

Trogocytosis and Acquired PD1 Receptor in Leukemia & Its Role in Cancer Immunology

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Abstract: ***Aim:** Cancer, one of the most dreaded diseases of human beings, has always been the cynosure of researchers. Everyday, new studies are being done to know more about the tumor cell - immune cell interactions that further lead to a generation of new techniques and therapies for cancer treatment. **Method:** The phenomenon that has caught everyone's eye is trogocytosis. The discovery of this unusual transfer mechanism has apprised the interest of oncologists and immunologists in cancer immunotherapy. Our study will focus on leukemia among other cancers. We will also analyze the role of ligand - receptor engagements and signaling pathways. **Conclusion:** In conclusion, we will review the role of trogocytosis in cancer immunology, how it inhibits immune cell responses, and its significance in non - immunological processes.*

Keywords: Leukemia, Trogocytosis, PD - 1, pMHC, Fc γ receptor, PI3K - AKT pathway

1. Introduction

Cancer is a group of diseases due to abnormal cell growth having the power to metastasize to other parts of the body. A major class among these cancers is leukemia, the cancer of blood cells that begins in the bone marrow. Being the most common type of cancer in children, leukemia is one of the biggest headaches in the developed world. Acute Myeloblastic Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), and Acute Lymphocytic Leukemia (ALL) are the most common types of leukemia. AML is caused by a clonal disorder that leads to abnormal proliferation and differentiation of myeloid (certain hematopoietic cells found in the bone marrow). Chemotherapy has been used as a standard treatment for AML but in the past few years, immunotherapy has become a better alternative for the treatment of AML and other liquid malignancies with betterment in the understanding of the immune system, immune checkpoints, and processes like trogocytosis in cancer progression [1].

This study aims to analyze the role of trogocytosis and the presence of immune checkpoint receptors like PD - 1 on immune cells in leukemia.

2. Trogocytosis

In the late 70s, some researchers reported the occurrence of a transfer mechanism in some microbes which includes gnawing of one cell by another. This process of cell nibbling was coined by Joly and Hudriser about 20 years from now and they called it trogocytosis. Trogocytosis is an active transfer phenomenon involving the transfer of plasma membrane fragments from the donor cell to the acceptor cell [2].

Trogocytosis is used for cell - cell interactions and cell killing in the immune system. It also plays a role in cellular remodeling during embryonic development and in the nervous system. Trogocytosis is considered an evolutionarily conserved process and it is also known to mediate cell clearance in multicellular organisms. Despite its widespread occurrence, the molecular mechanism of trogocytosis has not been well defined. Some say that it is essentially a failed phagocytosis. However, when a macrophage fails to perform phagocytosis, it does not ingest bites. Trogocytosis occurs in specific situations and it requires physical interaction between two live cells. The cell can live even after trogocytosis whereas phagocytosis is the complete ingestion of a dead cell target.

Trogocytosis involves PI3K and cytoskeletons like actins and myosin also. After recognizing the ligands on the target cells, the trogocytosis - associated acceptor cells undergo an energy - consuming process that includes actin rearrangements [3, 4, 5].

3. Trogocytosis in Non - Immune Cells

Trogocytosis was initially found in microbes as a defense mechanism in them. Although it has been widely studied in immune cells, it also plays equally important roles in non - immune cells.

Trogocytosis in Protozoa

As mentioned earlier, trogocytosis is being used by amoeba for a long time for attacking other cells. It was first reported in *Naegleria fowleri* which uses it for nibbling mammalian cells. Intestinal parasitic amoeba *Entamoeba histolytica* also uses trogocytosis for the lysis of the live epithelial cells.

