Blood Spotlight

Fcγ-receptor–mediated trogocytosis impacts mAb-based therapies: historical precedence and recent developments

Ronald P. Taylor and Margaret A. Lindorfer

Department of Biochemistry and Molecular Genetics, University of Virginia Health Sciences Center, Charlottesville, VA

A specialized form of trogocytosis occurs when Fcγ receptors on acceptor cells take up and internalize donor cell-associated immune complexes composed of specific monoclonal antibodies (mAbs) bound to target antigens on donor cells. This trogocytosis reaction, an example of antigenic modulation, has been described in recent clinical correlative studies and in vitro investigations for several mAbs used in cancer immunotherapy, including rituximab and ofatumumab. We discuss the impact of Fcγ-receptor–mediated trogocytosis on the efficacy of cancer immunotherapy and other mAb-based therapies. (*Blood*. 2015;125(5):762-766)

Introduction

Trogocytosis, as first described by Joly and Hudrisier,[1] is characterized by the transfer of cell surface molecules from a donor cell to an acceptor cell and proceeds in discreet steps[2-5]: firstly, the two cells form an immunologic synapse,[6,7] due to recognition of cognate ligands on the donor cell by cell-surface receptors on the acceptor cell. Secondly, in an energy-requiring process that includes actin polymerization, membrane remodeling, and signaling, portions of the plasma membranes of the cells merge, and chelated ligands on the donor cell along with sections of its plasma membrane are pinched off and taken up by the acceptor cell. Thirdly, captured material is then either displayed on the surface of the acceptor cell, or internalized, processed, and degraded. This reaction has been demonstrated for a variety of acceptor cells, including T and B lymphocytes, monocyte/macrophages, dendritic cells, neutrophils, and natural killer (NK) cells, and plays an important role in antigen presentation and processing. In all cases, only small portions of donor cells are taken up by acceptor cells, and because donor cells are only marginally perturbed, trogocytosis (gnawing or nibbling) is an apt description of the process.

We will focus our discussion on a specialized form of trogocytosis, mediated by Fcγ receptors (FcγR) on effector cells, including monocytes, macrophages, neutrophils, and NK cells, which manifests as an alternative reaction during monoclonal antibody (mAb)-based cancer therapy.[8] Reaction of a therapeutic IgG mAb with its target on a cancer cell would normally be expected to mediate cell killing by well-defined mechanisms: antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis, complement-dependent cytotoxicity, and/or direct killing (eg, programmed cell death).[9-11] The first two reactions require participation of effector cells expressing FcγR. However, evidence based on clinical correlative studies and in vitro investigations indicates that many immunotherapeutic mAbs can mediate either cell killing or trogocytosis of their targeted antigens in the presence of effector cells (Table 1).[8,12-16] In the later reaction, once the mAbs bind to target antigens on the donor cells, an immunologic synapse is formed. The acceptor cell then removes the mAb-antigen immune complexes from the donor cell and subsequently internalizes them.[13,17-19] Therefore, targeted donor cells can escape relatively unharmed from the intended cytotoxic immunotherapy.[8,12,20] Table 2 summarizes laboratory and clinical parameters used to define FcγR-mediated trogocytosis. Although our first studies with THP-1 acceptor cells indicated that FcγRI plays a major role in trogocytosis, it is now clear that depending upon the cell type, virtually all FcγR can promote trogocytosis.[21,22]

Biological and historical perspective

This escape mechanism provides insight into possible biological functions of FcγR-mediated trogocytosis. For example, during IgG immune responses to infectious microorganisms, trogocytosis could allow effector cells to remove IgG-opsonized pathogens from the surface of infected cells without destroying the cells. In the immune adherence reaction, complement (C3b)-opsonized polyclonal antibody/pathogen immune complexes are bound to primate erythrocytes via CR1, the receptor for C3b.[23] Both in vitro experiments and preclinical studies in nonhuman primate models have demonstrated that the immune complexes are transferred to and processed by acceptor macrophages, and this reaction represents another example of trogocytosis in immune defense.[23-26] A similar reaction has been demonstrated to remove CR2 (the receptor for C3d) and bound C3d-tagged immune complexes from opsonized B cells, and likely plays a role in immune responses mediated by complement.[27-29] Trogocytosis could also provide a means of removing small amounts of autoantibodies and self-antigens from weakly opsonized cells, allowing them to escape phagocytosis and destruction in conditions associated with autoimmune diseases.[30] Similarly, Griffin et al reported that when IgG-opsonized B cells were allowed to form immune complexed "caps," then the immune complexes could be removed by macrophages in a reaction that spared the B cells from phagocytosis.[31]

