Capture of Target Cell Membrane Components via Trogocytosis Is Triggered by a Selected Set of Surface Molecules on T or B Cells

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*J Immunol* 2007; 178:3637-3647; doi: 10.4049/jimmunol.178.6.3637

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The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Capture of Target Cell Membrane Components via Trogocytosis Is Triggered by a Selected Set of Surface Molecules on T or B Cells

Denis Hudrisier,2* Anne Aucher,* Anne-Laure Puaux,* Christine Bordier,* and Etienne Joly*

Key events of T and B cell biology are regulated through direct interaction with APC or target cells. Trogocytosis is a process whereby CD4+ T, CD8+ T, and B cells capture their specific membrane-bound Ag through the acquisition of plasma membrane fragments from their cellular targets. With the aim of investigating whether the ability to trigger trogocytosis was a selective property of Ag receptors, we set up an assay that allowed us to test the ability of many different cell surface molecules to trigger trogocytosis. On the basis of the analysis of a series of surface molecules on CD4+ T, CD8+ T, and B cells, we conclude that a set of cell type-specific surface determinants, including but not limited to Ag receptors, do trigger trogocytosis. On T cells, these determinants include components of the TCR/CD3 as well as that of coreceptors and of several costimulatory molecules. On B cells, we identified only the BCR and MHC molecules as potential triggers of trogocytosis. Remarkably, latrunculin, which prevents actin polymerization, impaired trogocytosis by T cells, but not by B cells. This was true even when the same Abs were used to trigger trogocytosis in T or B cells. Altogether, our results indicate that although trogocytosis is performed by all hematopoietic cells tested thus far, both the receptors and the mechanisms involved can differ depending on the lineage of the cell acquiring membrane materials from other cells. This could therefore account for the different biological consequences of Ag capture via trogocytosis proposed for different types of cells. *The Journal of Immunology, 2007, 178: 3637–3647.

In many situations, during their development, activation, and effector functions, T and B cells interact with other cellular partners. For instance, development of both CD4+ and CD8+ T cells is controlled via direct interactions with epithelial cells or APCs in the thymus (1). Their activation requires interactions with APC in secondary lymphoid organs and their effector functions depend on Ag recognition on APC or target cells. Even though B cells can recognize soluble Ags, they very often recognize (both during their development and/or activation) membrane-bound Ags or soluble Ag adsorbed on cell surfaces (2). These recognition processes largely determine the outcome of the target cell-effector cell interaction (i.e., activation or tolerance). Ag recognition by one Ag receptor is followed by receptor as well as Ag internalization by the lymphocyte, even when the Ag was initially bound to the membrane of another cell (3, 4). It is now clear that the internalization of membrane-bound Ag starts with its acquisition in a membrane-associated form by the effector cell, a process we termed trogocytosis (5, 6). Although trogocytosis is usually unidirectional, exceptions exist whereby membrane materials are transferred in both direction, for example between NK cells and their targets (7). In such cases, however, it is unclear whether this bidirectional transfer takes place within a single area of contact or via the formation of separate structures where membrane traffic follows antiparallel directions.

