Microvillar cell surface as a natural defense system against xenobiotics: a new interpretation of multidrug resistance

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Lange, Klaus, and Joachim Gartzke. Microvillar cell surface as natural defense system against xenobiotics: a new interpretation of multidrug resistance. Am J Physiol Cell Physiol 281: C369–C385, 2001.—The phenomenon of multidrug resistance (MDR) is reinterpreted on the basis of the recently proposed concept of microvillar signaling. According to this notion, substrate and ion fluxes across the surface of differentiated cells occur via transporters and ion channels that reside in membrane domains at the tips of microvilli (MV). The flux rates are regulated by the actin-based cytoskeletal core structure of MV, acting as a diffusion barrier between the microvillar tip compartment and the cytoplasm. The expression of this diffusion barrier system is a novel aspect of cell differentiation and represents a functional component of the natural defense system of epithelial cells against environmental hazardous ions and lipophilic compounds. Because of the specific organization of epithelial Ca²⁺ signaling and the secretion, lipophilic compounds associated with the plasma membrane are transferred from the basal to the apical cell surface by a lipid flow mechanism. Drug release from the apical pole occurs by either direct secretion from the cell surface or metabolism by the microvillar cytochrome P-450 system and efflux of the metabolites and conjugation products through the large multifunctional anion channels localized in apical MV. The natural microvillar defense system also provides a mechanistic basis of acquired MDR in tumor cells. The microvillar surface organization is lost in rapidly growing cells such as tumor or embryonic cells but is restored during exposure of tumor cells to cytotoxins by induction of a prolonged G₀/G₁ resting phase.

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membrane and cytoplasm, inhibits the entrance of hydrophilic and lipophilic xenobiotics via MV into the cytoplasm. Furthermore, the polarized organization of the epithelial secretory response to various external signals generates a basal-to-apical lipid flow that clears the plasma membrane from lipophilic xenobiotics. The same process also results in transmembrane “flipping” of lipid-bound xenobiotics from the inner lipid layer of the plasma membrane to the outer layer. Ultimately, all membrane-intercalated lipophilic compounds are swept to the apical pole of the cell to be either secreted from the cell surface or metabolized by the microvillar cytochrome P-450 system and released via anion channels located at the same site. This type of peripheral processing allows tissue cells to keep the cytoplasm free from toxic xenobiotics. Rapid cell division, due to carcinogenic transformation or during embryonic growth, abolishes this defense mechanism, but it is restored under conditions of restricted growth, e.g., in the presence of cytotoxic compounds.

The presented concept of xenobiotic defense is one of several cell-physiological consequences emerging from the hypothesis of microvillar regulation and signaling (57, 58, 62–69; reviewed in Refs. 59–61), which is briefly outlined below.

THE CONCEPT OF MICROVILLAR SIGNALING

Surface Organization of Differentiated Cells

Recent work on cellular regulation via microvillar pathways has yielded evidence for a common mechanism involved in regulation of various forms of transport and uptake processes. This type of regulation of cellular functions, including glucose transport, transmembrane ion fluxes, and Ca$^{2+}$ signaling, was shown to develop during growth arrest and differentiation when important integral membrane proteins such as transporters and ion channels are segregated into MV tips (63).

The tip compartments of MV form a multitude of small pericellular spaces on the cell surface, subsumed under the term “entrance compartment,” which can be entered without restriction by cellular substrates including hexoses (62, 63, 69) and ions (57, 64, 65). The entrance compartment, however, is separated from the cytoplasm by a tight bundle of actin filaments representing an effective diffusion barrier (Fig. 1) (57, 64, 65, 68, 69). The effectiveness of the microvillar diffusion barrier is regulated by a variety of physical effects as well as by receptor-mediated signaling (reviewed in Refs. 59–61).

Distribution of Integral Membrane Proteins on the Cell Surface

In undifferentiated, rapidly growing (embryonal or tumor) cells, functionally important membrane proteins and membrane lipids are continually inserted into the cell surface by exocytic events. Exocytic membrane components, originating from the endoplasmic reticulum and processed within the Golgi complex, are finally inserted into the cell surface (Fig. 2A). After fusion of trans-Golgi vesicles with the plasma membrane, they turn inside out, forming small spherical surface protrusion (blebbing). Subsequently, the inserted membrane domain is integrated into the plasma membrane, and the integral membrane proteins are scattered over the whole cell surface by lateral diffusion. Thus a great number of integral membrane proteins, essential for cellular metabolism and growth, are recruited to the surface of rapidly dividing cells, warranting maximal activity of life-preserving membrane functions such as uptake of glucose and amino acids and regulation of ionic influx and efflux. Uptake rates exclusively depend on metabolic demands and are limited by the activity of key enzymes. This state, also known as metabolic limitation (23), is typical for rapidly growing cells.

Under conditions of restricted growth or during cell differentiation, generally induced by depletion of metabolic substrates and serum factors at high cell density, membrane insertion by exocytosis proceeds in a different way. As shown in Fig. 2B, blebbing of exocytic vesicles is not followed by integration of the inserted membrane domain into the cell surface because a coat of proteoglycans (PGs) stabilizes the exocytic membrane domain (50, 72, 75). The transmembrane organization of the PGs and the associated surface coat inhibits lateral diffusion of other integral membrane proteins out of this membrane domain (63). Subsequent growth of microfilament bundles at specific submembrane nucleation sites elevates the newly inserted membrane patch to the tip of the growing MV.

The different fates of exocytic membrane domains in rapidly growing and differentiated cells are primarily due to the regulated activity of PG-associated ectoendopeptidases (of the plasminogen activator type) that are able to initiate degradation of the surface coat (50, 51). In differentiating or resting cells, these ectoendopeptidases are both less expressed and specifically inhibited (44, 52, 72, 75, 79, 119, 121).

Fig. 1. Schematic presentation of the microvillar structure. Functional membrane proteins are localized at the tip of the microvilli (MV). In the unstimulated state, the diffusion of ions and substrates from the tip compartment to the cytoplasm is impeded by the actin filament bundle of the shaft region, which acts as a diffusion barrier.
On the other hand, the formation of MV and their maintenance critically depends on the expression of specific linker proteins connecting functional membrane proteins with the actin cytoskeleton. Thus glucose transporters and ion channels or exchangers, recruited to the cell surface by exocytosis, are connected via ERM (ezrin, radixin, moeit) proteins and other linkers to the microvillar cytoskeleton. This intriguing functional aspect of the microvillar organization has been discussed in a recent review of microvillar ion channels (60).

