Review

Intercellular Trogocytosis Plays an Important Role in Modulation of Immune Responses

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Intercellular communication is an important means of molecular information transfer through exchange of membrane proteins from cells to cells. Advent of the latest analytical and imaging tools has allowed us to enhance our understanding of the cellular communication through the intercellular exchange of intact membrane patches, also called trogocytosis, which is a ubiquitous phenomenon. Immune responses against pathogens or any foreign antigens require fine immune regulation, where cellular communications are mediated by either soluble or cell surface molecules. It has been demonstrated that the membrane molecule transfer between immune cells such as dendritic and T cells can be derived through internalization/recycling pathway, dissociation-associated pathway, uptake of exosomes and membrane nanotube formations. Recent evidence implicates the trogocytosis as an important mechanism of the immune system to modulate immune responses. Exchange of membrane molecules/ antigens between immune cells has been observed for a long time, but the mechanisms and functional consequences of these transfers remain unclear. In this review, we discuss the possible mechanisms of trogocytosis and its physiological relevance to immune system, with special reference to T cells and the stimulatory or suppressive immune responses derived from T cells with acquired dendritic cell membrane molecules. *Cellular & Molecular Immunology*. 2008;5(4):261-269.

Key Words: cellular interaction, membrane molecule transfer, CTL response, immune suppression

Introduction

In order to discuss the phenomenon of trogocytosis and its importance in immune response, it is imperative to recall the events which occur in the immune system as a whole. The immune system is composed of different cell subsets which play distinct but entwined roles. For an effective immune response, series of complex cellular events must occur. As the first step, an exogenous antigen or pathogen must be identified as a foreign particle and if necessary, be processed by professional antigen presenting cells (APCs), then active T and B cells must come in contact with APCs or activated APCs. T-helpers must assist B cells and cytotoxic T cells, and there must be the process of proliferation and differentiation to generate and amplify the number of effector cells orchestrating humoral and cell-mediated immunity. In addition, it must generate memory T cells to elicit strong immune response on future exposure to the same antigen. Immune responses so generated must be regulated appropriately to maintain homeostasis and prevent autoimmunity or immuno-pathological conditions. This brief glimpse of immune response reveals the intricacy of immune system and raises an important question: how are the functions of different cell subsets regulated and coordinated? Immune subsets are characterized by the functions they perform. Protein molecules that are expressed on cell surfaces play a pivotal role in cellular functions and form the basis of cellular phenotypic characterization. For example, expression of CD3 indicates T cells and T cell receptor (TCR)/CD3 plus CD4 or CD8 define $CD4^+$ and $CD8^+$ T cells. Similarly, cell population expressing major histocompatibility complex (MHC) class II can be described as APCs, and natural killer (NK) cells could be characterized by killer Ig-like receptors (KIR) (1). When lymphocytes come in contact with target cells, many different molecules on APC and lymphocyte slide (like CD28/CD80 and LFA-1/ICAM-1) together and form an interface which is termed as "immunological synapse" (IS) and has been observed for T (2, 3), B (4) and NK cells (5). The immunological synapse, like T cell IS, is thought to be the seat of initiation of TCR signaling events (6) which lead to different lymphocyte functions such as proliferation and

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cytokine production. These cytokines coordinate and regulate cell to cell interaction necessary to elicit immune responses. Intercellular membrane or protein transfer noticed among immune cells, the immunological synapse is shown to facilitate this transfer (7).

What is trogocytosis?

About 35 years ago, some surprising findings were observed, in which protein molecules considered specific for one cell type were seen on the surfaces of other cell types (8, 9). For example, transfer of antigenic material from macrophages to lymphocytes (10), uptake of macrophage Fc receptors and MHC molecules by T cells (11), acquisition of recipient MHC class I and II molecules on donor thymocytes in bone-marrow chimaeras (12, 13), transfer of MHC class II proteins from splenic cells to allogenic T-cell clones (14) and capture of B-cell surface immunoglobulin by T cells (15, 16). To describe this phenomenon of intercellular transfer of membrane patches, containing membrane-anchored proteins from one cell type to another, following immunological synapse formation, Hudrisier and colleagues (17) coined the term "Trogocytosis" derived from the ancient Greek word "trogo", which means nibble.

Trogocytosis, a widespread phenomenon

Importance of trogocytosis was realized when in-depth studies began to understand the mechanisms of trogocytosis and was found to be a widespread phenomenon (9). Of late new reports of intercellular membrane transfer started pouring in. It was demonstrated that T cells can acquire not only the MHC class I and class II proteins (18, 19), but also the costimulatory proteins (20-22), the membrane fragments (23, 24) from APCs and the proteins from endothelial cells (25). Till recently, the protein transfer by trogocytosis is believed to be unidirectional in murine system (1). However, our recent work has provided the first evidence of bidirectional membrane molecule transfer between dendritic and T cells in murine system (26). Similarly, new findings on trogocytosis are acquired pertaining to NK cells showing that NK cells can capture the target cell-MHC class I protein both *in vitro* and *in vivo* (27-29) and the virus receptor (CD155) (30) and the membrane fragments (31) from the target cells. Both in human and mouse cells, it was shown that NK cell receptors for MHC class I protein can be transferred to target cells (32), demonstrating a bidirectional membrane transfer. B cells can capture membrane-associated antigens from target cells and the amount of antigen captured correlates with the affinity of B-cell receptor for the antigen (4, 33). In addition, γδ T cells have been shown to capture the membrane fragments from the tumor cells, such as Daudi cells (a B-lymphoblastoid cell line, derived from Burkitt's lymphoma) (34). Similarly, dendritic cells (DCs) have been shown to transfer the captured allogenic MHC class I and class II proteins *in vivo*, during transplantation (35, 36).

In addition, bidirectional transfer of membrane protein is noticed in the systems unrelated to immunity, such as the exchange of EpherinB proteins important in axon guidance, which takes place during detachment of neuronal growth cones (37). The glycosyl-phosphatidylinositol (GPI)-anchored proteins are transferred across the homotypic interactions between HeLa cells (38), and the transmembrane protein bride is internalized from one cell by contact with another during the eye development in *Drosophila melanogaster* (39). Thus, these studies conducted in various systems together present strong evidence that the cell surface proteins are commonly transferred between cells both *in vitro* and *in vivo* (9).