One primary consequence of trogocytosis is that not only the Ag but also other target cell surface molecules are acquired by effector cells (8, 9). Interestingly, although a portion of the acquired molecules is internalized, a fraction also remains on the cell surface of the effector, in an apparently functional conformation (4, 10). Although a clear in vivo demonstration of the physiological significance of trogocytosis is still lacking, the biological and pathological consequences envisaged for trogocytosis are very diverse and depend on the type of target and effector cells to a large extent (5, 6, 11). For instance, trogocytosis allows B cells to internalize membrane-bound ligands and to present them to CD4+ T cells in the context of MHC class II molecules (12). In the case of T cells, it is thought that, after internalization, the Ag-TCR interaction may be maintained to sustain T cell activation (13), whereas the Ag remaining on the surface of the T cells after acquisition may control T-T interactions and could contribute to activation of tolerance. The decision between activation or tolerance could also depend on the acquisition of costimulatory molecules together with the Ag (11). Apart from CD8+ T, CD4+ T, and B cells (14), other types of hematopoietic cells have been shown or suggested to perform trogocytosis. This is the case of γδT cells (15), NK cells (16), CD8αβ intraepithelial lymphocytes (10), dendritic cells (17, 18), and also very likely monocytes and macrophages. Even outside the immune system, transfer of membrane-bound ligands on recipient cells has been described and was shown to involve the capture of plasma membrane components (19–22). A common feature of the active capture of membrane-bound ligands is the engagement of receptors on recipient cells. On T cells, the use of anti-TCR blocking mAbs has strongly suggested the involvement of the TCR (4). Similarly, activatory and inhibitory receptors on NK cells (16, 23–25), the BCR on B cells (3, 14), and scavenger receptors on DC...
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<tr>
<th>Determinant</th>
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* Expression was assessed by flow cytometry using a mix of anti-mouse Ig-Alexa-647 plus anti-rat Ig-Alexa-488 to reveal lymphocytes preincubated with the indicated mAb. In B cells, for which anti-mouse Ig cannot be used, we also used directly labeled mAbs. +, −, and +/− refer, respectively, to strong, no, and very weak binding compared with the adequate isotype control.

** Boldface type represents the mean fold induction of trogocytosis (median fluorescence of PKH-67 on effector cell in the presence of the indicated mAb divided by the median fluorescence intensity in the absence of any Ab added) ± SD.

+ CD9 up-regulation and CD145 down-regulation were determined by flow cytometry after a 4-h coinoculation between effectors and P815 in the presence of the indicated mAb.

+ CD195 up-regulation, CD107 detection at the cell surface, and intracellular IFN-γ staining was determined by flow cytometry after a 4-h coinoculation between effector cells and P815 in the presence of the indicated mAb.

+ CD9 up-regulation and BCR down-modulation were measured by flow cytometry after a 4-h coinoculation between effector cells and P815 in the presence of the indicated mAb.
FIGURE 1. Principle and proof of concept of the redirected trogocytosis assay. A. Scheme representing the principle of the redirected trogocytosis assay. Previously labeled FcR-expressing target cells, such as P815, are bridged with effector cells (in our case T or B cells) via a mAb recognizing a cell surface molecule on the effector cells. The capacity of a given mAb to trigger trogocytosis is monitored by flow cytometric analyses, via the detection, on the effector cells, of the label initially incorporated in the plasma membrane of the P815 cell. B. Efficient redirected trogocytosis is triggered by the 145-2C11 anti-CD3 mAb on CD8$^+$ OT-I (left panels) and CD4$^+$ DO11.10 (right panels) T cells. Both types of activated T cells were mixed with PKH-67-labeled P815 in the presence of the 145-2C11 mAb (middle and lower panels) or its isotype control (top panels). Bottom panels, P815 cells were preincubated with the 2.4G2 anti-FcγRIII/A mAb. Histograms represent the PKH-67 fluorescence recorded on cells gated as CD8$^+$ or CD4$^+$, and the numbers provided correspond to the median fluorescence intensities (MFI) of the cells in the upper left quadrant. C. As in B except that P815 cells that had been prelabeled with biotin were used and that trogocytosis was detected using streptavidin-PE (SA-PE) staining. D. The indicated concentrations of the 145-2C11 mAb were used to coat either or both P815 and OT-I cells before mixing the cells and analyzing trogocytosis. The percentage of trogocytosis of CD8$^+$ cells is shown as a function of the concentration of mAb. Percentage of trogocytosis corresponds to the ratio of signal due to the capture of the PKH-67 fluorescent lipid by effector cells compared with that recorded on P815. It is calculated as $100 \times \frac{[\text{MFI of lymphocytes with mAb}] - [\text{MFI of lymphocytes without mAb}] \text{[MFI on P815 cells]}}{\text{[MFI on P815 cells]}}$. The results are representative of more than five independent experiments. E. Median fluorescence intensity for PKH-67 on gated effector cells. DO.11.10 CD4$^+$ T cells, OT-I CD8$^+$ T cells, or MD4 B cells were cocultured with P815 in the absence (●, in duplicates) or presence of isotype control Abs: mouse (○, IgG1, IgG2a, and IgG2b from left to right), rat (□, IgG1, IgG2a, and IgG2b from left to right) or hamster (◆, IgG1 and IgG2 from left to right).