The microvillar surface is a typical feature of cells during arrested growth in G0/G1 phases of cell cycle or in the differentiated state (9, 18, 30, 111). Under these conditions, newly synthesized integral membrane proteins are localized exclusively at MV tips. Thus the cellular metabolism is severely restricted because the function of transporters and channels is strongly limited by the cytoskeletal diffusion barrier within the microvillar shaft. Membrane limitation and membrane regulation of metabolism are characteristic of differentiated cells (23). In this state, substrate consumption is reduced, and the uptake of energy-providing substrates and structural components becomes subject to regulation by external signals.

Functional Significance of the Microvillar Surface Organization

The exclusive localization of functionally important integral membrane proteins on MV tips is a novel aspect of cellular differentiation (63), which implies a number of remarkable consequences. One of the most important results is the functional integration of the highly efficient oxidative ATP production into the framework of glycolysis, the original energy-providing system of primitive systems. Another characteristic feature of differentiated cells is their extremely low cytosolic Ca$^{2+}$ concentration, which is an absolute prerequisite for cellular Ca$^{2+}$ signaling. The maintenance of a steep [Ca$^{2+}$] gradient between the extracellular and intracellular space is essential for regulation of cellular functions by cytosolic Ca$^{2+}$. Again, the microvillar diffusion barrier provides for an effectively restricted activity of cation channels located on MV tips (57, 58, 64, 65, 76). As recently proposed, Ca$^{2+}$ influx via microvillar pathways is regulated by modulation of the actin-based cytoskeletal diffusion barrier (reviewed in Ref. 60). In this concept, the microvillar actin filaments play a dual role as receptor-operated high-affinity Ca$^{2+}$ store and regulated diffusion barrier inhibiting the influx of external Ca$^{2+}$ into the cell (66; reviewed in Ref. 59). Another function of the microvillar surface organization is to prevent uptake of hydrophilic and lipophilic toxins into the cytoplasm.

ROLE OF MICROVILLAR SURFACES IN CELL PROTECTION AGAINST CYTOTOXINS

In principle, environmental hazards should be more harmful during long-term exposure to static cell systems than to rapidly growing and regenerating sys-
tems. The low turnover rate of genetic and structural components should render resting cells much more susceptible to environmental hazards. In fact, the opposite is true. Thus handling of xenobiotics by resting cells may be one of those cellular features that has been extensively improved during evolution.

Two different types of xenobiotics must be considered, hydrophilic and lipophilic compounds, which can enter the cell via different pathways. Hydrophilic compounds are generally excluded from the cytoplasm because the central hydrophobic region of the plasma membrane effectively blocks the passage of hydrated chemicals. However, inorganic ions or ionic compounds can permeate the plasma membrane through ion channels. Hazardous compounds of this type belong to the group of toxic metal cations, toxic inorganic anions, and ionic organic compounds. Lipophilic toxins enter the cell through the plasma membrane, the entrance compartment for these compounds. Numerous lipophilic organic compounds readily enter the hydrophobic region of the membrane lipid bilayer and are distributed from the inner bilayer via the cytoplasm to intracellular membrane systems. Most of the cytotoxic drugs used in tumor therapy belong to this type of slightly water-soluble, weakly basic, lipophilic organic chemicals. For both hydro- and lipophilic xenobiotics, the microvillar cell surface represents an effective uptake barrier.

Mechanisms Preventing Uptake of Lipophilic Xenobiotics

The mechanisms of cellular resistance against lipophilic cytotoxins have been extensively investigated in connection with MDR in tumor chemotherapy. A vast amount of experimental data on long-term treatment of living cells with cytotoxic compounds has accumulated in this field. According to the present state of investigations, the epithelial defense system against lipophilic cytotoxins appears to be a complex mechanism, specifically designed for clearing the plasma membrane from lipophilic toxins of most different chemical structures. However, despite the large experimental effort to elucidate the mechanism(s) underlying MDR, a satisfying concept for integration of all presently known facts into a systematic framework is still lacking.

Reduced uptake of lipophilic compounds through microvillar surfaces. Uptake of lipophilic compounds into the cytoplasm occurs via incorporation into the hydrophobic region of the lipid bilayer and further redistribution into the cytosol, depending on the lipid/water partition quotient of the compound. This latter process, the drug transfer across the lipid/water interface at the cytoplasmic surface of the plasma membrane, is the rate-determining step for uptake of xenobiotics into the cytoplasm. The rate of drug entry at this interface largely depends on the size of this interface, which is large in cells with a smooth surface but significantly reduced by formation of microvillar surfaces. This interpretation contradicts the current opinion that MV increase the uptake of extracellular components by increasing the surface area, a view that has been severely challenged by the finding that the microvillar actin filament bundle acts as an effective diffusion barrier even for low-molecular-weight compounds (57, 62–65, 68, 69).

As shown in Fig. 3, the surfaces of freshly isolated rat hepatocytes are so densely covered by MV that the original cytoplasm-facing plasma membrane almost becomes invisible. In cells of this phenotype, only a small portion of the plasma membrane is directly exposed to the cytoplasm, whereas the bulk of the surface membrane covers microfilament bundles of MV and, thus, is separated from the cytoplasm by the cytoskeletal diffusion barrier. Cells exhibiting this type of surface morphology are still able to incorporate lipophilic compounds into surface membranes, but further delivery into the cytoplasm is severely restricted by the underlying cytoskeleton. Direct access of lipophilic compounds via the cytoplasm-facing membrane surface is also reduced because the intermicrovillar space is largely filled with surface coat components.

Role of the surface coat for uptake of lipophilic compounds. Differentiated epithelial cells including hepatocytes, glial cells, and adipocytes express a complex surface coat consisting of PGs and glycosaminoglycans (GAGs) that are tightly associated with microvillar cell surfaces. PGs are noncovalently bound to GAGs via cationic bridges, whereas GAGs are anchored by their transmembrane core proteins to the submembrane actin skeleton. Both types of coat components represent negatively charged polyelectrolytes resembling cation exchangers. The highly hydrated, swollen matrix of this surface coat acts as an additional diffusion barrier for lipophilic molecules, especially for cationic compounds, shielding even those regions of the plasma membrane that have direct access to the cytoplasm.

Moreover, cationic xenobiotics, by competing with the polyvalent cationic bridging components, should be
able to release noncovalently bound GAGs from the cell surface. Thus it can be assumed that basic xenobiotics are bound to the surface coat and released as a complex with surface coat components.

In contrast to resting and differentiated cells, dividing cells release PGs from the surface of exocytic blebs (39, 44, 50–52, 72, 75, 79, 119, 122), leaving the plasma membrane unprotected.

**Dynamics of membrane lipids on microvillar surfaces.** Besides the passive barriers against lipophilic toxins, MV, and surface coats, an additional, active mechanism of drug extrusion is operating on microvillar surfaces. As depicted in Fig. 2, actin filaments, nucleated at the inner face of exocytic blebs, initiate the formation of the microvillar shaft structure. Elongation of the shaft region, however, gives rise to a flow of membrane components from the plain cell surface into elongating MV. Thus growing MV represent lipid sinks on the cell surface into which phospholipids and lipid-associated membrane components are drafting as long as formation/elongation of MV proceeds (Fig. 4).