What are the mechanisms responsible for the intercellular membrane transfer?

Generally, there are several mechanisms by which the protein transfer takes place from one cell to another. Proteins are associated with cell surface by hydrophobic interactions, and the disruption of this hydrophobic interaction is necessary to initiate the intercellular transfer of proteins (9). Absorption, exosome uptake, internalization, and membrane nanotube formation are the probable mechanisms of intercellular membrane transfer (Figure 1) (16, 18, 20, 40-42).

Cell-to-cell interactions

There are three mechanisms of direct cell-to-cell contact-dependent intercellular transfer of proteins from APCs to T cells.

TCR-mediated internalization and recycling

T cell responses are initiated by TCR recognition of peptide/MHC (pMHC) on APCs (43, 44). Subsequent (within minutes) to specific interactions of T cells with APCs, TCR and MHC molecules are assembled at the centre of supramolecular activation clusters at the site of T cell contact (2, 45-47). TCR-down-regulation is observed following interactions of TCR with pMHC complexes (48-50) and T cell-APC interactions cause APC-derived surface molecules to adhere to the surface of T cells (51, 52). Thereafter, these clusters are internalized through TCR-mediated endocytosis and localized in endosomes and lysosomes, followed by recycling and expression of these molecules on T cell surfaces within 30 minutes (18). For efficient and specific acquisition of TCR-mediated pMHC complexes, a sustained TCR signaling is a pre-requisite and the possibility of involvement of perforin's cytolytic activity has been ruled out during membrane capture process (23). The peptide-MHC complexes transferred from APCs to T cells are the best studied examples of protein transfer that occurs *via* trogocytosis. Here, T cells can acquire both MHC class I and class II proteins from APCs (19, 23, 24, 53-55). Reports of intercellular transfer of membrane fragments from APCs to T cells (24, 54) and from target cells to NK cells (31), and even through homotypic interactions between cells like Daudi cells (56), are consistent with the membrane transfer

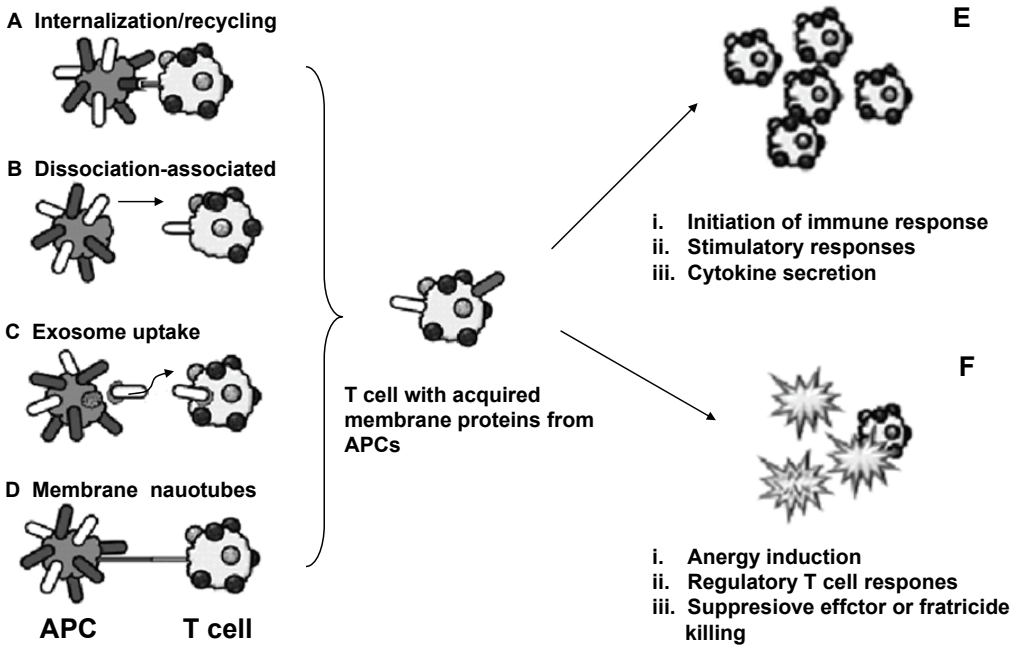

Figure 1. Mechanism for intercellular protein transfer (trogocytosis) between immune cells and its immunological consequences. (A) Internalization and recycling pathway; (B) Dissociation-associated pathway; (C) Exosome uptake; (D) Membrane nanotube formation. Trogocytosis has an important influence on the course of T-cell-mediated immune responses. (E) In some circumstances, the intercellular transfer of cell-surface proteins from APCs to T cells can amplify immune responses or broaden cellular stimulation or activate neighbouring effector cells leading to augmenting cytokine production. (F) In some other conditions, the trogocytosis may induce anergy or tolerance, and T cell function as regulatory T cells in subsequent immune modulation. In addition, the process of trogocytosis can dampen immune responses by fratricide killing, i.e., lysis of CTLs by neighbouring CTLs.

mechanism that involves the transfer of membrane fragments derived from the intercellular contact or IS (57). One possible mechanism of intercellular transfer of membrane fragment might be that the MHC proteins and the other APC ligands are pulled during T-cell-receptor internalization (9), and the created force might break the high-avidity protein-protein interactions.

Dissociation-associated pathway

Before the advent of this theory, the membrane protein capture is thought to depend on TCR-mediated internalization during the direct cell-to-cell contact (as described above) or the APC-derived exosome/vesicle transfer (which will be described below). Using fibroblasts expressing a GFP-tagged I-E^k molecule with covalently attached antigenic peptide, Wetzel et al. demonstrated a third mechanism, the cellular dissociation (58). With the help of live cell imaging, they showed that T cells, while spontaneously dissociating from APCs often capture MHC-peptide complexes directly from the immunological synapse. It was further shown that the MHC transfer is peptide specific and is enhanced by costimulation through CD28-CD80 interactions. T cells dissociated from the MCC:GFP cells were fully activated, expressing high levels of CD69. The activation phenotype is also relevant when considering the spontaneous dissociation of T cells from APCs. In two different studies using *in vitro* imaging, repeated association and dissociation of $CD4^+$ T cells from macrophages were observed (59) and the same was the case with dendritic cells in a three-dimensional collagen matrix (60). To explain this phenomenon, it was shown that the cells were interacting with multiple APC partners, accumulating the activation signals until fully activated. Alternatively, abortive activation event leaving the cells partially activated was explained for the spontaneous association and dissociation of T cells. Thus, Wetzel and colleagues implicated the activation of T cells to spontaneous association and dissociation from MCC:GFP cells, as T cells formed a mature IS, expressed high levels of CD69, and displayed significant TCR-down-regulation. Removal of specific MHC-peptide ligands from APCs would limit their availability for other T cells, which may be an important event in controlling an immune response (58). Such Ag stripping from dendritic cells is seen *in vivo,* suggesting that stripping would prevent lower affinity T cells to access Ag, thereby generating a higher affinity T cell response (61).

