Interactions of human monocytes with TMVs (tumour-derived microvesicles)

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Abstract
The tumour microenvironment represents a dynamic complex milieu, which includes tumour cells, cells of the immune system and other (cellular and non-cellular) components. The role of these particular ‘puzzle pieces’ may change substantially due to their mutual interactions. The present review concerns different opinions on interactions that occur between monocytes, tumour cells and TMVs (tumour-derived microvesicles).

Monocytes/macrophages in tumour progression: friend or foe?
Macrophages play an important role in many aspects of innate and adaptive immune response [6]. Moreover, they can alter their function whenever facing changes in the microenvironment. For example, macrophages recruited and activated by LPS (lipopolysaccharide), cytokines [e.g. TNF or IFNγ (interferon γ)] become M1 polarized type [6,7]. They produce high levels of TNF, IL-6, IL-12 and IL-23, but low levels of IL-10. M1 macrophages can eliminate micro-organisms and tumour cells [6,7]. On the other hand, M2 macrophages are oriented to scavenge cell debris, promote angiogenesis and adaptive immunity. Generally, M2 macrophages share an IL-12low, IL-23low, IL-10high phenotype, together with a high expression of scavenger and mannose receptors. M2 macrophages, depending on different stimuli used for their polarization, have been divided into three subtypes: M2a, stimulated by IL-4, IL-13; M2b, stimulated by immune complexes in combination with IL-1β or LPS (express IL-1R (IL-1 receptor) and TLR (Toll-like receptor)); and M2c, activated by glucocorticoids or IL-10 (high level of CD36, low HLA-DR expression) [6,7]. Recently, the M4 macrophages with specific pro-atherogenic capacities were described as an important population within atherosclerotic lesions [8].

Tumour-educated macrophages, usually described as TAMs (tumour-associated macrophages) share many similarities with M2 polarized cells [6,7]. In contrast with blood monocytes, which are cytotoxic to tumour cells, such TAM activity in the tumour bed is usually decreased. In vitro unresponsiveness of monocytes to repeated stimulation with tumour cells is referred to as selective deactivation of monocytes [9].

Within the tumour mass, other cells with immunosuppressive activity were also identified and named as MDSCs (myeloid-derived suppressor cells) [10]. Human MDSCs are composed of two subsets: monocytic MDSCs (CD14+ and granulocytic MDSCs (CD15+). Both are positive for CD11b and CD33, and negative/low for HLA-DR [11]. MDSCs and TAMs share several properties, yet also display distinct features, e.g. differences in phenotype and mechanism of action [12].
TMVs (tumour-derived microvesicles)

Interactions of monocytes/macrophages with tumour cells may proceed by a direct contact between cells, through engagement of various receptors (e.g. CD44), by juxtaepithelial interaction and via biologically active factors acting in paracrine or endocrine mode. In the tumour bed, similar to blood monocytes, macrophages may be exposed to TMVs, which interact with them in a ‘reccrining’ manner [13]. Generally, MVs (microvesicles) are defined as circular (spherical) fragments released from cytoplasmic or endosomal compartments during the cell’s lifespan. MVs are distinguished by size and origin into two groups: exosomes, which are smaller (30–100 nm), more homogeneous in size and released by the endosomal compartment, and ectosomes, also known as just microvesicles [14], which are larger (0.1–1 μm) and are released from the plasma membrane in a Ca2+-dependent manner [15,16]. Exosomes have a more homogeneous protein composition than MVs, generally do not express PS (phosphatidylserine) and are viewed as antigen-harbouring vehicles [15,17]. Exosomes are characterized by expression of CD9, CD63, CD81, CD82, heat-shock proteins (‘canonical’ markers) and other ‘cell-specific markers’ (summarized in [16]).

Tumour cells can release MVs (exosomes and TMVs) continuously, and the rate of MV shedding is increased in more malignant tumours [18]. Also, tumour cell activation by different stimuli, e.g. hypoxia, irradiation or exposure to activated complement components and to shear stress, results in increased TMV vesiculation [19].

TMVs display a broad spectrum of bioactive molecules originating from tumour cells, including growth factors, lipids, chemokines, membrane receptors, mRNA and regulatory miRNA (microRNA) [20,21]. The molecular content of TMVs mirrors parental tumour cells; however, in many cases, their expression level does not correlate with that on the cells they originated from (‘distorting mirror’). TMVs from different tumour cell lines (melanoma, glioma, breast, lung and pancreas) express a number of surface molecules, such as CD44, CEA (carcinoembryonic antigen), CD95l (CD95 ligand), EMMPRIN (extracellular matrix metalloproteinase inducer), EpCAM (epithelial cell adhesion molecule), MUC1 (mucin 1), Her-2 (human epidermal growth factor receptor 2/ neu, c-Met, MAGE-1 (melanoma-associated antigen 1), EGFRvIII (epidermal growth factor receptor variant III) and chemokine receptors [20,22–25], which are involved in tumour growth and its metastasizing potential.