The isolated rat hepatocyte (Fig. 3) is an excellent example for demonstrating the vast amount of microvillar lipid membrane located on the cell surface. The basal (and apical) surfaces of hepatocytes and other epithelial cells preferentially consist of tightly stacked MV. The maintenance of this surface organization is a highly dynamic process. Because of the action of growth factors, hormones, and other signaling molecules that stimulate exocytosis, formation and elongation of MV from exocytic blebs continuously convey lipid components of the plasma membrane into the microvillar shaft region. Lipophilic xenobiotics, associated with phospholipids or coat components of the plasma membrane, are carried along with these components from the cytoplasm-facing membrane to the much less hazardous location on MV. Subsequent release of membrane lipids, including their associated xenobiotics, may occur via vesiculation of MV or secretion with surface coat components as discussed below.

Because of the polarized organization of epithelial cells, the flow of lipids within the plasma membrane of this cell type follows different and rather complex dynamics.

**Lipid movement within the surface of stimulated epithelial cells.** The surface of polarized epithelial cells is specifically organized into three functionally different domains. The basal surface contains receptors, transporters, and ion channels used for uptake of metabolic substrates and hormonal regulation of cell functions. The apical side is part of the external body surface and represents the secretory site of epithelial cells. At the apical pole, waste products of cellular metabolism as well as xenobiotics and their metabolites are secreted.

Because of the functional compartmentation of epithelial surfaces, receptor-stimulated secretion results in a complex redistribution of membrane components from the basal to the apical pole. The main features of epithelial lipid transfer are summarized in Fig. 5. According to the present state of knowledge about epithelial surface organization and Ca²⁺ signaling, receptor-
stimulated exocytosis (secretion) proceeds as follows. 1) Receptor stimulation on the basal surface activates a phospholipase C-coupled pathway and initiates a delayed n-myo-inositol 1,4,5-trisphosphate (IP$_3$)-induced cytoplasmic Ca$^{2+}$ signal at the apical pole of the cell (71, 82, 85, 112). This polarized signal generation most likely is due to the absence of IP$_3$ receptors in the basal part of epithelial cells (57). 2) Apical Ca$^{2+}$ signaling stimulates exocytosis of trans-Golgi vesicles at this cell pole, the physiological site of secretion. Newly formed exocytic membrane domains (surface blebs) are starting points for the outgrowth of new apical MV. 3) Receptor stimulation on the basal cell surface and the subsequent disorganization of basal MV (58, 65, 69) cause membrane lipids (and proteins) of these MV to become integrated into the cell surface, while MV elongation at the apical pole draws lipid components into the apical direction. Thus loss of basal MV combined with formation and/or elongation of apical MV draws membrane lipids from the basal to the apical surface of epithelial cells, generating a flow of membrane components to the apical pole. 4) The basal-to-apical phospholipid flow carries membrane-associated xenobiotics from the basal to the secretory pole of the cell.

The general principle of basal-to-apical lipid fluxes results from the spatial separation and functional specialization of apical and basal surfaces in polarized epithelial cells. Receptors are preferentially localized on basal surfaces, whereas the primary Ca$^{2+}$ signal and exocytosis (secretion) are confined to the apical pole. The lateral membrane region adhering to the neighboring cells spatially and functionally separates basal and apical membrane domains. The relative size of basal-to-lateral-to-apical surfaces of hepatocytes amounts to 70:15:15 (Fig. 5).

Receptor-mediated activation at the basal surface results in the well-known shape change of MV forming ballooned surface protrusions that are integrated into the plasma membrane (58, 65, 69). This process activates a large amount of membrane lipids used at the apical pole for elongation of both newly inserted exocytic blebs and existing MV and for excretion of lipid-associated xenobiotics via vesiculation into the luminal space. At later stages of stimulation, the intracellular Ca$^{2+}$ signal spreads over the whole cytoplasm, and exocytosis with MV regeneration also occurs at the basolateral cell surface (37, 71, 82, 85, 109, 112, 113).

An excellent example of the enormous extent of the apical lipid transfer was published by Shepard et al. (97–99), who observed extreme MV elongation on the apical side of the neural tube epithelia after metabolic stress. Apical surfaces became rapidly covered with a dense meshwork of extremely long, thin MV (10 times the normal length). The morphological appearance of this meshwork, resembling a fleece of textile fibers, has given this effect the name “matting.”

The proposed mechanism of epithelial secretion is in accordance with the physiological function of this cell type in defense against xenobiotics. Several biochemical processes are involved in the vectorial transport of xenobiotics by epithelial cells.

The function of P-glycoprotein as ATP channel: xenobiotic signaling through ATP efflux. Acquired MDR is generally associated with enhanced expression of specific membrane glycoproteins that are also components of normal epithelial cell systems. These phosphorylated glycoproteins (P-glycoproteins) are involved in active drug extrusion of multidrug-resistant cells. However, the mechanism of this process remained unclear. Until now, two properties of the MDR-associated drug extrusion have been clearly established. First, drug extrusion depends on the presence of intracellular ATP. Second, P-glycoproteins expressed in MDR, such as the multidrug resistance-associated proteins MDR1, MRP1, and MRP2, are ATP-conducting anion channels (1, 11, 93). MRP2, identical to the hepatic canalicular multispecific organic anion transporter (cMOAT), is also involved in hepatic bile acid transport.

Considering these findings, the function of P-glycoprotein as a drug extrusion pump appears rather unlikely. Recent experimental data show that P-glycoprotein is able to equilibrate cytoplasmic ATP with the external milieu within seconds (1). Consequently, the presence of active P-glycoproteins would lower the ATP concentration immediately below the plasma membrane, which contradicts their postulated function as an ATP-driven drug pump. Therefore, some authors have proposed a purinergic paracrine or autocrine function for released ATP (116). For instance, Roman et al. (93) reported that a hepatoma cell line overexpressing MDR proteins accelerates (6 times) the regulatory volume decrease (RVD) by hypoxic hepatocyte swelling. These cells also exhibit threefold higher ATP efflux rates. Moreover, capturing of extracellular ATP or blockade of P2 receptors abolishes RVD in parental cells and reduces RVD in MDR-overexpressing cells.

ATP release was also observed in isolated hepatocytes in response to both osmotic swelling (116) and mechanical stimulation (95). Furthermore, released ATP can induce Ca$^{2+}$ signaling also in neighboring hepatocytes or bile duct cells via purinergic receptors of the basal surface (95). The authors concluded that “increases in cell volume lead to efflux of ATP through opening of a conductive pathway consistent with a channel and that extracellular ATP is required for recovery from swelling.”