Exosome uptake

Intercellular membrane transfer is mediated by the secretion and uptake of enclosed membrane bodies or vesicles such as exosomes (50-90 nm vesicles released by variety of cells) following the fusion of external endosomal membrane with plasma membrane. Their composition may slightly differ from bulk membrane (62). It was suggested that cells

perform this phenomenon to lose potentially harmful components as in case of the recovery of human neutrophils from complement attack by shedding membrane attack complex (63). Transfer of membrane material through vesicle shedding is heavily dependent on interactions between the plasma membrane and underlying cytoskeleton. Local disruption of the cytoskeleton is known to result in membrane blebbing (8). The generation of membrane vesicles or membrane evaginations requires cytoskeletal reorganization and membrane mobility. For example, shedding of adhesion receptors from the surface of activated platelets probably involves calpain action, with rupture of membrane-associated cytoskeleton and dissociation of membrane/cytoskeleton attachment (64). Recent advances in biophysics like, "optical tweezers" shed novel insight into the importance of membrane/cytoskeleton interactions (65). This technique allows a precise estimation of the force generated by small membrane tethers obtained by pulling microbeads bound to the cell surface with a force of a few pico newtons (pN). Thereby, it is possible to estimate intrinsic plasma membrane tension and energy of adhesion to cytoskeleton. The energy of adhesion to cytoskeleton accounts for ~75% of the apparent membrane tension (8). Transient disruption of cortical microfilament as a result of weakening in membrane-cytoskeleton interactions with a second messenger like, phosphotidylinositol 4,5 biphosphate (66) or cytosolic calcium increase (67) could facilitate local release of the plasma membrane, consequently the vesicle formation.

Studies have shown that APCs shed MHC class II glycoproteins which are acquired by T cells (14, 68). It has been suggested that APC-derived vesicles could be a possible mechanism of intercellular transfer of MHC class II glycoproteins and other APC-derived molecules from APCs to T cells. Exosomes (bearing class II MHC) of APCs might have been derived from MHC class II endocytic compartments (69, 70). Exosomes are formed by a process that involves invagination of the limiting MHC class II endocytic compartment vesicular membrane, resulting in a multivesicular compartment comprised of smaller vesicles within a larger vesicle. Upon fusion with surface membrane, exosomes may be released into extracellular spaces and are captured by T cells. Alternatively, APC surface membrane may vesiculate near the contact area between opposing APC-T cell conjugates and released to augment specific acquisition by cognate responders. Several studies have also shown that diverse APCs participate in the intercellular exchange of membranes (35, 71, 72). A critical factor for T-cell activation is TCR/CD28-mediated signaling rather than TCR/CD28-mediated adhesion, which was demonstrated to play a pivotal function in intercellular exchange of membrane (54). Vesicular or exosome mediated transport of antigen/MHC class II complexes from the professional APCs to T cells represent an important mechanism of cellular communications in the immune system (73).

Membrane nanotube formation

Intercellular exchange of proteins through membrane tubes, i.e., long membrane tethers, between cells provides another probable mechanism of cell-surface protein transfer between cells. Rustom et al. (74) demonstrated that rat neuronal PC12 cells or kidney cells were connected *via* membrane tunnels or nanotubes. These nanotubes were shown to facilitate the transfer of lipid organelles between cells through actin-dependent mechanism. Nanotube structures were also reported to connect a wide range of immune cells, such as T cells, B cells, NK cells, and monocytes (24, 75). In the event of disassembly of immunological synapse, formation of membrane nanotubes was observed between B cells and NK cells (76). Recently, the transmission of calcium fluxes between myeloid cells has been shown to take place by nanotube formation (41, 77, 78). Nanotube-mediated intercellular transfer of calcium fluxes induces phenotypic changes in distal DCs, which is reminiscent of response generally seen by direct antigenic stimulation. However, the molecular mechanism of calcium fluxes transmission by nanotubes is still elusive. Interestingly, the heterogenecity in the structure of membrane nanotubes connecting human macrophages is observed. Thicker nanotubes are made up of both F-actin and microtubules, whereas thinner ones contain only F-actin. It was shown that nanotubes containing microtubules transport vesicles over long distances, whereas, using a constitutive flow of nanotube surface, bacteria "surf" along nanotubes that lack microtubules (75). Surface transport along thin nanotubes was found to be dependent on ATP but independent of microtubules. However, transport of vesicles, like endosomes and lysosomes were only observed inside thicker nanotubes (containing microtubules) connecting macrophages. Recently, Sowinski et al. (79) have demonstrated that the formation of T-cell nanotubes between T cells can have important consequences for allowing rapid spread of HIV-1. In addition, it has been shown that the mitochondria can access thick nanotubes. Rescue of aerobic respiration in cells deficient in mitochondria was demonstrated by the intercellular transfer of whole mitochondria or mitochondrial DNA from normal cells, possibly involving membrane nanotubes formation (80). At present, the best datum for functional relevance of membrane nanotubules in immune-cell biology is the demonstration that they mediate communication of antigenic signals between myeloid cells (9, 77).

Trogocytosis: a functional relevance to immune responses

Trogocytosis has a broader impact in immunobiology. It is well established that costimulatory or other protein molecules (extracellular and intracellular) on the cell membrane have considerable impact on cellular function. Therefore, it is obvious that acquisition of different molecules (which is not normally transcribed) by lymphocytes or other cells through trogocytosis may directly or indirectly influence the phenotype and functions of immune subsets capturing these membrane proteins. Several studies demonstrated that trogocytosis has an important influence on the course of the

immune responses (either stimulatory or suppressive immune responses) (Figure 1) (9).