E. histolytica is sensitive to the complement system which lyses a cell by inflaming, opsonizing, and then killing it [6]. During their invasion, *Entamoeba* makes itself resistant to complement - mediated lysis by blocking the formation of complement Membrane Attack Complex (MAC). It achieves this by trogocytosis of CD59 and DAF proteins that are needed for MAC formation [7]. Amoebas use different mechanisms for trogocytosis and phagocytosis. They use trogocytosis on live epithelial cells but use phagocytosis for ingesting dead epithelial cells. The decision of whether to perform trogocytosis or phagocytosis is regulated by the lipid membrane composition of the cell [7]. Trogocytosis is also seen in the malarial pathogen *Plasmodium falciparum*. It entraps the infected RBCs into the microvessels of the brain in an actin - dependent manner, very similar to trogocytosis, leading to cerebral malaria [7].

Trogocytosis in Cell Remodeling

Coming up of techniques like light sheet fluorescence microscopy, correlative light and electron microscopy, and two - photon *in vivo* microscopy have made *in vivo* studies easier and revealed the importance of trogocytosis in cellular remodeling.

Neuronal Remodeling

Microglial cells are a type of fixed macrophage found in the CNS. They are highly motile glial cells and get rapidly activated in neuroglial diseases. They use trogocytosis to remodel neuronal cell synapses. Moreover, recent *in vivo* studies on *Xenopus laevis* show that these resident macrophages use trogocytosis for trimming retinal ganglion cell axons in their developing retinotectal circuit. Astrocytes also perform trogocytosis on neighbor neurons to remodel synapses connections and eliminate damaged organelles [7].

Embryonic Trogocytosis

During post - fertilization developments, some embryonic cells have also been found to use trogocytosis for cell remodeling. During gastrulation, the endodermal cells of *Caenorhabditis elegans* actively remove and digest the lobes formed by the primordial cells as shown in Figure 1. This process of cell cannibalism utilizes cytoskeletal systems like actins, dynamins, and SNXs hinting at a strong resemblance with trogocytosis [4, 7].

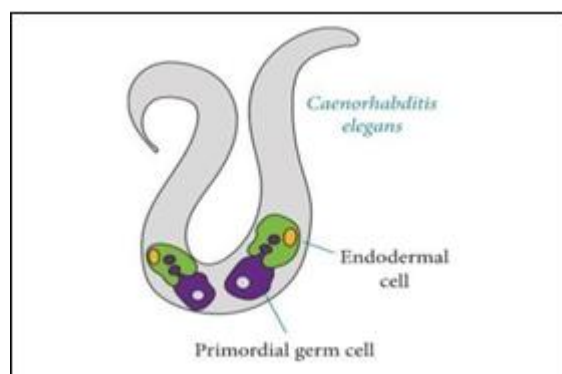


Figure 1: During development, *C. elegans* primordial cells connected to the endodermal cells form lobes that are removed by the endodermal cells by trogocytosis [7].

4. Role of NK Cells in Trogocytosis

Natural Killer cells or NK cells are a kind of innate immune cells that function against tumorigenic and virus - infected cells. They do so by physically interacting with the target cells by forming an immunological synapse, which acts as an interface between the target cell and the NK cell. NK cells also have inhibitory receptors that form inhibitory immunological synapses for protecting them from killing self - cells.

During the early 21st century some scientists found that the formation of immunological synapses led to the trogocytosis of some protein complexes like pMHC - I from target cells to NK cells. A peptide Major Histocompatibility Complex class - I (pMHC - I) is a heterodimeric glycoprotein complex that binds to the peptide fragments derived from antigens and displays them on the cell surface for recognition by defense cells [8]. This trogocytic transfer is mediated by the inhibitory NK cell receptors that recognize MHC - I molecules. The transfer of pMHC - I also accompanies the transfer of membrane fragments that depend on actin polymerization. Further studies showed that trogocytosis between NK cells and target cells is not a unidirectional but rather a bidirectional mechanism where inhibitory NK receptors are also transferred from NK cells to target cells [9].

