T Lymphocyte

Subjects: Immunology

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Exosomes are extracellular vesicles (EV) of endosomal origin (multivesicular bodies, MVB) constitutively released by many different eukaryotic cells by fusion of MVB to the plasma membrane. However, inducible exosome secretion controlled by cell surface receptors is restricted to very few cell types and a limited number of cell surface receptors. Among these, exosome secretion is induced in T lymphocytes and B lymphocytes when stimulated at the immune synapse (IS) via T-cell receptor (TCR) and B-cell receptor (BCR), respectively. IS formation by T and B lymphocytes constitutes a crucial event involved in antigen-specific, cellular and humoral immune responses. Upon IS formation by T and B lymphocytes with antigen-presenting cells (APC) the convergence of MVB towards the microtubule organization center (MTOC), and MTOC polarization to the IS, are involved in polarized exosome secretion at the synaptic cleft. This specialized mechanism provides the immune system with a finely-tuned strategy to increase the specificity and efficiency of crucial secretory effector functions of B and T lymphocytes. Since inducible exosome secretion by antigen-receptors is a critical and unique feature of the immune system this entry considers the study of the traffic events leading to polarized exosome secretion at the IS and some of their biological consequences.

Keywords: exosomes ; T lymphocytes ; B lymphocytes ; polarized secretion ; immune synapse ; T-cell receptor ; B-cell receptor ; multivesicular bodies ; diacylglycerol ; MHC-class II compartment

1. Introduction

To constitute an IS, T lymphocytes must recognize processed antigenic peptides loaded onto MHC molecules present on the cell surface of professional antigen-presenting cells (APC) or pathogen-infected cells. TCR interaction with peptide-MHC-I complexes (pMHC-I) induces naïve $CD8^+$ cytotoxic T lymphocytes (CTL) activation (priming), whereas TCR interaction with peptide-MHC-II complexes (pMHC-II) leads to $CD4^+$ Th lymphocyte activation (Figure 1) [1]. Primed CTL form IS with target cells resulting in a specific killing. In addition, mature IS formation can induce T lymphocyte anergy or activation-induced apoptosis (AICD)[2].

Figure 1. T lymphocyte—antigen-presenting cells (APC) immune synapse (IS) and polarized secretion. Stages 0 and 1 are common for both Th and cytotoxic T lymphocytes (CTL) IS. After the initial scanning contact of TCR with pMHC on APC, Th effector T lymphocytes (upper panel) form mature IS with antigen-presenting B lymphocytes within several minutes. This IS lasts many hours during which de novo cytokine (i.e., IL-2, IFN-γ) production and secretion occur, which require continuous T-cell receptors (TCR) signaling. Primed effector CTL (lower panel) establish more transient, mature IS after scanning their target cells (i.e., a virus-infected cell), and deliver their lethal hits within a few minutes. Secretory lysosomes (lytic granules) are very rapidly transported (within very few minutes) towards the microtubule organization center (MTOC) (in the minus "–" direction) and, almost simultaneously, the MTOC polarizes towards the central supramolecular activation complex (cSMAC) of the IS, an F-actin poor area that constitutes a secretory domain. MTOC translocation to the IS appears to be dependent on dynein anchored to the Adhesion and Degranulation Promoting Adapter Protein (ADAP) at the peripheral SMAC (pSMAC), which pulls MTOC in the minus direction. In both types of IS (lower zoom panel), the initial F-actin reorganization in the cell-to-cell contact area, followed by a decrease in F-actin at the cSMAC and an accumulation at the distal SMAC (dSMAC) appears to be involved in granule secretion. In stage 3, MVB fusion with the plasma membrane occurs in both types of IS and leads to TCR-containing exosome polarized secretion at the IS. The exosomes released in Th IS contain proapoptotic FasL and Apo2L and can induce target cell death or Th cell death (AICD). TCR–containing shedding microvesicles have been described in Th IS.

2. CTL-Target Cell Immune Synapse

CTL-target cell IS induces the rapid polarization (from seconds to few minutes) of CTL MTOC and lytic granules (secretory granules or secretory lysosomes-SL-with MVB structure) towards the central supramolecular activation cluster (cSMAC) at the IS (Figure 1). Lytic granules fusion with the plasma membrane (degranulation) induces the secretion of certain cytotoxic factors such as perforin and granzymes to the synaptic cleft, triggering target cell apoptosis [3]. Upon degranulation, FasL located at the secretory granule limiting membrane becomes exposed to the plasma membrane at the IS and induces target cell Fas crosslinking leading to target cell apoptosis [4][5][6][7].