Stimulatory effect on immune responses

The intercellular transfer of membrane molecules can provide signals for immune response. For example, membrane-tethered antigens are internalized by B cells for processing and subsequent presenting them to T cells (4, 33). Usually, APCs such as DCs can acquire antigens and subsequently present the processed peptide-MHC class I and II complexes to T cells (35). Acquisition of APC cell-surface MHC and associated molecules by T cells endows T cells with novel functions. We have recently demonstrated that during intercellular membrane transfer, $CD4^+$ T cells derived from the wild-type ovalbumin (OVA)-specific TCR transgenic OT II mice can not only acquire the synapse-comprised MHC class II and costimulatory molecules (CD54 and CD80), but also the bystander pMHC I complexes from OVA-pulsed DCs (DC_{OVA}) (81). This phenomenon is seen because the bystander pMHC I and the pMHC II complexes localize in the same immunological synapse formed between DCs and $CD4^+$ T cells (82). These $CD4^+$ T cells are type 1 helper T (Th) cells since they secrete IFN-γ, TNF-α and IL-2, but no IL-4. These $CD4^+$ T cells carrying acquired APC Ag-presenting machinery can act as $CD4^+$ Th1-APCs in stimulation of OVA-specific $CD8^+$ CTL responses (22, 81, 83). In addition, $CD4^+$ Th1-APCs also induce OVA-specific antitumor immunity in C57BL/6 mice against the OVA-expressing murine melanoma line $BL6\text{-}10_{OVA}$ cells. Interestingly, the stimulatory effect of $CD4^+$ Th1-APCs is mediated through its endogenous CD40L and acquired CD80 costimulation and IL-2 secretion (84). Importantly, the acquired pMHC I complexes on $CD4^+$ Th1-APCs play an important role in targeting the stimulatory effect of $CD4^+$ Th-APCs to naïve $CD8^+$ T cells *in vivo* (84). Similarly, we have further demonstrated that naïve $CD8^+$ cytotoxic T (Tc) cells also acquire pMHC I and costimulatory CD54 and CD80 molecules through DC_{OVA} stimulation, and act as Tc-APCs. These Tc-APCs can play both negative and positive modulations in antitumor immune responses by eliminating DC_{OVA} and neighbouring Tc-APCs, and by stimulating OVA-specific $CD8^+$ central memory T responses and antitumor immunity *via* targeting role of acquired pMHC I complexes (85). More recently, we have further shown that the exosome-targeted $CD4^+$ T cell vaccine using OVA-specific or non-specific $CD4^+$ T cells with uptake of OVA-specific DC-released exosomes expressing pMHC I complexes are capable of breaking $CD4^+25^+$ regulatory T (Tr) cell-mediated immune suppression and stimulating efficient antigen-specific $CD8^+$ CTL response (86, 87). However, $CD4^+$ T cells with uptake of exosomes without expression of OVA-specific pMHC I complexes are unable to stimulate OVA-specific $CD8^+$ CTL responses. These data clearly elucidate an important role of acquired pMHC I complex on $CD4^+$ T cells in targeting the stimulatory effect of $CD4^+$ T cells to $CD8^+$ T cells *in vivo*. In addition, it has also been demonstrated that MHC class II and CD80 which had been acquired from APCs by $CD4^+$ T cells could sustain T cell activation in the absence of APCs (88). Sustained activity of transcriptional factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP1) was seen in T cells with acquired CD80 molecules. T cells, upon CD80 acquisition could up-regulate the signal transducer and activator of transcription-5 (Stat5) in the absence of APCs or exogenous signal 1 (88). Furthermore, Brandes et al. (89) have demonstrated that human γδ T cells expressing MHC II and costimulatory molecules can also act as APCs and stimulate proliferation and differentiation of naïve γδ T cells.

Suppressive effect on immune responses

$CD4^+$ T cells that have captured agonist pMHC II complexes can subsequently present them to adjacent $CD4^+$ T cells, and these T cells can proliferate in response to T cell-mediated presentation (83), but as the number of activated cells increases, this T-T cell interaction can result in apoptosis or the induction of anergy or tolerance or regulatory T cells (90-92). These mechanisms may serve to limit the clonol expansion (90). The adoptive antigen-specific $CD4^+$ regulatory T cells including Tr1 and Th3 play an important role in immune suppression of autoimmune diseases and antitumor immunity (93). However, the molecular mechanism for antigen-specificity acquisition of adoptive $CD4^+$ Tr cells is elusive. We have recently demonstrated that the tolerogenic OVA-pulsed DC_{OVA} expressing the immune suppressive cytokine IL-10 were able to *in vitro* and *in vivo* induce responses of type 1 regulatory T (Tr1) cells secreting IL-10 and IFN-γ (94). These $CD4^+$ Tr1 cells acquired pMHC I by tolerogenic DC_{OVA} activation and efficiently inhibited immunogenic DC_{OVA}-mediated $CD8^+$ CTL responses and antitumor immunity. Importantly, the acquired pMHC I complexes on $CD4^+$ Tr1 cells lead to an enhanced suppression by 7-fold relative to analogous $CD4^+$ Tr1 cells without acquired pMHC I, indicating that the antigen specificity acquisition of adoptive $CD4^+$ regulatory T cells is *via* acquired pMHC I complexes. Interestingly, the non-specific $CD4^+25^+$ Tr cells can also become antigen-specific and more immunosuppressive in inhibition of antigen-specific $CD8^+$ CTL responses after uptake of antigen-specific DC-released exosomal pMHC I complexes. These data indicate that the antigen-specificity acquisition of $CD4^+$ Tr cells *via* acquiring DC's pMHC I may be an important means in augmenting $CD4^+$ Tr cell's suppression.