(18) have been proposed to be implicated in trogocytosis. With a view to reach a better understanding of the type of receptors involved in triggering trogocytosis on αβ T and B cells, we set up a versatile screening assay inspired from the classical redirected cytolytic approach (26).

Materials and Methods

Cell lines and mice

The plasmacytoma cell line P815 of the H-2d MHC haplotype was used as target cell. The CD8$^+$ T cells specific for OVA257-264 presented by I-Ad were obtained from DO11.10 TCR-transgenic mice (27). CD4$^+$ T cells specific for OVA323–339 presented by I-A$^d$ were obtained from DO11.10 TCR-transgenic mice (28); MD4 mice, expressing a transgenic BCR specific for hen egg lysozyme (HEL)3 served as a source of B cells (29). T cells were placed in culture in the presence of the corresponding peptide (0.1 μM) for OVA257–264 and 1 μM for OVA323–339. MD4 B cells were used either ex vivo or after an overnight culture in complete culture medium. Splenic T and B cells were obtained from naive B6 and BALB/c animals.

Reagents and Abs

Peptidites were synthesized using an Applied automated synthesizer. All peptides were HPLC purified (>98%), and their identity was confirmed by mass spectrometry. The fluorescent lipid PKH-67, cytochalasin D, and soluble HEL were from Sigma-Aldrich. Biotinylation reagent (sulfo-N-hydroxysuccinimidyl ester-long chain biotin) was from Pierce. Latrunculin B was from Calbiochem. Fluorescently labeled mAbs against mouse CD4 (GK1.5 or RM4-4), CD8α (53.6.7.2), CD8β (53.5.8), the DO11.10 TCR (KJ1.16), B220 (RA3-6B2), CD154 (MR1), CD107 (ABL-93), IFN-γ (XMG1.2), CD69 (H1.2F3), CD3ε (2C11), κ chain (187.1) as well as fluorescent streptavidin were all purchased from BD Pharmingen. Fluorescent anti-mouse and anti-rat IgG were from Molecular Probes. Unlabeled mAbs listed in Table I were either obtained from BD Pharmingen or purified in our laboratory from hybridoma supernatant.

Target cell staining

For PKH-67 staining of P815 cells, 50 million cells were washed in PBS, and the pellet was resuspended in 2.5 ml of diluent C (Sigma-Aldrich) in a 15-ml tube. PKH-67 was diluted at a final concentration of 2–4 μM in 2.5 ml of diluent C in a 15-ml tube, and the solution was rapidly added to cells and incubated for 5 min at room temperature with occasional agitation. Five milliliters of FCS were then added, and after 1 min of incubation at room temperature, cells were washed three times in complete culture medium. For surface biotinylation, 50 million live cells were resuspended in PBS containing 0.2 mg/ml sulfo-N-hydroxysuccinimidyl ester-long chain biotin (Pierce). After 10 min at room temperature, cells were washed twice in culture medium, then incubated for 10 min at 4°C in PBS containing 100 mM glycine, and then washed twice again. In all cases, target cells were left 1 h at 37°C and then washed once again in complete culture medium.

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3 Abbreviations used in this paper: HEL, hen egg lysozyme; β2m, β2-microglobulin.
before resuspending them at 5 million/ml. Stained cells were then used in functional assays or in trogocytosis experiments.