Recent studies on murine (mouse) NK cells confirmed that PD - 1 is acquired from tumor cells by trogocytosis. To get this result, the researchers used an RMA cell marked with a Thy - 1.1 marker. They found that with PD - 1 and Thy1.1, NK cells also acquired several other proteins and lipids that were not present in them before, using a transfer mechanism that requires cell - cell contact just like trogocytosis. It was reconfirmed by using NK cells pretreated with sodium azide and latrunculin A which resulted in the reduction of PD - 1 acquisition. Both these chemicals prevent ATP synthesis and actin polymerization respectively. Using RMA - S - *Pd11* cells the researchers further verified the antitumor inhibitory activities of PD - 1 on NK cell responses post - trogocytosis [10].

5. Role of Ligand - Receptor Interactions

Trogocytosis in immune cells, in most cases, is mediated by ligand - receptor interactions. These are a major class of protein - protein interactions that play a key role in many biological processes such as metabolism, neurotransmission, and signal transduction pathways [11].

FcγR - MEDIATED TROGOCYTOSIS

Recent evidence indicates that trogocytosis impacts several immunologic phenomena including the action of CAR - T cells (Chimeric antigen receptor T - cells) and checkpoint inhibitors. The acceptor cells form immunological synapses with the IgG - opsonized cells, thus allowing for the transfer of cell - bound IgG and associated antigen and membrane fragments to the acceptor cells [12]. Nelson described the immune adherence phenomenon in 1953 where he demonstrated that bacteria opsonized with antibodies could activate covalent tagging of the immune complex by C3b. The C3b - tagged immune complex could then be

immobilized on the surface of erythrocytes (E) via multivalent interactions and then transferred to acceptor neutrophils and macrophages again via other multivalent interactions. This immune adherence phenomenon is the first description of Fc γ R - mediated trogocytosis [12].

IgG4 has often been selected for certain therapeutic applications including antibody – drug conjugates because it is presumed to weakly interact with Fc γ R. But a study demonstrated that even IgG4 can also mediate trogocytosis. HIV - specific antibodies also mediate trogocytosis of some glycoproteins and immunoglobulins that can be abrogated by blocking the Fc γ RIIa or Fc γ RIIb receptors [12]. Inhibitory receptors like Fc γ RIIb2 (only Fc γ R on LSEC in the liver) are also known to perform trogocytosis and internalize small immune complexes [12].

In previous examples, the substrate donor cells escaped alive from the effect or cells after trogocytosis. This pattern is not followed when breast cancer cells are opsonized with an anti - HER2 mAb like trastuzumab (TRA). Macrophages and neutrophils also mediate trogocytosis of the TRA/HER2 complexes bound to breast cancer cells, but in these cases, the donor cells are killed. Trogocytosis leading to cell death has been termed trogoptosis. One reason behind this can be excessive damage caused to the cell membrane leading to cell death or if the HER2 protein is critical for cell functioning then also the cell cannot live without it [12].

TCR – Mediated Trogocytosis

CD4⁺T cells are known to capture pMHC – II complexes but they also acquire the by stander pMHC - I complexes that are located close to the pMHC – II complexes. Similarly, CD8⁺T cells also acquire both the pMHC molecules though they are known to capture cognate pMHC - I complexes, in general [9]. pMHC - II acquired CD4⁺T cells further interact with cognate CD4⁺T cells leading to negative regulations thereby inducing either apoptosis or anergy of CD4⁺T cells and ceasing proliferation of memory CD4⁺T cells. Something similar happens with pMHC - I - acquired CD8⁺T cells and increases CD8⁺T cell fratricide after trogocytosis [9].

Patients - derived CAR T cell therapy is more efficient against refractory B cell malignancies, where chemotherapy doesn't do well. But insufficient infusion of CART cells causes trogocytosis of target antigens thereby reducing their density on the tumor cells and ultimately leading to reduced efficacy of CAR T cell therapy [9].