Vesiculation of TMVs may be viewed as an efficient removal mechanism of redundant or even harmful molecules. Thus more cautious approaches are advised when analysing biological fluids, as some of them may be in a TMV membrane-bound form [26]. Release of TMVs may be responsible for chemoresistance of cancer cells [27]. TMVs may also help tumour cells to escape from apoptosis (by releasing caspase 3) and complement-induced lysis (by releasing C5b-9 complex) [28,29]. On the other hand, TMV uptake may enhance tumour progression as a result of an aggressive phenotype acquisition [27]. TMVs/exosomes may affect cells directly by delivering biologically active compounds, such as chemokines [30], growth factors [31] and proteases, including metalloproteinase-2 and -9, which may result in an increased growth and invasiveness of cancer cells [18]. The number of TMVs increases in bodily fluids during tumour advancement [18,25], but it is difficult to judge whether it is only a consequence of tumour growth (cell number increase) or other mechanisms, e.g. tumour progression. This implies that TMVs may affect the target cells (including cells of the immune system), not only locally, but also systemically.

Interaction of TMVs with monocytes

TMVs were described to interact with many cell types and modulate miscellaneous processes, including migration [22,30], differentiation [32], angiogenesis [13,31,33] and metastasizing potential [34]. Also, monocytes respond to TMVs, as shown in the in vitro model [35]. It is likely that TMVs released in vivo interact with monocytes in blood and with resident macrophages, e.g. in the tumour bed. Biochemical properties of TMVs and circumstances of the interactions between monocytes and TMVs are crucial for monocyte responses to tumour cells.

Activate, suppress, differentiate

On the basis of our findings, TMVs (a heterogeneous population of vesicles released by tumour cells) activate monocytes, as shown by the increased cytokine (TNF, IL-10 and IL-12), chemokine (CXCL8, CCL2, CCL3, CCL4 and CCL5) and ROI production [30,35]. Similar, but stronger, activation was observed when monocytes were stimulated with tumour cells [9]. Also, tumour-derived exosomes may induce a slight enhancement in IL-10 secretion together with a marked induction of TNF, IL-6 and TGFβ (transforming growth factor β) secretion by differentiating monocytes [36]. TMVs, but not tumour exosomes, enhanced expression of HLA-DR on monocytes [20] and changed their morphology [32]. The mechanism(s) by which TMVs induce production of different mediators by monocytes is not clear. However, hyaluronan or other CD44 ligands carried by TMVs may be involved in monocyte–TMV interactions, by analogy to monocyte–tumour cell interactions [37]. Hyaluronan has recently been found to induce potent pro-inflammatory responses in dendritic cells and macrophages via CD44 or TLR [38]. The role of CD44 molecules was confirmed by the blocking experiments that resulted in the inhibition of TNF secretion by monocytes stimulated with TMVs [35].

Our previous study showed that subpopulations of monocytes defined according to CD14 and CD16 expression, interact with TMVs in different manners [39]; however, the pattern of the response (cytokine and ROI profile) resembles that induced by tumour cells [4]. TMV-activated CD14++CD16− monocytes produce more ROIs and IL-10 compared with the CD14+CD16+ subset. CD14+CD16++ monocytes, following TMV stimulation, showed an increased release of TNF, IL-12p40 and RNIs [39]. This is in agreement with the
fact that CD14+CD16++ monocytes are mainly involved in antitumour response [4]. Engulfment of TMVs is mainly performed by CD14++CD16− cells which are precursors of professional phagocytes (CD64+ cells) [39].

On the other hand, tumours may 'educate' monocytes/macrophages to support its growth [5]. This process results in M2-type polarization of macrophages or their deactivation [9]. In the in vitro model, monocytes pre-exposed to tumour cells and then restimulated with malignant cells showed a significant decrease in the production of TNF and IL-12 and an increase in IL-10 (mRNA and protein release). A similar observation was noted when monocytes were briefly (1–2 h) pre-incubated with TMVs followed by stimulation with tumour cells (M. Baj-Krzyworzeka, unpublished work). This observation may suggest a role for TMVs in deactivation or polarization of monocytes to cells with decreased anti-tumour potential. Thus a short contact of monocytes with TMVs alters the balance in the profile of cytokines produced following tumour cell contact [9].

Engulfment of TMVs is a dose-dependent phenomenon, and the local production in vivo will determine their final output. However, the possibility cannot be excluded that the presence of TMVs in blood and other body fluids may cause not only local, but also systemic, modulation of monocyte function.