Antigenic modulation, first described in the pre-mAb era,[32] occurs when treatment of cells with specific antibodies promotes upregulation

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Table 1. Important examples of trogocytosis mediated by mAbs

Target Epitope	mAb	Key observations
CD3	Muromonab-CD3 (OKT3)	Early clinical report of antigenic modulation. In vitro experiments demonstrated requirements for monocytes and intact IgG.[36,37]
CD5	T101	Loss of CD5 was induced by treatment of patients with CLL or cutaneous T-cell lymphoma with mAb T101. In vitro studies indicated FcγR on monocytes and intact IgG were required.[38-44,75,76]
CD8	Hit8a	When whole blood samples are probed with CD8 mAbs for standard flow cytometry analyses, CD8 is transferred to and internalized by monocytes and neutrophils.[19,21,30]
CD20	RTX (Rituxan) OFA (Arzerra)	Both mAbs promote in vivo and in vitro trogocytosis.[8,12-14,17,18,20,22,52,55-58,61,64,67]
	CD20-6	This type II CD20 mAb promotes trogocytosis.[57]
CD22	Epratuzumab	Treatment of SLE patients with epratuzumab resulted in trogocytosis of CD22 and other cell surface proteins from circulating B cells.[15,66]
CD25	Daclizumab (Zenapax)	Treatment of patients with multiple sclerosis with daclizumab resulted in trogocytosis of CD25 from T cells.[15]
CD33	Gemtuzumab ozogamicin (Mylotarg)	Trogocytosis, mediated by FcγRIIb on LSECs, may explain why some patients who receive Mylotarg therapy experience severe liver damage.[22,68-70,72]
EGF-R	Cetuximab (Erbitux)	Cetuximab mediates trogocytosis by monocytes of the EGF receptor on 3 different cell lines in vitro.[18,77]
Her2/Neu	Trastuzumab (Herceptin)	Trastuzumab mediates trogocytosis by monocytes of the Her2/Neu antigen on a breast cancer cell line in vitro.[18]

CLL, chronic lymphocytic leukemia; EGF-R, epidermal growth factor receptor; LSEC, liver sinusoidal endothelial cells; OFA, ofatumumab; RTX, rituximab; SLE, systemic lupus erythematosus.

of resistance of the cells to cytotoxicity mediated by the antibodies. Several mechanisms can mediate this resistance, including endocytosis by the cell of both the antibody and the targeted cell-surface antigen (including CD22 and CD40[33]), or capping of the complexes on the cell surface.[34,35] Therefore, it is not surprising that trogocytosis mediated by therapeutic mAbs was first described as "antigenic modulation" more than 30 years ago, when specific mouse mAbs were under investigation as immunosuppressive agents following renal transplants[36,37] and as immunotherapeutic agents for cancer.[38] The reaction is well-documented for IgG2a mAb T101 (CD5-specific), which was tested for treatment of chronic lymphocytic leukemia (CLL) and cutaneous T-cell lymphoma.[39-44] The mAb infusion initially promoted clearance of substantial numbers of cells. However, within hours, more cells entered the bloodstream, and many of these cells had considerably reduced CD5 levels. In vitro investigations revealed that some direct internalization of mAb T101 by B cells occurred[41,42] (as noted above, one of the first mechanisms for antigenic modulation), but the major mechanism of CD5 loss was indeed mediated by FcγR on monocytes, in a reaction identical to FcγR trogocytosis, but not so named, some 30 years ago.