Another consequence of degranulation is ILV secretion as nanosize EV at the CTL-target cell synaptic cleft, first described by Peters et al. [8]. Although the vesicles secreted by CTL were not referred at that time as exosomes, their formation and mode of exocytosis justifies such a nomenclature [8][9].

Subsequent publications demonstrated that T lymphocyte stimulation of T lymphoblasts (including $CD4^+$ and $CD8^+$ lymphocytes) with activation agonists produced non-directional secretion of nanosize EV (quoted as microvesicles) carrying pro-apoptotic FasL and Apo2L [10] via MVB-mediated degranulation [11], providing an alternative mechanism of TCR-controlled AICD that does not necessarily imply cell-to-cell contact [11][12]. Moreover, it was shown that upon TCR triggering T lymphoblasts secrete exosomes [12][13] containing TCR/CD3 [13], extending the early observations obtained in CTL forming synapses [8]. CTL MTOC reorientation is initially guided by a diacylglycerol (DAG) gradient centered at the IS [14], generated by TCR-stimulated phospholipase C (PLC). DAG phosphorylation by diacylglycerol kinase α(DGKα) is involved in the spatiotemporal control of the DAG gradient [15][16] and MTOC polarization to the IS in CTL [14]. DAG activates, among others, several members of the PKC and PKD families [17], such as PKCδ, which is necessary for the polarization of lytic granules and cytotoxicity in mouse CTL [18][19].

3. Th Immune Synapse

Polarized secretion upon Th IS formation has been less studied than polarized CTL secretion [20]. Th IS are more stable and longer (from minutes up to several hours) than CTL IS (few minutes) [21][22]. Th IS are required for both directional and continuous cytokine secretion [21][22]. These cytokines are contained in secretory vesicles and IL-2, IFN-γ-containing secretory vesicles undergo polarized traffic to the F-actin poor area at cSMAC [23][24][25][26] as CTL lytic granules. Although the identity of the cytokine-containing secretory vesicles has not been characterized yet [27][28] they, most probably, are not MVB [28] (Figure 1).

Early reports in $CD4^+$ Jurkat cells and T lymphoblasts demonstrated that stimulation with activation agonists [10] or anti-TCR [12][13] induced exosome secretion. Stimulation with a heterologous receptor agonist, that mimics TCR-derived signals leading to full T cell activation [29] and AICD [30], also induced exosome secretion in $CD4^+$ Jurkat cells [12], suggesting that exosome secretion is a general consequence of T lymphocyte activation. IS formation by $CD4^+$ Jurkat cells and superantigen-coated Raji B cells acting as an APC, which constitutes a well-established IS model [31][32][33], induces polarized MVB traffic towards the IS, MVB degranulation and exosome release [34][35] (Figure 1, right side panel). In this Th-APC IS model, a positive role of TCR-triggered DAG and its regulator DGKα [15], in polarized MVB traffic towards the IS was demonstrated [34][36][37]. As DGKα also controls late endosomes polarized traffic during invasive

migration [38], and MTOC and lytic granules polarized traffic in CTL (described above), DAG and DGKα can be considered as general regulators of polarized traffic . DAG-activated PKCδ is needed for cortical actin reorganization at the IS, MTOC and MVB polarization to the IS and exosome secretion in this IS model [39]. Overall, this leads us to hypothesize that an altered actin reorganization at the IS may underlie the deficient MVB polarization occurring in PKCδ-interfered T cell clones [39]. In this model, DAG-activated PKD1/2 regulates MVB maturation and polarization leading to exosome secretion [40], suggesting that several regulatory points in exosome secretion are controlled by DAG.

Microvesicles or ectosomes budding from the Th cell plasma membrane and accumulating at the IS have been described [41] (Figure 1, right side panel). These shedding vesicles were enriched in TCR and capable to trigger B-lymphocyte signaling via pMHC-II stimulation [41][42]. Thus, it appears that distinct types of EV from Th lymphocytes are secreted at the IS. Further research will be necessary to establish whether these subtypes of EV trigger different Th effector responses or, on the contrary, redundantly or synergistically trigger the same responses.

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