6. Signaling Pathways Such SS PI3K – AKT and regulation of CD84 Targeting Immune Signaling Check Points

The PI3K – AKT signaling pathway is a signal transduction pathway that promotes survival and growth in response to extra cellular signals. It is highly conserved and its activation is controlled via a multistep process – regulated receptor. A fully active PI3K - AKT pathway mediates numerous cellular functions including angiogenesis, metabolism, growth, proliferation, survival, protein synthesis, transcription, and apoptosis. The impact of PD -

L1 manipulations has been studied in the PI3K - AKT pathway using western blotting. It showed that cutting down PD - L1 decreased the PI3K and p - AKT expressions in KG - 1a cells (promyeloblast macrophage cell line isolated from the bone marrow of a 53 - year - old white male AML patient) [1, 13].

Chronic Lymphocytic Leukemia (CLL) is a type of cancer in which the bone marrow makes too many lymphocytes with limited ability to exert anti - tumor responses. CD84 is a member of the SLAM family that connects CLL cells with their microenvironment. It is a cell - surface protein that forms homophilic dimers by self - association. CD84 controls PD - L1 expression on CLL cells and their stroma and an increase in CD84 stimulation elevated AKT and pAKT levels suggesting that CD84 up - regulates PD - L1 through activation of AKT. A decrease in CD84 can reduce PD - L1 expression and increase CLL cell lysis hence blocking CD84 expression can be used as an effective therapy against CLL [1, 14].

7. Role of the Microenvironment in Helping Trogocytosis in Leukemia

A tumor microenvironment (TME) is the environment around a tumor to which the tumor is related closely and interacts constantly. It plays an important role in developing, growing, and surviving lymphoid malignancies. Moreover, trogocytosis is depicted to be involved in establishing and maintaining the tumor microenvironment. Thus, knowing TME – tumor cell interactions may improve cancer treatment strategies. This has been studied in CLL and Hodgkin lymphoma (cHL), a kind of cancer affecting the body's lymphatic system.

The B - cell receptor pathway is a central mechanism by which CLL cells maintain their interaction with the TME. PI3K constitutes a crucial component of this pathway and its inhibitor can be used for CLL treatment. PD - L1 is highly up regulated in Hodgkin and Reed - Sternberg (HRS) cells and hence it seems that an anti - PD - 1 antibody would be strongly effective against cHL [15, 16].

The tumor microenvironment contains a variety of potential targetable pathways. Antibodies interrupting the CXCL12/CXCR4 interaction have demonstrated anti - CLL activity *in vitro* and mouse models. Anti - BAFF - R antibody blocked protective survival signaling in CLL cells and enhanced antibody - dependent cellular cytotoxicity *in vitro* [15].

8. Limitations

In this study, many functions of trogocytosis have been described but it seems that trogocytosis is a process of far more significance and may involve events and fields other than immune functions. Most of the studies show trogocytosis as a foe but it can also act as a friend by trogocytosing some molecules to the immune cells that enhance antitumor response under certain circumstances but no exclusive study has been done on this till now. Its mechanism of transfer is not well – defined scientists

stillarenotsurewhethertrogocytosisoccursselectivelyornotand whetherthetransferofbystanders follows some pattern or it's completely random.

9. Conclusion

In this study, we analyzed and evaluated the role of trogocytosis and immune checkpoints in leukemia. A lot of progress has been made to understand their complex mechanism in regulating immune and non - immune cell functions and their significance in cancer immunotherapy. Despite numerous ongoing research, the therapeutic techniques that include trogocytosis and therapeutic targets like PD - 1 remain at an elementary stage and need some remarkable technical advancements before it is fully applicable. Application of genomic and cellular techniques like single - cell genome sequencing, single - cell cytokine analysis, and mass cytometry can reveal additional functions of trogocytosis and immune check point receptors like PD - 1inthetumormicroenvironment [17]. Hence, it is well convincing that in the future with more technical and biological advancements, trogocytosis will be very important in therapeutics against leukemia and other cancers. Moreover, it is also likely that anti - PD - 1 immunotherapy to emerge as an effective cancer treatment.

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Conflicts of Interest

The authors declare no conflict of interest.

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