Intercellular transfer of proteins from T cells to APCs might also balance the immune responses, as anergic or regulatory T-cell-derived vesicles have been shown to induce a tolerogenic phenotype in APCs (95). It was shown that $CD8^+$ T cells which had acquired cognate pMHC I complexes became susceptible to antigen-specific lysis or fratricide killings, thereby contributed to effector clearance (18, 23, 96). Recently, Mostbock and colleagues (97) demonstrated that acquisition of antigen presentasome (APS), an MHC/costimulatory (CD80 molecules) complex, was an important factor for memory T-cell homeostasis. They suggested that acquisition of APS by memory T cells could

lead to negative regulatory consequences, as it activated BAX/BAK and perforin pathways leading to cell death of CD4/CD80 acquired T cells. In another recent study, it was reported that acquisition of the bystander MHC class I-peptide complexes by $CD4^+$ Th cells made them become targets for specific CTL killing (98). This study suggested that the mechanism of Ag-specific $CD4^+$ T cell regulation may have important roles during the immunopathology of viral infection such as HIV. Intercellular transfer of proteins can also regulate NK cell functions. Acquisition of MHC class I molecules by NK cells from tumor cells resulted into a reduced NK cytotoxic function in mice (28). In addition, contact between NK cells and target cells, which express NKG2D and MIC respectively, led to intercellular exchange of NKG2D and MIC that correlated with reduction in NKG2D-dependent NK cell cytotoxicity in subsequent interactions (41).

Intercellular membrane transfer and its consequences discussed above are important from quantitative standpoint, providing either positive or negative modulation. It was suggested that if trogocytosis involves unusual (i.e., rarely expressed) and/or functionally atypical molecule, then it may induce qualitative changes in the phenotype and functional characteristics of a particular cell. An example of this specialized regulatory role is the expression or the acquisition of HLA-G. HLA-G is a non-classical HLA class I molecule characterized by a strong immunosuppressive function. It is expressed in some types of cancers, transplantations, auto-immune diseases, inflammatory conditions and viral infections. HLA-G was found to inhibit functions of NK cells and CTLs (99), induce regulatory cells (100-102), inhibit allogenic responses (100, 101) and DC maturation (102), and up-regulate inhibitory receptor expression (103). HLA-G has been shown to transfer from APCs to T cells resulting in functional consequences. LeMoult et al. (104) suggested that the HLA-G-associated trogocytosis could have a major impact on immune responses, with which highly efficient regulatory T cells could be generated by reversing the function of effector immune cells. They have emphasized the need for monitoring HLA-G expression in pathologic context and incorporation of HLA-G blocking strategies into immunotherapies.

Uncharacteristic phenotypes and negative consequences

Intercellular transfer of proteins not normally transcribed by the cells might endow the cells with properties not normally associated with that of particular cell type. It has been shown that the intercellular transfer of GPI-anchored prion proteins might be important in the pathogenesis of the prion proteins (105). Development of multidrug resistance in tumors has been demonstrated to be due to the intercellular transfer of P-glycoproteins that can pump many chemotherapeutic agents out of tumor cells (106). Furthermore, the intercellular transfer of viral receptor can facilitate Epstein-Barr virus (EBV) infection in NK cells (107). Similarly, the intercellular transfer of the chemokine receptor and HIV co-receptor CC-chemokine receptor 5 (CCR5) can render cells susceptible to HIV infection *in vitro* (108). Thus, there is considerable evidence showing that the intercellular protein transfer can contribute to several pathologies.

Perspectives

It is well known that the actions of individual immune cells are independent. However, the new science opened up by the recent research developments discussed above is that through direct exchange of proteins and membrane patches as well as specific intercellular connections, individual immune cells become such physically integrated. Theoretically, these integrations and connections can be considered to be a challenge to the central doctrine of cell theory (109). Clearly, more and more evidence demonstrates that the cell-surface proteins can transfer between cells both *in vitro* and *in vivo*, and this widespread intercellular transfer of cell-surface proteins has an important role in modulation of immune responses. Now the most challenging question in this fascinating area of immunology is to establish the functional consequences of intercellular membrane transfer *in vivo*. For example, we need to devise the methods and ways for improvement of intravital imaging and automated detection of nanotubes (110), precisely delineate the process of trogocytosis and determine its consequences *in vivo*. A better understanding of intercellular membrane transfer will thus help to translate this knowledge into therapeutic interventions, and as a diagnostic tool, given its significant influence in diverse immunological and pathological circumstances.

References

1. Caumartin J, Lemaoult J, Carosella ED. Intercellular exchanges of membrane patches (trogocytosis) highlight the next level of immune plasticity. Transpl Immunol. 2006;17:20-22.
2. Grakoui A, Bromley SK, Sumen C, et al. The immunological synapse: a molecular machine controlling T cell activation. Science. 1999;285:221-227.
3. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. Nature. 1998;395:82-86.
4. Batista FD, Iber D, Neuberger MS. B cells acquire antigen from target cells after synapse formation. Nature. 2001;411:489-494.
5. Vyas YM, Mehta KM, Morgan M, et al. Spatial organization of signal transduction molecules in the NK cell immune synapses during MHC class I-regulated noncytolytic and cytolytic interactions. J Immunol. 2001;167:4358-4367.
6. Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. Nat Immunol. 2000;1:23-29.
7. Bossi G, Trambas C, Booth S, Clark R, Stinchcombe J, Griffiths GM. The secretory synapse: the secrets of a serial killer. Immunol Rev. 2002;189:152-160.
8. Hudrisier D, Bongrand P. Intercellular transfer of antigen-presenting cell determinants onto T cells: molecular mechanisms and biological significance. FASEB J. 2002;16: 477-486.
9. Davis DM. Intercellular transfer of cell-surface proteins is common and can affect many stages of an immune response.