Redirected trogocytosis

After target cell labeling, cells were placed in U-bottom 96-well plates (0.5 \times 10^6 cells/well in 100 \mu l final volume). Effector cells were placed in V-bottom 96-well plates (0.2 \times 10^6 cells/well in 100 \mu l final volume) and incubated with 5 \mu g/ml (or the indicated concentrations) of the different unlabeled mAbs for 30 min at 4°C. After two washes in culture medium, cell pellets were resuspended with 100 \mu l of culture medium of which 50 \mu l were removed for FACS staining and 50 \mu l were added to target cells, centrifuged for 30 s at 900 rpm to promote conjugate formation, and then left at 37°C for 1 h. Conjugates were then dissociated by washing cells twice in PBS containing 2 mM EDTA, before staining with anti-CD8, anti-CD4, or anti-B220 mAb. Cells were then analyzed on a FACSCalibur (BD Biosciences). Effector cells were then gated positively according to their staining with lineage-specific markers (CD8 for CTL, CD4 for Th cells, and B220 for B cells). Trogocytosis was measured directly by the detection of PKH-67 on the effectors. When biotinylated target cells were used, flow cytometric detection of trogocytosis was performed using a second step involving staining with streptavidin conjugated to PE together with CD8^+, CD4^+, or B cell markers. In some experiments, T and B cells were treated with latrunculin B (2–10 \mu g/ml) or cytochalasin D (10 \mu M) for 30 min at 37°C before conjugate formation, and the drug was left throughout the assay.

**Determination of surface receptor expression**

The expression of receptors on T and B cells was assessed by flow cytometry on a sample of T or B cells preincubated with unlabeled mAbs prepared for redirected trogocytosis assay. These samples were incubated with a second layer of anti-mouse Ig coupled to Alexa-488 and anti-rat Ig coupled to Alexa-647. This combination allowed the binding to mouse, rat, and hamster mAbs. In the case of B cells where the use of anti-mouse Ig mAbs is not possible due to BCR expression, directly labeled mAbs were utilized.

![FIGURE 2. Selective sets of cell surface molecules on CD4^+ T CD8^+ T and B cells trigger trogocytosis upon engagement with mAb. Redirected trogocytosis by DO11.10 CD4^+ T, OT-I CD8^+ T, and MD4 B cells was determined in the presence (white histograms) or absence (gray histograms) of the indicated mAb. Histograms were obtained with cells gated as positive for CD4 (A) CD8 (B), or B220 (C). Results are representative of at least three independent experiments. Additional results are presented in Table I. FL-1H, Fluorescence.](http://www.jimmunol.org/)

![FIGURE 3. Effect of the dose of mAb on Ag binding and trogocytosis. A, OT-I cells were incubated with the indicated concentrations of the various mAbs and either analyzed by flow cytometry for mAb binding using a mix of fluorescently labeled anti-rat plus anti-mouse IgG (○) or used in redirected trogocytosis (●). For those mAbs binding to effector cells and triggering trogocytosis, the signals recorded for mAb binding to effector cell (binding) or of PKH-67 capture in a coculture with P815 (redirected trogocytosis) were both normalized so that they could be represented on the same graphs. Normalizations were conducted using the formula 100 \times [(MFI exp) − (MFI neg)]/(MFI max) − (MFI neg)] where (MFI exp) refers to the median fluorescence intensity for a given concentration of an Ab, (MFI neg) in the absence of mAb, and (MFI max) in the presence of a saturating concentration of mAb. For the anti-CD71 mAb, which binds to effector cells but does not trigger trogocytosis (lower right panel), median fluorescence intensities obtained in binding experiments (left axis values) and redirected trogocytosis (right axis values) could not be normalized and are therefore represented directly as median fluorescence intensities for both events. B, As in A but MD4 effector B cells were used. For both A and B, similar results were obtained in two independent experiments.](http://www.jimmunol.org/)
Redirected T and B cell activation

Redirected activation of T and B cells was measured by flow cytometry after 4 h of coculture at 37°C of either population of effector cells with P815 cells (E:T 1:5) in the presence of the indicated mAbs. We performed a double staining to assess CD69 up-regulation on lineage marker-positive cells (CD4 or CD8 or B220). In addition, T cell degranulation was measured by flow cytometry using published procedures for the detection of CD107 (30) or CD154 expression (31) at the cell surface of CD8+ cells (Fig. 1C). These levels are comparable with those we obtained in at least two independent experiments.