These findings suggest the existence of an autocrine mechanism involved in the volume response of hepatocytes. As shown by Sukhorukov et al. (105), hyposmotic cell swelling is functionally coupled to shortening of MV. When physicochemical methods were used, three different cell lines yielded similar results, indicating that hyposmotically stressed cells obtain the necessary membrane lipids by using material from MV. As a consequence of the lowered microvillar diffusion barrier, efflux of cytoplasmic ATP through anion (MDR/CFTR/Cl$^{−}$, where CFTR is the cystic fibrosis transmembrane conductance regulator) channels occurs. Subsequent purinergic stimulation by external ATP completely opens the microvillar diffusion pathways, allowing rapid RVD by massive K$^{+}$ and Cl$^{−}$ efflux as recently discussed (61).
This scheme of a self-amplifying mechanism in RVD, triggered by cell swelling, is further supported by the finding that P-glycoproteins are expressed in differentiated hepatocytes (3, 14, 70, 96) and by the demonstration of a 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS)-sensitive ATP efflux pathway in vesicles isolated from MV of hepatocytes (57). Thus, as proposed by Wang et al. (116) and Schlosser et al. (95), released ATP may function as a physiological coupling factor between swelling-induced mechanical membrane stress and the activation of the Ca\(^{2+}\) signal pathway necessary for an optimal activation of K\(^+\) and Cl\(^-\) channels during RVD.

A quite similar mechanism of coupling between the action of xenobiotics and cellular Ca\(^{2+}\) signaling is proposed below.

**ATP as coupling factor between the membrane action of lipophils and the export mechanism.** Scanning electron microscope studies have shown that short-term exposure (1–2 min) of isolated rat hepatocytes to low concentrations of organic lipophilic compounds such as toluene and styrene results in activation of the microvillar structure in a manner quite similar to that of Ca\(^{2+}\) signaling by vasopressin receptors (34). The observed morphological changes (ballooning of MV) on the hepatocyte surface are characteristic for the abolishment of the microvillar diffusion barrier for external ions and other low-molecular-weight compounds that can leave or enter the microvillar tip compartment via transporters or channels (57, 58, 65, 67–69). The same microvillar shape changes are produced by the lipophilic tumor promoters and Ca\(^{2+}\) store effectors phorbol myristate acetate and thapsigargin (58, 65). In some cases (e.g., toluene, ethanol), these effects are reversible. Within 1 h of the continued presence of the lipophils, the cell surface is restored to almost the original state (34).

Various reports document the activation of Ca\(^{2+}\) signaling induced by lipophilic xenobiotics (5, 6, 16, 27, 38, 46, 49, 55, 81, 83, 89, 92, 110, 120), indicating that the nonspecific action of lipophilic compounds is constitutively coupled with Ca\(^{2+}\) signaling. The effects of aromatic compounds on the microvillar morphology are rapid, occurring within a minute after addition. Thus changes of physical membrane properties due to intercalation of lipophilic compounds into the lipid membrane, as proposed by Engelke et al. (25, 26), may be responsible for the observed opening of the microvillar influx pathways for cations and anions. Uptake of lipophils into the hydrophobic region of the microvillar membranes most likely represents the primary process of xenobioteic action on the cell. Subsequent detachment of the swollen and fluidized lipid bilayer from the microvillar actin filament bundle opens influx and efflux pathways for Ca\(^{2+}\) and ATP via microvillar cation and anion channels, respectively. Pathways for both ions have been detected in MV of hepatocytes and hamster insulinoma cells (57, 64). Thus, like swelling-induced RVD, the action of lipophilic chemicals may be coupled via ATP efflux and autocrine activation of purinergic receptors to Ca\(^{2+}\) signaling. Several recent reports about the microvillar localization of P-glycoprotein (41, 106) and CFTR (28, 107) support the assumption that ATP efflux through microvillar pathways is possible.

Proceeding from these findings, the most plausible events following xenobiotic action on the plasma membrane are the initial partial opening of the microvillar ionic pathways, as indicated by the observed detachment of the microvillar lipid membrane from the microfilament bundle, followed by ATP efflux and autocrine purinergic stimulation on the basal cell surface. Subsequent apical exocytosis inserts functional ATP channels (P-glycoprotein) into the apical pole of the cell. Apical ATP secretion (13, 94) further potentiates and prolongs the secretory Ca\(^{2+}\) signal via apical receptors (37). Finally, termination of the xenobioteic response mechanism depends on the restoration of the apical diffusion barrier system.

Some of the basic requirements for this mechanism have been established in isolated rat hepatocytes: the activating effect of lipophilic compounds on the microvilli of hepatocytes was demonstrated (34); an ATP efflux pathway that is sensitive to the anion channel blocker DIDS is present in microvillar membranes of isolated rat hepatocytes (57); and ATP activates Ca\(^{2+}\) signaling in hepatocytes via purinergic receptors on the basal (20, 42) and apical hepatocyte surface (Fig. 6B) (37). As shown in Fig. 6B, rapid shape changes of MV occur on the basal cell surface of isolated rat hepatocytes after short exposure to extracellular ATP (0.1 mM). These shape changes closely resemble vasopressin effects on hepatocytes (58) in that basal MV are transformed to ballooning membrane protrusions within a few minutes after addition of external ATP (Fig. 6B). MV recovered within 1 h of prolonged ATP exposure, exhibiting significantly elongated appearance (Fig. 6C). MV elongation may be due to entry of external ATP into the microvillar tip compartment and ATP-induced elongation of microfilaments at their fast-reacting barbed ends.

The events initiated by the action of lipophilic xenobiotics at the basal cell surface may be summarized as follows.

**TRIGGER MECHANISM.** Because of the uptake of lipophilic xenobiotics, the physical properties of the basal microvillar membranes are changed (fluidity, volume). These changes cause detachment of the membrane from the microvillar actin filament bundle, accompanied by loss of the diffusion barrier function (Fig. 7A).

**SIGNAL AMPLIFICATION.** Cytosolic ATP is released through ATP-conducting anion channels of the basal microvillar membranes (Fig. 7A). Released ATP activates basal purinergic receptors and stimulates exocytosis at the apical pole of the cell via the Ca\(^{2+}\) signaling pathway (Fig. 7A). Insertion of new P-glycoprotein/ATP channels into the apical surface gives rise to ATP efflux at the apical pole (Fig. 7B). Apical ATP 1 stimulates apical purinergic receptors and 2 increases MV length, most likely by entering the tip compartment of existing MV via anion channels (Fig. 7B). Elongation of existing MV and newly inserted exocytic blebs draw
lipid components from the basal to the apical pole of the cell (Fig. 7C).

TERMINATION. The process is terminated when, in the absence of lipophilic xenobiotics, regular basal and apical MV are restored and ATP efflux is decreased (Fig. 7C).

