

Trogocytosis with

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What is Trogocytosis?

Denis Hudrisier and Etienne Joly: We coined the word trogocytosis from the ancient greek trogo which means nibble (1). It refers to a cellular process whereby a recipient cell rapidly and actively 'nibbles' sizeable quantities of plasma membrane fragments from a donor cell following specific receptor-ligand interactions. This mechanism explains how some recipient cells can capture transmembrane or membrane-bound ligands from donor cells in a receptor-dependent manner. This is now well documented for cells of the lymphoid lineage such as T and B lymphocytes, natural killer cells and dendritic cells (1) but could also concern other types of cells that have been shown to acquire transmembrane ligands (e.g. R8 cells acquiring boss during drosophila eye development (2) or ephrin acquisition in the course of different cellular processes (3,4)). Trying to decipher trogocytosis gives rise to fascinating questions regarding i) the nature of the alterations on the donors cell's membrane that can lead to their piecemeal capture

Are there any problems using this method?

Denis Hudrisier and Etienne Joly: Compared to other methods for detecting active lymphocytes, the main problem of our method remains one of background leading to poor noise to signal ratio in some cases. Indeed, we find that mixing two cell types together almost always leads to some exchange of materials from the surface of one cell type to the other. This does result in the acquisition by lymphocytes of a detectable portion of the fluorescent compo-

by recipient cells; ii) the identity, topology and fate of the acquired molecules; iii) the physiological or pathological roles that acquired molecules might play once transferred to recipient cells (see below). Whilst actively pursuing answers to these various questions, we have found that it is also possible to make use of trogocytosis to identify, quantify, characterize and purify lymphocytes reacting against a given antigen (5). Our method is based on co-culture between pre-labelled target cells and effector cells. Labelling of targets can be achieved either with biotin or with fluorescent membrane markers chosen among a series of commercially available ones. During the co-culture, effector cells recognizing the antigen on the labelled targets will acquire detectable amounts of the marker and will become distinguishable from non-reactive cells by flow cytometry. Commonly, other fluorescent markers are used in order to stain various populations or subpopulations among effector cells.

nents initially incorporated in the membrane of the target cells, even in the absence of antigen. In the presence of antigen, however, the acquisition of membrane fragments from the target cells increases very significantly above background recorded when the antigen is missing, or with other controls such as blocking signalling in T cells with PP2. We are currently exploring novel approaches with a view to lower this background. Other problems that we are

What advantages does this method have against previous ones?

Denis Hudrisier and Etienne Joly: In our eyes, the main advantages are that this method is cheap and simple to set up, and can be used for all kind of lymphocytes. Furthermore, those lymphocytes may be recovered alive afterwards, for further amplification, characterisation, or even conceivably for therapeutic purposes. A further advantage is that detection by trogocytosis can also be combined with all the other available methods based on flow cytometry. As far as we know detection via trogocytosis is the only method where the reactivities of CD4⁺ T, CD8⁺ T, B, $\gamma\delta$ T or NK lymphocytes can all be assessed concomitantly, using a single experimental setup. For instance MHC-peptide multimers can be used to trace CD8⁺ T cells and sometimes CD4⁺ T cells. CD107 detection can be applied to NK or CD8⁺ T cells and CD154 to

facing are not specific to the trogocytosis method: the detection by flow cytometry of very rare active lymphocytes, or of lymphocytes with low reactivity/avidity also remain limiting factors for all the other methods available such as staining with tetramers (6), for cytokine secretion or for CD107 (7).

CD4⁺ T cells. Compared to multimers, or to the capture of GFP-tagged MHC molecules (8), a major advantage of our method is that it does not involve the lengthy and costly steps of producing specific recombinant materials. In fact, the precise knowledge of the antigenic peptide and of its presenting MHC molecule is actually dispensable. Furthermore, whereas staining with multimers will not distinguish between reactive and anergic lymphocytes, capture of GFP-tagged MHC molecules will only become detectable with target cells coated with very high levels of antigen. Via our method, only reactive lymphocytes will be detected, and we have shown that, for CD8 T cells, the sensitivity of these reactive lymphocytes to the doses of antigen is comparable to that needed to activate cytotoxicity (9).

What are the molecular mechanisms of trogocytosis?

Denis Hudrisier and Etienne Joly: To date, very little is known concerning the actual mechanisms of trogocytosis. Although, for T cells, it is absolutely clear that both the actin cytoskeleton and intra-cellular signalling are required, this is not necessarily the case for all the other types of lymphocytes (our unpublished observations). Another burning question is whether a transient membrane continuity does occur between effector and target cells. On the one hand, some studies based on morphological data have described the presence of membrane bridges (10) or of nanotubes (11) between lymphocytes and their targets. In contrast, others have

argued that the principal vectors of trogocytosis are membrane vesicles (12) or torn pieces of membrane (13). Although all these mechanisms are by no means mutually exclusive, we perceive that their respective roles will have important consequences regarding the nature of the components being transferred as well as their topology and thus their potential biological activity within the recipient cells. Finally, the fate of the components after their transfer remains largely unknown. We do know that certain acquired molecules can be maintained at the cell surface, and that they can be either well integrated in the lymphocyte's membrane or

loosely attached; Alternatively, a certain proportion of the captured molecules will be internalized and targeted either towards degradation (14) or to signaling compartments (15).

Is trogocytosis of actual relevance in B and T cell biology?

Denis Hudrisier and Etienne Joly: Biological consequences of antigen capture by T cells have been recently covered in depth in an excellent review (16). We are currently exploring whether molecules other than the antigen that have been acquired by lymphocytes can end up in the appropriate compartment, where they can be biologically active. For example, do some of the molecules adsorbed on the cytoplasmic side of the plasma membrane of the target cells end up in the cytoplasm of the lymphocytes? If we find that it is the case, we will then have to investigate to what extent this can participate in the biology of lymphocytes either in a pathological or a physiological way. For instance, antigen capture by T cells could subsequently lead either to activation or toler-

ance of T cells having the same antigen specificity, depending on the fact that co-stimulatory molecules are co-captured or not with the antigen.

From a pathological point of view, we fully expect that many pathogens have found various ways to exploit this cellular event for their own benefit either in terms of dissemination or replication. In this regard, the transfer either of viral genomes, or of viral receptors to lymphocytes could explain how such lymphocytes could become infected by viruses to which they are not normally permissive.

Alltogether, within a few years, we expect that progress in this rapidly evolving field of research will tell us whether important physiological and pathological processes rely on the evanescent process of trogocytosis.

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