Results

Redirected trogocytosis as an assay to test receptor-mediated trogocytosis

With the aim of identifying cell surface receptors able to trigger trogocytosis, in other words, unidirectional capture of membrane fragments, we evaluated the possibility of using anti-receptor mAbs in a redirected trogocytosis assay, inspired from the commonly used in redirected cytolytic assays. Occurrence of trogocytosis was then determined by the ability of various mAbs to trigger the capture, by the effector cells, of membrane fragments from P815 cells, which both perform trogocytosis when stimulated in an Ag-specific manner (14). On the basis of previous knowledge that Ag recognition by the TCR triggers trogocytosis, we tested the stimulatory 145-2C11 anti-CD3ε mAb or its hamster IgG1 isotype control in our initial experimental system. As shown in Fig. 1B, 145-2C11 but not its isotype control triggers the capture of membrane fragments from PKH-67-labeled P815 by both OT-I and DO11.10 T cells. Trogocytosis triggered by 145-2C11 was abolished when P815 cell were pretreated with the 2.4G2 anti-FcγRIII/II mAb indicating that binding to the FcγR on P815 was necessary. Similar results were obtained with biotinylated P815 cells (Fig. 1C) or with other FcγR+ targets such as A20 cells (not shown). Capture was unidirectional given that unlabeled P815 did not acquire lipids from PKH-67-labeled OT-I cells in the presence of anti-CD3 mAb (not shown). Preincubation of the effector cells with the mAb followed by washing or leaving the mAb in contact with both effector and target cells gave the same extent and dose dependence of trogocytosis. In contrast, preincubation of the mAb with P815 and washing the excess Ab off before effector cell addition leads to much lower levels of trogocytosis, probably due to the weak mAb-FcR binding (Ref. 32 and Fig. 1D). The levels of fluorescent signal recorded at the end of the redirected trogocytosis assays usually corresponded to 2–3% of those present on the P815 target cells (Fig. 1D). These levels are comparable with those we routinely obtain in Ag-driven trogocytosis (8, 14).

Identification of surface receptors triggering trogocytosis on activated T and naive B cells

Having obtained the proof of concept that trogocytosis mediated by TCR recognition of its Ag can be mimicked by anti-CD3ε mAbs in a redirected trogocytosis assay, and with the objective to
understand whether other surface molecules on effector cells could trigger trogocytosis, we tested a series of surface molecules for which mAbs of the IgG isotype were available (Table I). The expression of all these receptors on T and B cells was determined by flow cytometry (see Table I). When used in redirected trogocytosis assay, the isotype controls for each of the anti-receptor mAbs led to levels of PKH-67 staining that were not higher than those recorded in the absence of any mAb added (Fig. 1E). Initially, to manage large numbers of samples, the mAbs were added to wells containing both P815 and effector cells and were not washed away during the assay. Because P815 were labeled with PKH-67 in batches for the whole experiment, the fluorescence intensity of PKH-67 on the P815 in all samples was identical, which was convenient for comparing the extent of trogocytosis triggered by the various mAbs. Those were tested in redirected trogocytosis assay against activated CD4+ DO11.10, activated CD8+ OT-I, and native MD4 B cells, which can all exhibit strong trogocytosis activity when activated via their respective cognate Ags (14). As shown in Fig. 2 and Table I, on each type of effector cells, we identified a selected set of surface receptors capable of triggering trogocytosis upon engagement. Whereas on T cells a relatively large number of cell surface molecules triggered trogocytosis, a very limited set was identified for B cells. On CD4+ and CD8+ T cells, cell surface molecules triggering trogocytosis included the TCR and CD3e as well as CD28. These observations are in good agreement with previous reports documenting the capture of peptide-MHC complexes triggered via the TCR and of CD80/86 via CD28 (33). In addition, we found that engagement of lineage-specific coreceptors (CD8α or β for OT-I cells and CD4 for DO11.10 cells) and costimulatory molecules such as CD2 and CD27 also triggered trogocytosis. Low but significant levels of trogocytosis were triggered by anti-CD9 or anti-CD5 mAbs on both types of T cells (Table I). The intensities of the signals recorded with all these Abs are provided in Table I for information, expressed as the fold induction in median fluorescence intensity compared with those obtained in the absence of any mAb added. None of the other mAbs against activation molecules, adhesion molecules (see CD18 in Fig. 2), or various chemokine, cytokine, or other types of receptor (see CD71 in Fig. 2) triggered trogocytosis. On B cells, trogocytosis was very strongly triggered by anti-BCR κ chain and anti-MHC class II mAbs and by one of two anti-BCR μ chain mAbs. An anti-CD81 mAb also triggered trogocytosis, albeit at low levels. Interestingly, mAbs neither against other components functionally associated with the BCR such as CD19, CD21/35 or CD79b (Igβ) nor against molecules known to transduce important signals on B cells such as CD80, CD86, or CD40 triggered...
trogocytosis. At least one mAb of each isotype was found to be able to trigger trogocytosis on at least one type of effector cell, indicating that the identification of surface receptors triggering trogocytosis was not biased by limitations in mAb binding to the FcR on P815 target. Altogether, our results show that a selected set of cell-type specific surface molecules trigger trogocytosis upon engagement.