TMVs are perceived to be reservoirs of pro-angiogenic factors, e.g. IL-8 and sphingomyelin [32,35]. Additionally, they also stimulated monocytes to produce pro-angiogenic chemokines [35]. However, the connection of such 'pro-angiogenic' monocytes with Tie2-expressing monocytes [42] has not been investigated to date.

Tumour-derived exosomes may impair monocyte differentiation into dendritic cells [36]. CD14+ monocytes treated with IL-4 and GM-CSF (granulocyte/macrophage colony-stimulating factor) in the presence of tumour exosomes promote the generation of a myeloid immunosuppressive subset from monocytes. MDSCs were characterized by CD14+HLA-DR−/low, CD80low, CD86low expression and TGFβ secretion [11,36]. MDSCs are a functional subpopulation of myeloid cells that resemble monocytes and granulocytes with immunosuppressive characteristics which are executed through different mechanisms. A defect in monocyte differentiation into more potent antigen-presenting cells is a tumour-related phenomenon, as exosomes from other cells did not affect this function [36].

Our data widen this observation on TMVs, which can push monocytes (CD14+CD11b+) in the in vitro culture to produce significant amount of TGFβ (M. Baj-Krzyworzeka, unpublished work).

**Mechanism of interaction**

There is no unique mechanism of TMV–monocyte interaction. Rather, several potential mechanisms of bidirectional interactions should be considered, including adhesion to cell membrane via determinants or lipids they carry, engulfment (endocytosis) and fusion with plasma membrane. Adhesion of TMVs to plasma membrane has been documented previously [20]; however, specific molecules involved in this process are unknown. Expression of integrins (e.g. CD29, CD49e or CD49d) and the presence of hyaluronan on TMVs may suggest their role in this process [20,43]. Integrins expressed on phagocyte membrane can associate with tetraspanins, markers of exosomes [44].

Monocytes are professional phagocytes and are able to engulf apoptotic cells through the recognition of PS by molecules called 'phosphatidylserine receptors'. PS-positive TMVs and exosomes may be engulfed by monocytes in a PS-dependent manner; however, there is an open question as to how other, i.e. PS-negative, TMVs are engulfed. Our results may indicate endocytosis as a mechanism for the uptake of the other TMVs, as their engulfment is diminished at lower temperature and after treatment with an inhibitor of actin polymerization, cytochalasin D (M. Baj-Krzyworzeka, unpublished work). Koppler et al. [41] also described that the transfer of TMVs to monocytes is dependent on the presence of Ca2+ ions and requires an intact cytoskeleton. However, the role of pinocytosis and/or macropinocytosis in TMV engulfment cannot be excluded, as the size of TMVs is equivalent to the size range of particles ingested in the macropinocytosis process.

Fusion with the cell membrane seems to be more important in the case of non-professional phagocytes and when MVs are smaller, of the exosome size. Valenti et al. [36] confirmed the fusogenic nature of the tumour exosome–monocyte interaction, as it was inhibited by the Ca2+-chelating agent EDTA, but not by cytochalasin D or low temperature.

**Conclusions and perspectives**

TMVs and tumour-derived exosomes are attractive candidates for clinical purposes. TMVs bearing tumour-specific markers (e.g. MUC1) may enable early detection of cancer [48]. Also, in patients with a high risk of cancer, miRNA profiling could be an informative tool [23]. Moreover, TMV numbers may be a useful parameter in monitoring cancer progression and the efficacy of anticancer treatment [25,45]. Currently, commercially available tests that use exosomal protein analysis may be potentially applicable for disease detection, e.g. prostate cancer [46]. Also, tumour-derived exosomes may represent a novel source of antigens with high-level immunogenicity for use in acellular vaccines, which in turn are used to load dendritic cells [47]. Although current clinical studies are concentrated on dendritic cells, it is
also possible to use macrophages pulsed by TMVs/tumour exosomes to present tumour-associated antigens [48]. This idea may be beneficial in tumours whereby the infiltrating macrophages are considered as prognostic indicator (e.g. uterine cancer or gastric cancer) [49].

To summarize, monocytes and the macrophages derived from them are heterogeneous cells with great plasticity that respond to a variety of different stimuli and undergo distinct physiological changes in response to the environment. A growing tumour is an example of such a microenvironment that alters their functional properties. Moreover, changes are ‘tumour-specific’ as different tumours may give rise to macrophages with distinct properties. In this network of interactions, a prominent place belongs to MVs which may activate monocytes, but also switch their polarization upon long-lasting contact (‘tumour education’). Depending on the cellular origin and the molecular composition, MVs may either stimulate or inhibit the immune response, so generalization of the biological effects evoked by certain vesicles should be treated with caution.

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References

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