Modern times and mechanisms

Ten years ago, our laboratory reported that when patients with CLL were treated with the usual 375 mg/m^2 doses of rituximab (RTX), the following patterns were observed: firstly, after infusion of only 30 mg of RTX, the circulating cells were well-opsonized, and a large fraction of cells was rapidly cleared from the circulation.[12] Then, soon after completion of the infusion, circulating CLL cell counts increased considerably, reaching levels close to or exceeding pre-infusion values. This observation would appear to present a contradiction: if 30 mg of RTX mediates clearance of a large burden of cells, why does the remainder of infused RTX (~500 to 700 mg) not clear more cells? The key to resolving this question was revealed by measuring CD20 levels on circulating cells before, during, and after mAb infusion. CLL cells in the bloodstream at the end of the infusion largely represent cells that had re-equilibrated from other compartments; initially, these CLL cells had levels of CD20 comparable to that of circulating cells before RTX infusion.[45] After RTX infusion, the cells had only 5% to 10% of the levels found on cells before RTX infusion. This rapid reduction in CD20 is most likely a consequence of trogocytosis of re-equilibrated cells in the circulation, due to the action of FcγR-expressing cells, which may include Kupffer cells as well as liver sinusoidal endothelial cells (LSECs).[17,46-49]

An important question must focus on the conditions that favor killing of targeted cells (the preferred outcome) vs escape via trogocytosis. In almost all of these treated CLL patients, complement titers in the bloodstream were reduced considerably after CD20 mAb infusions, indicating this effector function was exhausted. Although RTX weakly promotes complement-dependent cytotoxicity on binding to CLL cells, it can activate and consume complement, thus covalently tagging cells with C3b/iC3b,[8,12] and this will facilitate clearance based on recognition of C3b/iC3b-opsonized cells by complement receptors on tissue macrophages.[8,26] Additional reports indicate that other mAb-promoted cytotoxic effector functions, including NK-cell–mediated antibody-dependent cellular cytotoxicity and phagocytosis by macrophages, can also be exhausted after CD20 mAb infusion under conditions of high CLL cell burdens.[50-53] Thus, trogocytosis takes over as an alternative reaction after the body's normal cytotoxic effector functions are exhausted. We have extended and generalized these studies to include ofatumumab (OFA), a next generation CD20 mAb. We have recorded virtually identical patterns of CD20 down-modulation for more than 65 CLL patients treated with either RTX or OFA, at doses between 100 mg and 1 g.[8,12,20,54] Finally, certain mAbs may be designed to simply block an active site on a cell and not promote cell killing by effector functions. Under these conditions, trogocytosis can occur even in the absence of effector function exhaustion.

Trogocytosis is rapid; in vitro investigations in model systems, reported by both our laboratory and several other laboratories reveal that at 37°C, monocytes and macrophages as well as NK cells and neutrophils, remove IgG immune-complexed substrates from mAb-opsonized cells in 20 to 30 minutes. We have found that trogocytosed CLL cells can be identified in blood samples taken from CLL patients within 0.5 to 2 hours of initiating CD20 mAb infusions.[8,12,14,18,22,55-58] Finally, in another example of antigenic modulation, CD20 mAbs, along with CD20, can also be internalized directly by B cells, but in vitro, this internalization process is considerably slower than trogocytosis.[14,59,60]

Table 2. Criteria for identifying FcγR-mediated trogocytosis

Criteria are as follows:
In vitro experiments with cell lines or with primary cells
• In the presence of acceptor cells that express FcγR, the mAb promotes transfer of its targeted antigen from the donor cell to the acceptor cell
• Both the mAb, its target antigen, small portions of the plasma membrane of the donor cell, and the operant FcγR of the acceptor cell are internalized by the acceptor cell
• The reaction is fast, going to completion in less than 1 hour at 37°C
• The $F(ab')_2$ fragments of the mAb have little or no activity in mediating trogocytosis, and inhibitors of FcγR, such as specific mAbs or high concentrations of human IgG, inhibit the reaction
• In vitro culture of trogocytosed cells in the absence of mAb and acceptor cells leads to re-expression of the trogocytosed antigen
Clinical correlative measurements specific for CLL
• Infusion of the mAb first promotes rapid clearance of a substantial fraction of circulating CLL cells
• Within 1 to 24 hours, additional cells appear in the circulation, but they express much lower levels of the mAb-targeted antigen.
• Trogocytosis of the targeted antigen continues for days to weeks until the plasma concentration of the mAb falls to a very low level (eg, <1 ug/ml). At this point, the level of the targeted antigen returns to close to pre-infusion levels on circulating cells.