Nat Rev Immunol. 2007;7:238-243.
10. Bona C, Robineaux R, Anteunis A, Heuclin C, Astesano A. Transfer of antigen from macrophages to lymphocytes. II. Immunological significance of the transfer of lipopolysaccharide. Immunology. 1973;24:831-840.
11. Lee ST, Paraskevas F. Macrophage--T cell interactions. I. The uptake by T cells of Fc receptors released from macrophages. Cell Immunol. 1978;40:141-153.
12. Sharrow SO, Ozato K, Sachs DH. Phenotypic expression of I-A and I-E/C subregion determinants on murine thymocytes. J Immunol. 1980;125:2263-2268.
13. Sharrow SO, Mathieson BJ, Singer A. Cell surface appearance of unexpected host MHC determinants on thymocytes from radiation bone marrow chimeras. J Immunol. 1981;126:1327-1335.
14. Lorber MI, Loken MR, Stall AM, Fitch FW. I-A antigens on cloned alloreactive murine T lymphocytes are acquired passively. J Immunol. 1982;128:2798-2803.
15. Hudson L, Sprent J, Miller JF, Playfair JH. B cell-derived immunoglobulin on activated mouse T lymphocytes. Nature. 1974;251:60-62.
16. Hudson L, Sprent J. Specific adsorption of IgM antibody onto H-2-activated mouse T lymphocytes. J Exp Med. 1976;143:444-449.
17. Joly E, Hudrisier D. What is trogocytosis and what is its purpose? Nat Immunol. 2003;4:815.
18. Huang JF, Yang Y, Sepulveda H, et al. TCR-mediated internalization of peptide-MHC complexes acquired by T cells. Science. 1999;286:952-954.
19. Arnold PY, Davidian DK, Mannie MD. Antigen presentation by T cells: T cell receptor ligation promotes antigen acquisition from professional antigen-presenting cells. Eur J Immunol. 1997;27:3198-3205.
20. Hwang I, Huang JF, Kishimoto H, et al. T cells can use either T cell receptor or CD28 receptors to absorb and internalize cell surface molecules derived from antigen-presenting cells. J Exp Med. 2000;191:1137-1148.
21. Baba E, Takahashi Y, Lichtenfeld J, et al. Functional CD4 T cells after intercellular molecular transfer of OX40 ligand. J Immunol. 2001;167:875-883.
22. Tatari-Calderone Z, Semnani RT, Nutman TB, Schlom J, Sabzevari H. Acquisition of CD80 by human T cells at early stages of activation: functional involvement of CD80 acquisition in T cell to T cell interaction. J Immunol. 2002;169:6162-6169.
23. Hudrisier D, Riond J, Mazarguil H, Gairin JE, Joly E. Cutting edge: CTLs rapidly capture membrane fragments from target cells in a TCR signaling-dependent manner. J Immunol. 2001;166:3645-3649.
24. Stinchcombe JC, Bossi G, Booth S, Griffiths GM. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity. 2001;15:751-761.
25. Brezinschek RI, Oppenheimer-Marks N, Lipsky PE. Activated T cells acquire endothelial cell surface determinants during transendothelial migration. J Immunol. 1999;162:1677-1684.
26. He T, Tang C, Liu Y, et al. Bidirectional membrane molecule transfer between dendritic and T cells. Biochem Biophys Res Commun. 2007;359:202-208.
27. Carlin LM, Eleme K, McCann FE, Davis DM. Intercellular transfer and supramolecular organization of human leukocyte antigen C at inhibitory natural killer cell immune synapses. J Exp Med. 2001;194:1507-1517.
28. Sjostrom A, Eriksson M, Cerboni C, et al. Acquisition of external major histocompatibility complex class I molecules by natural killer cells expressing inhibitory Ly49 receptors. J Exp Med. 2001;194:1519-1530.
29. Zimmer J, Ioannidis V, Held W. H-2D ligand expression by Ly49A$^+$ natural killer (NK) cells precludes ligand uptake from environmental cells: implications for NK cell function. J Exp Med. 2001;194:1531-1539.
30. Fuchs A, Cella M, Giurisato E, Shaw AS, Colonna M. Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). J Immunol. 2004;172:3994-3998.
31. Tabiasco J, Espinosa E, Hudrisier D, Joly E, Fournie JJ, Vercellone A. Active trans-synaptic capture of membrane fragments by natural killer cells. Eur J Immunol. 2002;32:1502-1508.
32. Vanherberghen B, Andersson K, Carlin LM, et al. Human and murine inhibitory natural killer cell receptors transfer from natural killer cells to target cells. Proc Natl Acad Sci U S A. 2004;101:16873-16878.
33. Fleire SJ, Goldman JP, Carrasco YR, Weber M, Bray D, Batista FD. B cell ligand discrimination through a spreading and contraction response. Science. 2006;312:738-741.
34. Espinosa E, Tabiasco J, Hudrisier D, Fournie JJ. Synaptic transfer by human γδ T cells stimulated with soluble or cellular antigens. J Immunol. 2002;168:6336-6343.
35. Russo V, Zhou D, Sartirana C, et al. Acquisition of intact allogeneic human leukocyte antigen molecules by human dendritic cells. Blood. 2000;95:3473-3477.
36. Herrera OB, Golshayan D, Tibbott R, et al. A novel pathway of alloantigen presentation by dendritic cells. J Immunol. 2004;173:4828-4837.
37. Zimmer M, Palmer A, Kohler J, Klein R. EphB-ephrinB bi-directional endocytosis terminates adhesion allowing contact mediated repulsion. Nat Cell Biol. 2003;5:869-878.
38. Anderson SM, Yu G, Giattina M, Miller JL. Intercellular transfer of a glycosylphosphatidylinositol (GPI)-linked protein: release and uptake of CD4-GPI from recombinant adeno-associated virus-transduced HeLa cells. Proc Natl Acad Sci U S A. 1996;93:5894-5898.
39. Cagan RL, Kramer H, Hart AC, Zipursky SL. The bride of sevenless and sevenless interaction: internalization of a transmembrane ligand. Cell. 1992;69:393-399.
40. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature. 2002;419:734-738.
41. Roda-Navarro P, Vales-Gomez M, Chisholm SE, Reyburn HT. Transfer of NKG2D and MICB at the cytotoxic NK cell immune synapse correlates with a reduction in NK cell cytotoxic function. Proc Natl Acad Sci U S A. 2006;103:11258-11263.
42. Hwang I, Sprent J. Role of the actin cytoskeleton in T cell absorption and internalization of ligands from APC. J Immunol. 2001;166:5099-5107.
43. Germain RN. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell. 1994;76:287-299.
44. Davis MM, Boniface JJ, Reich Z, et al. Ligand recognition by αβ T cell receptors. Annu Rev Immunol. 1998;16:523-544.
45. Monks CR, Kupfer H, Tamir I, Barlow A, Kupfer A. Selective modulation of protein kinase C-θ during T-cell activation. Nature. 1997;385:83-86.
46. Dustin ML, Olszowy MW, Holdorf AD, et al. A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. Cell. 1998;94:667-677.
47. Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A. T

