monocyte showing an epithelial-like shape. Anti-bcl2 mAb, Peroxidase-anti-peroxidase (PAP) method, DAB as chromogen and Mayer's haematoxylin counterstaining. c. Chamber slide preparation of human epithelial cells showing the typical shape. Anti-bcl2 mAb, Peroxidase-anti-peroxidase (PAP) method, AEC as chromogen and Mayer's haematoxylin counterstaining. x 1,000

Table 2. Involvement of cytoskeleton in various cellular functions.

CELLULAR FUNCTION	ASSOCIATED CYTOSKELETAL PROTEINS	REFERENCES
Localized membrane growth	actin ERM proteins	Drubin and Nelson, 1996 Paglini et al., 1998
Directional cell migration	ERM proteins (via association with CD42, CD43, CD2, ICAM 3)	Del Pozo, 1996; Tsukita et al., 1997
Vectorial transport	fodrin, α-catenin, ankyrin	Bennet, 1990; Marrs, 1995
Cell adhesion	α-actinin, talin, vinculin, zysin, paxillin ERM proteins	Clarke and Brugge, 1995 Takeuchi et al., 1994
Response to growth factors (EGF)	F-actin	Den Hartigh et al., 1992
Cell cycle progression	actin	Lew and Reed, 1995
NK activity	ezrin	Helander et al., 1996

1986). Notably, in these couples, the cytoskeleton inside the helper T cells is unidirectionally oriented to face the bound hybridoma cells (Kupfer et al., 1986), further suggesting that the cytoskeleton-driven polarization of T cells is a key phenomenon during antigen presentation. Last but not least, the redistribution of adhesion receptors (i.e. ICAM 1-3, CD44) within a lymphocyte uropod (del Pozo et al., 1996) is a crucial part in the multistep regulatory system leading to adhesiveness to cell and/or substrates. Particularly, polarization of adhesion molecules on lymphocyte uropods, as well as on other uropod-forming leukocytes, may be involved in both physiological [antigen presentation (Kupfer et al., 1994; Stowers et al., 1995), transendothelial migration (Burgio et al., 1995; Del Pozo et al., 1996) and persistent and sustained cell-to-cell stimulation (Del Pozo et al., 1996)] and pathological phenomena [cell-to-cell viral (Fais et al., 1995) (Fig. 6) or bacterial infection (Aroeti et al., 1998) and polykarion formation (Fais et al., 1996)].

Epithelial cells

Epithelial cells may be considered as the prototype of cellular polarization (Drubin and Nelson, 1996; Aroeti et al., 1998). The structural asymmetry of these cells is distinctive. In epithelial cells, subsets of membrane and cytoskeletal proteins localize to functionally and structurally distinct membrane domains, termed apical and baso-lateral, and microtubules and sorting compartments of the secretory apparatus are asymmetrically distributed in the cytoplasm. This polarized organization is the basis for the function of these cells in vectorial transport of ion and solutes across the epithelium. As for leukocytes, adhesion receptor proteins are particularly polarized in epithelial cells (Drubin and Nelson, 1996). Moreover, by analogy with leukocytes, specialized cytoskeletal and signaling networks assemble around the adhesion receptors and position other cytoskeletal complexes and protein-sorting compartments relative to a spatial cue (Drubin and Nelson, 1996). Subsequently, a complex cascade of events occurs, that are initiated by cell adhesion (Drubin and Nelson, 1996). Of interest is that lymphocytes (Fig. 7a) and monocytes (Fig. 7b), may acquire a morphological feature *in vitro* that is very similar to that of typical epithelial cells (Fig. 7c). This in turn suggests that the cytoskeletal-driven polarization may be considered a sort of temporarily-defined "epithelization" of leukocytes. On the other hand, epithelial cells may express some leukocyte antigens (Fais and Pallone, 1989) and may exert functions, such as antigen presentation (Mayer and Shlien, 1987) and macropynocytosis (Falzano et al., 1993), that are professionally carried out by monocyte/macrophages. It is therefore conceivable that cell polarization is a common phenomenon that allows interactions between membrane proteins, cytoskeleton and signaling networks that form a sort of template for propagating signals for cellular functions from the membrane cue. Cytoskeleton-membrane receptor interaction is the key to the regulation of some important functions of epithelial cells. As an example, remodelling of the actin cytoskeleton occurs in many systems, including the epithelial cells, in response to growth factors (Aplin and Juliano, 1999). The Epidermal Growth Factor-Epidermal/Growth Factor-receptor pathway is one of the best characterized (Rijken et al., 1991; van Bergen et al., 1992). In fact, a direct association of the EGFR to F-actin has been shown (den Hartigh et al., 1992) and one of the effects of the EGF-induced signal transduction is a rapid polymerization of F-actin (Rijken et al., 1991, 1998). This event plays a role in the regulation of receptor catalytic activity via phosphorylation of EGFR in Thr654, required for the negative feedback regulation of EGFR signaling (Rijken et al., 1998). Moreover, EGF stimulates the tyrosine phosphorylation of focal adhesion kinase (p125FAK) and the cytoskeletal protein paxillin (Tapia et al., 1999). Treatment with cytochalasins, that selectively hamper actin polymerization, inhibits the EGF-stimulated tyrosine phosphorylation (Rijken et al., 1998; Tapia et al 1999). Moreover, several EGFR substrates, such as PKCγ1 (Diakonova et al., 1995; Bedrin et al., 1997), phosphatidylinositol kinase, phosphatidylinositol phosphate kinase, diacylglycerol kinase (Payrastre et al., 1991), calpactin (Akiyama et al., 1986), spectrin, ezrin, (Bretscher, 1989) and others, are directly associated with membrane cytoskeleton (Bedrin

et al., 1997).

Thus, the cytoskeleton could play an important role, not only in the organization of cellular structure and polarization, but as an integral part of the signal transduction system.

Conclusion and perspectives

In conclusion, cell polarity is the ultimate reflection of complex mechanisms that establish and maintain functionally specialized domains in the plasma membrane and cytoplasm. The spatial arrangement and protein composition of these domains facilitate various cellular processes (Table 2) in a number of eukaryotic cells, in yeast and in bacteria. Embedment-free electron microscopy has revealed an internal cell structure with an elaborate and dynamic structural network throughout the cytoplasm and nuclei (Penman, 1995). In resinless ultrathin sections a staggering network of interconnected cytoskeletal filaments appears with polyribosomes, mitocondria and a myriad of unidentified small structures attached to the cytoskeleton (Penman, 1995). Using the same technique, the nuclear space appears as a complex network of core filaments that connect with the nuclear lamina, and the chromosomes appear attached on spindle fibers, which are interconnected through numerous thin filaments (Penman, 1995). None of these structures are visible using the conventional resin embedding technique. The importance of these findings are further emphasized by the recent demonstration that mechanical connections exist between integrins, cytoskeletal filaments and nucleoplasm that stabilize the nuclear structure (Maniotis et al., 1997), suggesting that the nuclear structure may change following cytoskeletal-driven cellular polarization. In fact, important evidences have been provided for a direct interaction between membrane, cytoplasm and the nucleus through cytoskeletal filaments (Djabali, 1999). Future scientists have to take carefully into account the membrane-cytoskeleton-nucleus interactions in rethinking cell structure and, as a direct consequence, in the reappraisal of the studies on cellular functions and signal transduction.

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