Although we have no direct evidence that CD20 mAbs promote trogocytosis of cells in tissues, we demonstrated that RTX mediated rapid loss of CD20 from human Z138 cells growing in the lungs of SCID mice.[61] Also, 30 years ago, mAb T101 was found to promote CD5 loss from CLL cells in tissues in treated CLL patients.[39,44] By analogy, it is likely that CD20 reductions in the tissues would accompany CD20 mAb therapy, and anecdotal reports suggest that this may occur.[62,63] Moreover, CD20 (or other trogocytosed targets) is not restored on targeted cells until the "pressure" mediated by the specific mAbs in the circulation is eliminated due to natural clearance of the mAbs from the bloodstream.[40,43,54,56] Indeed, we have found that for CLL patients who receive doses of 300 mg and 1 g of OFA on days 1 and 8, CD20 remains considerably depressed on day 29, as OFA will still be present in the bloodstream.[8]

Alternative dosing paradigms

Given that 30 mg doses of RTX (or OFA) are adequate to clear ~80% of circulating cells, we reasoned that trogocytosis could be substantially reduced, and effector functions allowed to better recover, by infusing considerably less mAb during each infusion (~20 mg/m^2), and thus reducing the number of mAb-opsonized cells that would be circulating (and subject to trogocytosis) after effector functions were exhausted. Thrice-weekly low-dose infusions over an extended time period should allow for multiple opportunities to efficiently target and eliminate malignant cells. Three recent low-dose trials, based on intravenous RTX alone, subcutaneous infusion of RTX alone, or low-dose RTX combined with alemtuzumab and pentostatin, although all limited in scope, provide quite favorable clinical and correlative evidence in favor of this paradigm.[52,54,64] We suggest that modified dosing schedules with these, or other mAbs now in development, have the potential to address the issue of trogocytosis, and thus enhance mAb efficacy, and may provide a revolutionary paradigm shift in the treatment of cancer patients with certain immunotherapeutic mAbs.[53] In contrast, the results of a dose-escalation trial for CLL indicated a higher level of efficacy for single agent RTX at (higher) weekly doses of 2250 mg/m,[2,65] although all remissions were partial. It is possible that the very high RTX doses in part blocked Fcγ receptors[13,19,21,22] and thus had the net effect of decreasing trogocytosis.

Trogocytosis of CD22 and CD25, and loss of innocent bystander proteins

Epratuzumab, specific for CD22 on B cells, is being investigated for the treatment of B-cell lymphomas, as well as for systemic lupus erythematosus (SLE).[15,66] Daclizumab, specific for CD25, a component of the IL2-receptor on T cells, is under investigation for treatment of multiple sclerosis.[16] We note that neither of these mAbs is particularly effective at promoting killing of targeted cells. Both mAbs have advanced to clinical trials, and correlative studies in these trials have revealed that these mAbs promote in vivo downregulation of their respective targets on circulating cells. Moreover, in vitro investigations indicated that acceptor monocytes could promote trogocytosis of CD22 and CD25 from B cells and T cells, respectively.[15,16]