lymphocyte costimulation mediated by reorganization of membrane microdomains. Science. 1999;283:680-682.
48. Valitutti S, Muller S, Cella M, Padovan E, Lanzavecchia A. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature. 1995;375:148-151.
49. Cai Z, Kishimoto H, Brunmark A, Jackson MR, Peterson PA, Sprent J. Requirements for peptide-induced T cell receptor downregulation on naive $CD8^+$ T cells. J Exp Med. 1997;185: 641-651.
50. Preckel T, Grimm R, Martin S, Weltzien HU. Altered hapten ligands antagonize trinitrophenyl-specific cytotoxic T cells and block internalization of hapten-specific receptors. J Exp Med. 1997;185:1803-1813.
51. Antigen presentation functions of the MHC. Keystone symposia on molecular and cellular biology. March 5-11, 1992. Abstracts. J Cell Biochem Suppl. 1992;16D:1-84.
52. Nepom JT, Benacerraf B, Germain RN. Acquisition of syngeneic I-A determinants by T cells proliferating in response to poly (Glu60Ala30Tyr10). J Immunol. 1981;127:888-892.
53. Puaux AL, Campanaud J, Salles A, et al. A very rapid and simple assay based on trogocytosis to detect and measure specific T and B cell reactivity by flow cytometry. Eur J Immunol. 2006;36:779-788.
54. Patel DM, Mannie MD. Intercellular exchange of class II major histocompatibility complex/peptide complexes is a conserved process that requires activation of T cells but is constitutive in other types of antigen presenting cell. Cell Immunol. 2001;214:165-172.
55. Patel DM, Arnold PY, White GA, Nardella JP, Mannie MD. Class II MHC/peptide complexes are released from APC and are acquired by T cell responders during specific antigen recognition. J Immunol. 1999;163:5201-5210.
56. Poupot M, Fournie JJ. Spontaneous membrane transfer through homotypic synapses between lymphoma cells. J Immunol. 2003;171:2517-2523.
57. Davis DM. Intrigue at the immune synapse. Sci Am. 2006;294: 48-55.
58. Wetzel SA, McKeithan TW, Parker DC. Peptide-specific intercellular transfer of MHC class II to $CD4^+$ T cells directly from the immunological synapse upon cellular dissociation. J Immunol. 2005;174:80-89.
59. Underhill DM, Bassetti M, Rudensky A, Aderem A. Dynamic interactions of macrophages with T cells during antigen presentation. J Exp Med. 1999;190:1909-1914.
60. Gunzer M, Schafer A, Borgmann S, et al. Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. Immunity. 2000;13:323-332.
61. Kedl RM, Schaefer BC, Kappler JW, Marrack P. T cells down-modulate peptide-MHC complexes on APCs *in vivo*. Nat Immunol. 2002;3:27-32.
62. Van Blitterswijk WJ, De Veer G, Krol JH, Emmelot P. Comparative lipid analysis of purified plasma membranes and shed extracellular membrane vesicles from normal murine thymocytes and leukemic GRSL cells. Biochim Biophys Acta. 1982;688:495-504.
63. Morgan BP, Dankert JR, Esser AF. Recovery of human neutrophils from complement attack: removal of the membrane attack complex by endocytosis and exocytosis. J Immunol. 1987;138:246-253.
64. Fox JE. Shedding of adhesion receptors from the surface of activated platelets. Blood Coagul Fibrinolysis. 1994;5:291-304.
65. Sheetz MP. Cell control by membrane-cytoskeleton adhesion. Nat Rev Mol Cell Biol. 2001;2:392-396.
66. Raucher D, Stauffer T, Chen W, et al. Phosphatidylinositol 4,5-bisphosphate functions as a second messenger that regulates cytoskeleton-plasma membrane adhesion. Cell. 2000;100:221-228.
67. Richelme F, Benoliel AM, Bongrand P. Dynamic study of cell mechanical and structural responses to rapid changes of calcium level. Cell Motil Cytoskeleton. 2000;45:93-105.
68. Yu DT, McCune JM, Fu SM, Winchester RJ, Kunkel HG. Two types of Ia-positive T cells. Synthesis and exchange of Ia antigens. J Exp Med. 1980;152:89s-98s.
69. Raposo G, Nijman HW, Stoorvogel W, et al. B lymphocytes secrete antigen-presenting vesicles. J Exp Med. 1996;183:1161-1172.
70. Geuze HJ. The role of endosomes and lysosomes in MHC class II functioning. Immunol Today. 1998;19:282-287.
71. Russo V, Tanzarella S, Dalerba P, et al. Dendritic cells acquire the MAGE-3 human tumor antigen from apoptotic cells and induce a class I-restricted T cell response. Proc Natl Acad Sci U S A. 2000;97:2185-2190.
72. Denzer K, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, Geuze HJ. Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. J Immunol. 2000;165: 1259-1265.
73. Arnold PY, Mannie MD. Vesicles bearing MHC class II molecules mediate transfer of antigen from antigen-presenting cells to $CD4^+$ T cells. Eur J Immunol. 1999;29:1363-1373.
74. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. Science. 2004;303:1007-1010.
75. Onfelt B, Nedvetzki S, Benninger RK, et al. Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria. J Immunol. 2006;177:8476-8483.
76. Onfelt B, Nedvetzki S, Yanagi K, Davis DM. Cutting edge: Membrane nanotubes connect immune cells. J Immunol. 2004;173:1511-1513.
77. Watkins SC, Salter RD. Functional connectivity between immune cells mediated by tunneling nanotubules. Immunity. 2005;23:309-318.
78. Baluska F, Hlavacka A, Volkmann D, Menzel D. Getting connected: actin-based cell-to-cell channels in plants and animals. Trends Cell Biol. 2004;14:404-408.
79. Sowinski S, Jolly C, Berninghausen O, et al. Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. Nat Cell Biol. 2008;10:211-219.
80. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci U S A. 2006;103:1283-1288.
81. Xiang J, Huang H, Liu Y. A new dynamic model of $CD8^+$ T effector cell responses *via* $CD4^+$ T helper-antigen-presenting cells. J Immunol. 2005;174:7497-7505.
82. He T, Zong S, Wu X, Wei Y, Xiang J. $CD4^+$ T cell acquisition of the bystander pMHC I colocalizing in the same immunological synapse comprising pMHC II and costimulatory CD40, CD54, CD80, OX40L, and 41BBL. Biochem Biophys Res Commun. 2007;362:822-828.
83. Game DS, Rogers NJ, Lechler RI. Acquisition of HLA-DR and costimulatory molecules by T cells from allogeneic antigen presenting cells. Am J Transplant. 2005;5:1614-1625.
84. Umeshappa K, Huang H, Xie YF, Xiang J. $CD4^+$ Th-APC with acquired pMHC I and II complexes stimulate type I helper $CD4^+$ and central memory $CD8^+$ T cell responses. J Immunol. 2008; in press.