Together, the proposed series of events establishes a complete mechanistic strategy of xenobiotic defense, initiated by the action of lipophilic xenobiotics at the basal cell surface, followed by Ca\(^{2+}\) signaling, apical exocytosis of functional ATP channel proteins, and...

Fig. 6. SEM of the surface of isolated rat hepatocytes. A: control. B: hepatocyte exposed to 0.1 mM ATP for 5 min. Shape changes of MV closely resemble those observed during vasopressin-induced Ca\(^{2+}\) signaling (58). C: hepatocyte exposed to 0.1 mM ATP for 1 h.

Fig. 7. A: basal epithelial surface. Change of bilayer volume and fluidity by intercalation of lipophilic compounds results in detachment of the swollen membrane from the microfilament bundle of MV and opens the pathway for ATP efflux via anion channels. IP\(_3\), d-myo-inositol 1,4,5-trisphosphate. B: apical surface showing exocytosis induced at the apical pole by stimulation of purinergic receptors on the basal surface. Insertion of ATP-permeable anion channels results in ATP release at the apical surface that, in turn, activates apical purinergic receptors and enhances exocytosis and MV formation and elongation at this side. C: scheme of the total proposed epithelial defense mechanism against the action of xenobiotics on the basal cell surface.
apical ATP efflux. Subsequent formation and elongation of apical MV generate a lipid draft to the apical pole of the cell from where lipid-associated xenobiotics are excreted into the bile fluid (Fig. 7C). According to this scheme, expression of P-glycoprotein/ATP channels in differentiated epithelial cells serves to establish an autocrine/paracrine mechanism to amplify basal-to-apical lipid fluxes in response to xenobiotics.

Final steps of detoxication involve metabolism of the xenobiotics, followed by secretion of lipids and surface coat components together with xenobiotics or their derivations from the apical pole of the cell.

Secretion of membrane lipids and associated xenobiotics from the apical surface. The process of apical lipid secretion has been extensively studied in hepatocytes. The following findings are clearly established: experiments with altered MDR genes in cells and animals have shown that secretion of phospholipids into the bile duct completely depends on the expression of P-glycoproteins (24, 86, 87, 101, 114); secretion of apical lipids and other membrane component occurs by formation of lipid vesicles at the apical surface of hepatocytes (2, 15); and these vesicles contain apical marker proteins (2) such as alkaline phosphatase (2, 17, 21, 22) and P-glycoprotein itself (3).

Electron microscope studies have demonstrated that these vesicles are formed by detachment of the exoplasmic leaflet of the canalicular membrane bilayer in hepatocytes. These findings strongly suggest that phospholipids as well as integral membrane proteins can be secreted directly from apical cell surface, most likely via vesiculation membrane components. This process is accelerated by the detergent-like bile fluid (15). The observed diameter of secreted vesicles of 60–70 nm closely corresponds to the diameter of MV of isolated rat hepatocytes (see Fig. 6A) (58).

Thus a constitutive plasma membrane clearing mechanism can be postulated for epithelial cells. Membrane phospholipids with different ionic and hydrophobic binding properties are used as molecular transport vehicles to convey a variety of chemical compounds such as cholesterol, bile components, organic anions, cations, and neutral amphipaths from the basal cell surface into the bile fluid (19, 88, 102). On the other hand, the same mechanisms can be activated by lipophilic xenobiotics on the apical surface alone. In this case, the secretion process is initiated by activation of the apical, instead of the basal, ATP efflux via MRP/ABCC channels. However, apical MV are considerably stabilized by the presence of membrane microfilament linker proteins such as villin, fimbrin, and myosin I. Consequently, they exhibit increased morphological resistance against lipophils compared with their basal counterparts.

ATP-dependent transbilayer flipping of phospholipids during MV formation: properties of lipid bilayers with high curvature. Another novel aspect has been introduced into the discussion of MDR mechanisms by the observation that phospholipids, originally located at the inner leaflet of the plasma membrane, are translocated to the outer leaflet of the plasma membrane in an ATP-dependent manner. Because the transfer of phospholipids from one bilayer to the other is an energetically unfavorable process, an ATP-consuming enzymatic mechanism for driving this process was postulated. Accordingly, P-glycoprotein was supposed to function as a “phospholipid transferase” or “flippase,” externalizing drugs by an ATP-dependent cotransfer with lipids. However, the mechanism of P-glycoprotein-mediated phospholipid flipping has remained just as unclear as the original assumption of a drug pump. The microvillar lipid-flow mechanism offers a simple explanation for the observed transbilayer movement of phospholipids, which turns out to be an intrinsic feature of the MV formation.

MV formation/elongation is a process that forces lipid bilayers into energetically higher conformational states. During the elongation of MV on the cell surface, phospholipids are to be transferred from the even surface with relative low curvature into the microvillar shaft region with an extremely high membrane curvature. As depicted in Fig. 8, transition of the nearly planar surface of the plasma membrane (thickness: 5 nm) into the microvillar shaft region with an outer diameter of 35–50 nm (determined on isolated hepatocytes) causes spatial crowding of phospholipids at the inner leaflet. According to the difference between the circumference of the outer leaflet and that of the inner leaflet, between 10 and 15% of the phospholipids of the inner bilayer have to change to the outer layer to match the spatial requirements of this structure. Most likely, this lipid transfer occurs at the bases of growing MV at which the shaft diameters appear to be smaller than at the upper region. Within this transition region, the original bilayer order of the membrane phospholipids becomes highly disturbed because of the transformation of the original, almost even, plasma membrane into a complicated topology consisting of two high-curvature components rectangularly oriented to each other.
Ultimately, ATP that is used for elongation of the microvillar microfilament bundle provides the energy necessary for lipid translocation. Thus the whole process may be characterized as ATP-dependent phospholipid translocation from the inner to the outer membrane leaflet. On the other hand, any process that impairs the microvillar structure such as, for instance, receptor-mediated signaling, is known to reverse the asymmetrical phospholipid distribution.

Most likely, some phospholipids, especially amino-phospholipids, are preferentially retained at (or transferred to) the inner face of the microvillar membrane because they are involved in binding to microfilaments (115). Negatively charged phospholipids including phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine are linked by divalent phosphoinositols, phosphatidylinositol anchors, phosphatidylcholine, and phosphatidylethanolamine are linked by divalent cation bridging (via Mg$^{2+}$ or Ca$^{2+}$) to the negative surface of microfilaments (35, 103, 104). Conspicuous experimental evidence for this type of interaction comes from the formation of planar paracrystalline arrays of actin filaments on the surface of lipid sheets in the presence of micromolar concentrations of Ca$^{2+}$ or Mg$^{2+}$ as demonstrated by electron microscopy (35, 103, 104). The preference of acidic phospholipids at the inner leaflet of microvillar membranes implies that phospholipids associated with weakly basic xenobiotics may remain within or translocated to the outer leaflet of the microvillar membrane.