Trogocytosis of CD22 on B cells mediated by epratuzumab also induces loss of other B-cell markers, including CD19, CD20, CD21, and CD79b. That is, CD22 is not the only protein removed from the B-cell surface, but a microdomain surrounding CD22 is also taken up, thus giving rise to an innocent bystander reaction in which sections of B-cell membranes containing CD22-epratuzumab immune complexes are removed together with nearby cell-surface proteins. This finding recapitulates clinical correlative measurements indicating that these markers, along with CD22, were reduced on B cells of SLE patients treated with epratuzumab, and it was suggested that these changes might therefore reduce autoimmune complications in SLE.[15] Likewise, in vitro binding of RTX to B cells promoted the transfer of CD20 and substantial amounts of CD19 to acceptor monocytes and neutrophils.[55,67] We reported that B-cell proteins, including CD19 and CD55, are partially removed from Z138 cells, along with CD20, during trogocytosis mediated by RTX.[13] We also observed reduced levels of CD19 in our correlative studies, but the CD19 gate was set broadly enough to include CLL B cells with considerably lower CD19 levels.[8,12] Thus, as a consequence of trogocytosis, altered levels of critical phenotypic markers could confound enumeration of specific subpopulations during the course of mAb-based therapy. In fact, Masuda et al[30] reported that CD3 and its ligand TCRαβ are also transferred to neutrophils during CD8 mAb-mediated in vitro trogocytosis, and found that this could give rise to false-positive flow cytometry results because granulocytes would appear to be CD8$^+$.[19]

Can trogocytosis impact therapies based on antibody-drug conjugates (ADC)?

Mylotarg (gemtuzumab ozogamicin), a humanized IgG4, CD33-specific mAb conjugated to calicheamicin, is an ADC that was used to treat acute myeloid leukemia. When this ADC is internalized by targeted CD33-positive cells, hydrolysis releases calicheamicin from the mAb, thus allowing it to intercalate into cell DNA and induce apoptotic death.[68,69] In ~10% of treated patients, Mylotarg

therapy has been associated with severe liver toxicity that can be fatal, and poisoning of the LSECs due to internalization of the ADC is a very likely pathologic mechanism.[70-72] The internalization of Mylotarg by $CD33^+$ cells is relatively slow (takes many hours), and this could allow enough time for circulating Mylotarg-opsonized cells to transfer their deadly cargo to LSECs via trogocytosis when they circulate through the liver. FcγRIIb on LSECs can clear circulating immune complexes,[46,47,49] and acceptor cell-associated FcγRIIb can promote trogocytosis and remove RTX (human IgG1)-CD20 complexes from opsonized B cells.[22] The affinity of FcγRIIb for IgG4 is modestly lower than for human IgG1,[73] but it is quite reasonable to expect that aggregated Mylotarg complexes bound to circulating $CD33^+$ cells could reach the multivalent avidity threshold necessary for chelation by a cluster of FcγRIIb on the LSEC, thereby allowing trogocytosis. Because only ~10% of patients treated with Mylotarg experience severe hepatic injury, it would be important to determine if subtle differences in FcγR activity on their LSECs, or other factors related to immune complex processing, or the unusual properties of human IgG4 antibodies in the circulation,[74] could identify those individuals most susceptible to adverse reactions. Also, because Mylotarg was removed from the market,[72] it may not be possible to unambiguously determine how it induces liver pathology. At the least, we strongly suggest that future ADC should be examined in appropriate in vitro model systems to determine if they are susceptible to potentially pathologic trogocytosis. This test would especially apply to ADC designed to target circulating cells.

In conclusion, FcγR-mediated trogocytosis is associated with and complicates a variety of mAb-based therapies, likely substantially reducing efficacy in cancer immunotherapy. In view of the continuing interest in the use of mAbs in virtually all aspects of biomedicine, it is very likely that other examples of trogocytosis will be discovered and reported to compromise mAb efficacy in future clinical trials. We recommend that this issue should first be addressed in the laboratory or in preclinical investigations, with a particular emphasis on ADC. In the case of mAb-based immunotherapies for cancer, alternative dosing paradigms may be quite effective in reducing trogocytosis, and thus enhancing the efficacy of antitumor mAbs. Future efforts to specifically suppress trogocytosis without impacting on other FcγR-mediated activities may be most rewarding.

Authorship

Contribution: R.P.T. and M.A.L. wrote the paper.

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Correspondence: Ronald P. Taylor, Department of Biochemistry and Molecular Genetics, Box 800733, University of Virginia Health Sciences Center, Charlottesville, VA 22908-0733; e-mail: rpt@virginia.edu.

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