85. Xia D, Hao S, Xiang J. $CD8^+$ cytotoxic T-APC stimulate central memory $CD8^+$ T cell responses *via* acquired peptide-MHC class I complexes and CD80 costimulation, and IL-2 secretion. J Immunol. 2006;177:2976-2984.
86. Hao S, Yuan J, Xiang J. Nonspecific $CD4^+$ T cells with uptake of antigen-specific dendritic cell-released exosomes stimulate antigen-specific $CD8^+$ CTL responses and long-term T cell memory. J Leukoc Biol. 2007;82:829-838.
87. Hao S, Liu Y, Yuan J, et al. Novel exosome-targeted $CD4^+$ T cell vaccine counteracting $CD4^+25^+$ regulatory T cell-mediated immune suppression and stimulating efficient central memory $CD8^+$ CTL responses. J Immunol. 2007;179:2731-2740.
88. Zhou J, Tagaya Y, Tolouei-Semnani R, Schlom J, Sabzevari H. Physiological relevance of antigen presentasome (APS), an acquired MHC/costimulatory complex, in the sustained activation of $CD4^+$ T cells in the absence of APCs. Blood. 2005;105:3238-3246.
89. Brandes M, Willimann K, Moser B. Professional antigen-presentation function by human γδ T Cells. Science. 2005;309: 264-268.
90. Tsang JY, Chai JG, Lechler R. Antigen presentation by mouse $CD4^+$ T cells involving acquired MHC class II:peptide complexes: another mechanism to limit clonal expansion? Blood. 2003;101:2704-2710.
91. Carlin LM, Yanagi K, Verhoef A, et al. Secretion of IFN-γ and not IL-2 by anergic human T cells correlates with assembly of an immature immune synapse. Blood. 2005;106:3874-3879.
92. Lombardi G, Sidhu S, Batchelor R, Lechler R. Anergic T cells as suppressor cells *in vitro*. Science. 1994;264:1587-1589.
93. Zou W. Regulatory T cells, tumour immunity and immuno-therapy. Nat Rev Immunol. 2006;6:295-307.
94. Hao S, Yuan J, Xu S, et al. Antigen specificity acquisition of adoptive $CD4^+$ regulatory T cells *via* acquired pMHC I complexes. J Immunol. 2008;181:2428-2437.
95. Nolte-'t Hoen EN, Wagenaar-Hilbers JP, Peters PJ, Gadella BM, van Eden W, Wauben MH. Uptake of membrane molecules from T cells endows antigen-presenting cells with novel functional properties. Eur J Immunol. 2004;34:3115-3125.
96. Hudrisier D, Riond J, Garidou L, Duthoit C, Joly E. T cell activation correlates with an increased proportion of antigen among the materials acquired from target cells. Eur J Immunol. 2005;35:2284-2294.
97. Mostbock S, Catalfamo M, Tagaya Y, Schlom J, Sabzevari H. Acquisition of antigen presentasome (APS), an MHC/ costimulatory complex, is a checkpoint of memory T-cell homeostasis. Blood. 2007;109:2488-2495.
98. Cox JH, McMichael AJ, Screaton GR, Xu XN. CTLs target Th cells that acquire bystander MHC class I-peptide complex from APCs. J Immunol. 2007;179:830-836.
99. Carosella ED, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. Adv Immunol. 2003;81:199-252.
100. LeMaoult J, Krawice-Radanne I, Dausset J, Carosella ED. HLA-G1-expressing antigen-presenting cells induce immuno-suppressive $CD4^+$ T cells. Proc Natl Acad Sci U S A. 2004; 101:7064-7069.
101. Le Rond S, Azema C, Krawice-Radanne I, et al. Evidence to support the role of HLA-G5 in allograft acceptance through induction of immunosuppressive/regulatory T cells. J Immunol. 2006;176:3266-3276.
102. Ristich V, Liang S, Zhang W, Wu J, Horuzsko A. Tolerization of dendritic cells by HLA-G. Eur J Immunol. 2005;35:1133-1142.
103. LeMaoult J, Zafaranloo K, Le Danff C, Carosella ED. HLA-G up-regulates ILT2, ILT3, ILT4, and KIR2DL4 in antigen presenting cells, NK cells, and T cells. FASEB J. 2005;19:662-664.
104. LeMaoult J, Caumartin J, Daouya M, et al. Immune regulation by pretenders: cell-to-cell transfers of HLA-G make effector T cells act as regulatory cells. Blood. 2007;109:2040-2048.
105. Liu T, Li R, Pan T, et al. Intercellular transfer of the cellular prion protein. J Biol Chem. 2002;277:47671-47678.
106. Levchenko A, Mehta BM, Niu X, et al. Intercellular transfer of P-glycoprotein mediates acquired multidrug resistance in tumor cells. Proc Natl Acad Sci U S A. 2005;102:1933-1938.
107. Tabiasco J, Vercellone A, Meggetto F, Hudrisier D, Brousset P, Fournie JJ. Acquisition of viral receptor by NK cells through immunological synapse. J Immunol. 2003;170:5993-5998.
108. Mack M, Kleinschmidt A, Bruhl H, et al. Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immuno-deficiency virus 1 infection. Nat Med. 2000;6:769-775.
109. Baluska F, Volkmann D, Barlow PW. Cell bodies in a cage. Nature. 2004;428:371.
110. Hodneland E, Lundervold A, Gurke S, Tai XC, Rustom A, Gerdes HH. Automated detection of tunneling nanotubes in 3D images. Cytometry A. 2006;69:961-972.