The systematic coupling of MV formation with transbilayer movements of phospholipids integrates a further well-established aspect of MDR into the novel concept. This mechanism enables the cell to get rid of even those xenobiotics that have reached the inner face of the plasma membrane and are specifically bound to certain acidic phospholipids. The proposed mechanism may have relevance for drug export in both polarized and nonpolarized cell types.

**Secretion of xenobiotics complexed with GAGs.** Besides excretion of xenobiotics via membrane vesiculation, an additional type of export mechanism may occur at the apical surface of epithelial cells. As mentioned earlier in this paper, the surface coat of the luminal brush border is composed of several types of negatively charged (sulfated) high-molecular-weight polysaccharides. The molecular properties of this surface coat closely resemble those of strong cation exchangers able to bind cationic or polar molecules with high affinity and to protect the plasma membrane against compounds of this type (7). In addition, the surface coat acts as a diffusion barrier for large molecules in a molecular sievelike manner (77).

Surface coat components are connected with the cell surface in at least three different ways. 1) PGs are anchored to the cell surface by their transmembrane core proteins. The cytoplasmic domains of these proteins allow the attachment of actin filaments, stabilizing the whole membrane domain by forming a cage-like structure. 2) Another type of PG is connected with the cell surface via lipid (phosphatidylinositol) anchors. 3) In contrast to PGs, GAGs are noncovalently attached to components of the surface coat by bridging divalent cations, such as Ca$^{2+}$, Mg$^{2+}$, or organic polyamines.

Release of anchored coat molecules can be achieved by proteolytic cleavage of the core protein or hydrolysis of the phosphatidylinositol anchors by surface lipases via receptor-mediated signaling. In contrast, release of GAGs merely needs exchange of the polyvalent ionic bridges for other cationic compounds with lower bridging power. In this case, cationic xenobiotics that have reached the external leaflet of the microvillar membrane may cause their own shedding as a complex with surface GAGs (Fig. 9). The whole process is accelerated by specific signaling events activating ectoproteases and ectolipases.

Apical secretion of xenobiotics complexed with PGs and GAGs adds a further mechanistic feature to the delineated physiological export pathway for lipophilic and basic xenobiotics.

**The integrated concept of xenobiotic processing: possible role of the cytochrome P-450 system.** Several putative microsomal proteins, such as the insulin-sensitive glucose transporters of adipocytes, the IP$_3$-sensitive Ca$^{2+}$ store, and some ion pathways, have been detected in microvillar membranes of different cell types (57, 58, 62–66). With the use of the classic subcellular fractionation techniques (by Teflon-glass homogenizer), most of these microvillar proteins have been found in microsomal fractions and, therefore, are generally believed to be components of intracellular membrane systems. In contrast, microsomal fractions are lacking microvillar components when the low-force shearing of MV from the cell surface precedes conventional cell fractionation (57, 62). These findings strongly suggest that still other systems, usually assigned to intracellular membranes, may be, in fact, of microvillar origin. One candidate is the drug-metabolizing cytochrome P-450 (CYP) system. It appears to be widely accepted knowledge that the functional relevant pool of the drug-metabolizing CYP system is localized in intracellular membranes of the endoplasmic reticulum. However, if one considers that the essential
function of these enzymes is protection of sensible intracellular (cytoplasmic, ribosomal, and nuclear) target systems against toxins, this location appears to be rather unfavorable.

A critical examination of the relevant literature revealed that the exclusive intracellular function of these enzymes has never been soundly proved and that, in fact, the microvillar localization (and function) of CYPs and related enzymes has been demonstrated in a series of publications. In a very early study, Bruder et al. (12) detected cytochrome b$_5$ and CYP in plasma membranes isolated from MV of the chick and rat intestinal epithelium. The authors concluded that the cytochrome-containing redox systems are general components of the cell surface and suggested a possible function of these enzymes in plasma membranes. Similar results were published by Gimenez-Gallego et al. (36) and Garcia et al. (32) for renal brush-border membranes, by Takesue and Sato (108) in MV of intestinal mucosa cells, and by Naganuma et al. (84) and Kitawaki et al. (53) using immunoelectron microscopy to localize aromatase CYP in MV of human placental syncytiotrophoblasts. Cell surface labeling (NHS-LC-biotin) was used by Amarn and Simpson (4) to demonstrated the presence of aromatase CYP, 17α-hydroxylase CYP, and NADPH:CYP reductase on the surface of cells in which these enzymes were expressed either endogenously or after transfection. Applying immunoelectron microscopy, Lester et al. (73) demonstrated the occurrence of CYP on apical MV of the rainbow trout hepatocytes, and Loepfer et al. (74) showed that various isozymes of CYP are localized and functionally active on MV of human hepatocytes. The latter study was initiated because CYP antibodies are present in several forms of autoimmune hepatitis, indicating that CYP isoforms are present on the plasma membrane of hepatocytes.

These findings suggest a novel scheme of xenobiotic handling that combines the proposed drug extrusion by vectorial lipid flow with drug metabolism into an integrated system of xenobiotic defense: Lipophilic compounds associated with plasma membrane are conveyed on the shortest possible way, without leaving the membrane, to apical MV in which they are processed by drug-metabolizing enzymes. Oxidative and conjugative steps transform lipophilic xenobiotics into negatively charged water-soluble compounds that can leave the microvillar compartment through the large anion channels such as the multispecific organic anion transporter (MOAT = MRP2) present in MV of the apical cell surface.

The complete scheme of xenobiotic handling by epithelial cells is shown in Fig. 10. Lipophilic xenobiotics in the plasma membrane are conducted by the induced lipid flow to the apical pole of the cell. Because of transmembrane flipping, phospholipid-bound xenobiotics are transferred from the inner bilayer of the plasma membrane to the outer bilayer of the microvillar membrane and subsequently secreted as a complex with surface coat components. Xenobiotics remaining at the inner face of the microvillar membrane are oxidized by microvillar CYPs and released as anionic oxidation or conjugation products via microvillar anion channels into the external milieu.

To get rid of unwanted compounds, peripheral drug metabolism would be a much more plausible defense strategy than intracellular processing. The peripheral localization of the CYP system beyond the microvillar diffusion barrier would protect sensible cellular systems such as DNA, RNA, and protein synthesis, the main targets of cytotoxic attack, against cytoxins and their sometimes more toxic oxidation products.

Two main components of the proposed integrated xenobiotic defense system, the lipid flow mechanism and the apical localization of functional CYP enzymes, are clearly supported by the experiments of Gan et al. (31). Using polarized Caco-2 cells, they studied the polarized transport of cyclosporin A and formation of its metabolite. The authors found that during apical exposition of cyclosporin A, the amount of the accumulating metabolite was much greater on the apical pole than on the basal side, and the formation of the metabolite on the apical side of the monolayers was much greater during apical exposition than during basal exposition. Gan et al. interpreted their results as “P-glycoprotein pump-mediated efflux of the metabolite to the apical side.” Preferred basal as opposed to apical drug transport was also observed by Yamaguchi et al. (121) in the same cell model.

Anion channels or transporters play an essential role in the proposed mechanism of drug metabolism. To protect the cell against toxic drugs, their water-soluble metabolism products must be able to leave the microvillar tip compartment immediately after they have been generated. Recent studies have begun to clarify the function of some members of the MRP family. To
date, six different homologues of MRP (i.e., MRP1–
MRP6) have been identified. All these MRPs are or-
geanic anion transporters; i.e., they transport anionic
drugs and neutral drugs conjugated to acidic ligands,
such as glutathione, glucuronic acid, or sulfate. MRP1
acts synergistically with the conjugating glutathione
S-transferase and UDP-glucuronosyltransferase and
confers resistance to the toxicities of some electrophilic
drugs and carcinogens. MRP1 (as well as MRP2 and
MRP3) can also cause resistance to neutral organic
drugs that are not known to be conjugated to acidic
ligands, presumably by transporting these drugs to-
gether with free GSH. MRP2 (cMOAT) has been local-
ized to the apical membrane of several polarized epi-
thelia and, particularly, to the canalicular membrane
of hepatocytes. The absence of functionally active
MRP2 glycoprotein from this membrane domain pre-
vents the secretion of many anionic conjugates into
bile. MRP2-mediated conjugate export represents a
decisive final step in the detoxification of drugs, toxins,
and endogenous substances. MRP3 mediates the trans-
port of anionic conjugates, particularly that of glu-
curonides and sulfon conjugates, across the basolateral he-
patocyte membrane into sinusoidal blood. MRP4,
expressed in high levels in prostate tissue, probably
functions as anion efflux pathway whose substrate
range includes glutamate and phosphate conjugates.
MRP5 and MRP6 have been found on the apical (lumi-
nal) side of brain microvessels endothelial cells. This
orientation implies xenobiotic transport from brain
to blood.

The general function of MRPs as anion channels or
transporters clearly supports the proposed scheme of
peripheral drug metabolism. The great diversity of
this protein family further points to the importance
of the MRP-dependent anionic excretion pathways in
both normal xenobiotic handling and acquired MDR.
The proposed concept of xenobiotic processing on mi-
crovillar cell surfaces provides, for the first time, a
systematic explanation for the role of MRPs in xenobi-
otic defense.

According to the central role that cell cycle control
and differentiation plays in expression of MDR, further
MDR-regulated proteins may exist that are compo-
ents of their control systems. Examples of this type of
MDR-regulated protein are the topoisomerase that is
(inversely) regulating G-phase length and the lipopro-
tein receptor-related protein that is probably involved
in inhibition of surface coat proteolysis.

The Microvillar Cell Surface as a Barrier Against
Hydrophilic Cytotoxins

In addition to lipophilic cytotoxins, cells have to
resist a great variety of hydrophilic toxins. For most
of the hydrophilic hazardous compounds, the lipid
plasma membrane represents an effective barrier.
However, toxic anions and cations can use ion channels
of the cell surface to enter the cytoplasm. The microvi-
llar cell surface represents an effective barrier against
this type of intoxication.

In recent publications (57, 64), influx pathways for
anions and cations have been identified as components
of the microvillar tip membrane in differentiated cells.
The activities of these channels generally depend on
the properties of the underlying cytoskeletal diffusion
barrier of microfilaments (reviewed in Ref. 60). The
cation exchanger properties of the microvillar actin
filament bundle (exhibiting external low-affinity bind-
ing sites for monovalent and divalent cations) impart
to this polymeric structure differential resistance prop-
erties that largely depend on the charge number of the
cation. Thus, although slightly permeable to K+,
microvillar pathways are impermeable to divalent cat-
ions, such as Ca2+ and Mg2+, as well as to highly
cytotoxic polyvalent heavy metal ions. However, all
cations that can pass the microvillar cation channels
readily enter the cell body when the diffusion barrier
system is abolished, e.g., during Ca2+ signaling.

Sensitization is an important consequence of the
concept of microvillar regulation. Sensitization to toxic
compounds accompanies not only receptor-mediated
activation but also several other types of microvillar
activation, including membrane stretch by external
shear forces or hyposmotic cell swelling, hyperthermia,
irradiation, and lipophilic xenobiotics, which are all
known to affect the structural organization of MV.
Similarly, dedifferentiation or stimulation of growth is
invariably associated with increased sensitivity to ionic
and lipophilic toxins. Enhanced sensitivity of embry-
onic tissue and cancer cells to cytotoxins is a physio-
logical consequence of the presented concept.

ACQUIRED MDR: A COMPLEX CELLULAR STRATEGY

Mechanism of Volume and Surface Reduction

Early experimental evidence for MDR on the cellular
level has suggested that MDR is due to reduced accu-
mulation of cytotoxic drugs in the cell by both de-
creased uptake and increased efflux.

Experiments such as those represented in Fig. 11
belong to the most frequently cited results document-
ing the function of a drug extrusion mechanism. The
main results of these experiments can be characterized

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Fig. 11. Acquired multidrug resistance decreases drug influx and
increases ATP-dependent drug efflux. The scheme shown comprises
the results of different studies.
as follows. Drug-sensitive cells take up lipophilic drugs to a high intracellular level. In contrast, resistant cells with intact ATP production exhibit a lower rate of drug uptake and a lower final amount. ATP-depleted resistant cells exhibit uptake kinetics identical to those of drug-sensitive cells. Reactivation of ATP production in ATP-depleted resistant cells preloaded with a drug initiates a rapid drug efflux, reducing the intracellular amount to nearly the same final value as that observed in resistant cells without prior ATP depletion.

How are these findings to be reconciled with the presented mechanistic concept? As shown in Fig. 4, rat hepatocytes, as typical drug-resistant and drug-metabolizing epithelial cells, are densely covered with MV. The differentiation-dependent change of the surface morphology has also been demonstrated for 3T3-L1 cells, lacking MV as undifferentiated fibroblasts but exhibiting microvillar surfaces when differentiated to adipocytes (69). The consequences of extensive MV formation for cell volume and cytoplasmic surface are depicted in Fig. 12.

ATP-dependent formation and elongation of MV is accompanied by salt and water extrusion from the cytoplasm and a corresponding volume reduction. On the other hand, ATP depletion leads to a reduced length, number, and shape of MV (45, 47, 56); consequently, salt and water uptake results in cell swelling (105). Thus, in the absence of metabolic inhibitors, the slower rate of drug uptake is due to the reduced interface between cytoplasm and lipid membrane, and the smaller amount of drug taken up at equilibrium is the result of a reduced cytoplasmic water space.

Reactivation of ATP production restores the microvillar surface morphology, decreasing cell volume and total drug content again. Starting from resistant cells preloaded with the drug under conditions of inhibited ATP production, restitution of ATP synthesis is followed by drug extrusion according to the reduced water space. Although this type of drug extrusion is accompanied by salt and water extrusion from the cytoplasm and a corresponding volume reduction, the overall result is that of an ATP-dependent outward pumping process, in which ATP is used for the growth of microvillar actin filaments.

Sukhorukov et al. (105) studied the specific relation between cell volume and MV morphology for several cell types. Using physical methods, the authors found that hyposmotically stressed cells obtain the membrane area necessary for swelling by using material from MV. Roman et al. (93) demonstrated the close relation between cell volume regulation and drug resistance. These findings underscore the specific ability of resistant cells to undergo rapid ATP-dependent volume changes in response to changes in intracellular and extracellular ATP. Thus the results of classic MDR experiments, as depicted in Fig. 11, can be completely explained by the specific properties of cell types with microvillar surfaces.

Acquired Cell Cycle Properties of MDR Cells

The expression of microvillar cell surfaces occurs during differentiation or growth arrest (9, 63, 69). MV are formed in the G_0/G_1 predifferentiation state and after the onset of differentiation. The G_0/G_1 phase is characterized by restricted cell growth and limited protein synthesis but a high rate of phospholipid synthesis (reviewed in Ref. 43). The time periods that cells can stay in G phases are rather long and extremely variable, reaching from hours to months and even years for nerve cells.

Cycling is actively controlled at two restriction points in G_1 and G_2 phases. At the G_1 restriction point, general cellular conditions necessary for a successful entry of the cell into a new cycle are checked. These conditions comprise cell contact to growth substrate, the presence of growth factors and nutrients, and a certain rate of protein synthesis. If one or several of these factors are lacking, cells enter the G_0 phase in which the production of components of the cycle control system, cyclin-dependent kinases and cyclins, is stopped, and cells are prevented from reentering the mitotic cycle. Even after the addition of growth factors, it takes several hours for the cycle to pass the G_1 restriction point. Another restriction point, G_2, also contributes to the overall duration of the mitotic cycle. At G_2, internal preconditions for mitosis, such as completion and correctness of DNA replication, are checked.

Thus cycle control largely depends on the main targets of cytotoxic compounds such as nucleotide and protein synthesis. Consequently, cells exposed to a toxic environment generally stay in the quiet G_1/G_0 state in which microvillar surfaces were formed (9). In this state, the existence of a P-glycoprotein-independent but density-dependent state of drug resistance in cultured cells has been observed (33, 117, 118). Under these conditions, the cells are able to clear their plasma membranes from lipophilic toxins and are less sensitive to their action because several drug-sensitive cellular processes such as DNA, RNA, and protein synthesis are resting.

Clonal selection of cell types with prolonged G phases (as observed in neuronal cells) may further contribute to acquired drug resistance of cancer cells treated with cytotoxins. Thus prolongation of the quiet states may be part of the mechanism of acquired MDR in tumor cells.
Several recent findings support this picture of drug resistance. MDR is induced by various differentiating agents or conditions (29, 33, 41, 78, 91) such as butyrate (40), dimethyl sulfoxide (80), dimethylformamide (80), dexamethasone (14, 96), and heat shock (90). In dense cultures of a colon cancer cell line, a P-glycoprotein-independent drug resistance develops, which is accompanied by an accumulation of the cells in the G0/G1 phase. This type of resistance is reduced when cells are forced to enter the S and M phases of the cell cycle (33). Growing multicellular prostate tumor spheroids develop quiescent cell subpopulations in central regions with features of intrinsic multilcell-mediated drug resistance (117, 118). MDR is a constitutional property of differentiated hepatocytes (70) and epithelial cells (54, 91). Expression of P-glycoprotein is positively correlated with differentiation in tumor cells (10, 48, 54, 91). Reduced growth rate and decreased tumorigenicity correlate with a high level of MDR in many cell lines (8).

CONCLUSION

The proposed hypothesis of xenobiotic defense reflects MDR as a complex cellular strategy to avoid environmental hazards in differentiated and resting cells. The involved mechanisms are based on the previously proposed principle of influx and efflux regulation via microvillar cell surfaces. Resistance against environmental lipophilic (and hydrophilic) xenobiotics is a constitutive property of differentiated epithelial cells that is lost in rapidly growing cells. During prolonged exposure to lipophilic cytotoxins, tumor cells restore the original state of resistance by development of a cell population with prolonged G phases, in which toxin-sensitive synthetic processes are delayed and drug extrusion is active.

The proposed principle provides a systematic basis for the interpretation of a wide variety of experimental data for which, until now, neither plausible mechanisms nor internal relations could be detected. Even such different and contradicting notions as the ATP-dependent membrane pump, the “vacuum cleaner” mechanism, the “lipid flipase,” the ATP channel function of P-glycoprotein, and the P-glycoprotein-independent forms of MDR are all integral parts of the presented mechanistic concept. Moreover, for the first time, a reasonably sensible integration of the drug-metabolizing CYP system into the epithelial xenobiotic defense system is possible.

Finally, the concept also leads to some important practical consequences. Because drug resistance is a property of normal tissue cells, all therapeutic maneuvers to abolish MDR lead to reduced resistance of normal tissue cells as well. The obvious consequence is an increase in the toxicity of the applied tumor drug, which inevitably affords reduced therapeutic dosages. Thus the advantage of an anti-MDR treatment is low unless the drug can be locally applied to the tumor alone. A similar feature of drug resistance is the phenomenon of sensitization, also called “collateral sensivity,” which, in the present concept, is to be expected under all conditions that activate the microvillar pathway. Thus sensitization occurs not only during receptor-mediated stimulation of ion and substrate fluxes but also under certain physical and chemical conditions such as metabolic depletion (2-deoxyglucose), mechanical membrane stress (hypertonic swelling), heat exposure (hyperthermia), magnetic field exposure, ultraviolet radiation, and exposure to lipophilic chemicals, all of which are known tumor sensitizers and effectors of the microvillar structure. The use of physical effects may be an especially promising way to override MDR because heat, field, and light or high-energy radiation can be easily focused on localized regions of the human body. Another technique of tumor sensitization may be cell cycle synchronization. Because tumor cells are highly sensitive in the S phase, drug application during S-phase accumulation (33) should be a promising way to affect a maximum number of tumor cells and to override the effect of G-phase prolongation.

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