The extent of trogocytosis triggered by a given mAb and the extent of its binding to Ag are strictly correlated

Next, we evaluated the ability of increasing concentrations of mAbs working in redirected trogocytosis to bind to surface Ag and to trigger trogocytosis. For that, effector cells were preincubated with increasing concentrations of the indicated mAbs, washed, and then split into two halves: one was used in subsequent staining with anti-mouse and anti-rat IgG and flow cytometry analysis; and the other was tested in our redirected trogocytosis assay. There is no correlation between the expression level of a given cell surface molecule and its ability to trigger trogocytosis. Indeed, certain strongly expressed molecules do not trigger trogocytosis (see the examples of CD71 on OT-I and MD4 cells in Fig. 3, A and B, respectively). When a receptor mediates trogocytosis, again there is no strict correlation between the extent of trogocytosis triggered and the density of receptor expression. In contrast, the only strict rule is that there is no case in which trogocytosis is triggered without detectable staining of the lymphocyte with the corresponding mAb. In line with this, the S19 mAb against β₂-microglobulin, which stains only OT-I cells (H-2b²), triggered strong trogocytosis only on these cells. The mAb concentration giving half-maximal binding was always closely correlated with that giving half-maximal trogocytosis, independently of what mAb or effector cell was used (Fig. 3).

Costimulatory effects in redirected trogocytosis

When used concomitantly with submaximal concentrations of anti-CD3 mAb on OT-I cells, mAbs triggering trogocytosis on their own led to an increase of the extent of trogocytosis caused by the anti-CD3, whereas adding mAbs that did not trigger trogocytosis on their own did not affect anti-CD3-mediated trogocytosis (Fig. 4A). Similar results were obtained when anti-CD3 was replaced by suboptimal concentrations of anti-CD2 (Fig. 4B) or anti-CD27, -CD28, -CD8 or -H2Kb (not shown). Finally, with mAbs that could not trigger trogocytosis on their own, we could not find any combination of two such mAbs that would trigger trogocytosis (Fig. 4C). On MD4 B cells, similar results were obtained with submaximal concentrations of anti-κ mAb (Fig. 4D) or anti-MHC class II mAb (Fig. 4, E and F).

In summary, when two mAbs are combined, we find an additive effect on the levels of trogocytosis recorded.

Trogocytosis triggered by surface determinants other than Ag receptors did not lead to full T or B cell activation

AgR-mediated activation of T or B cells can be mimicked by aggregating some cell surface molecules (34) or by aggregating membrane microdomains (35). We therefore investigated how trogocytosis triggered by receptors other than the TCR and the BCR in our redirected assay correlated with their ability to mimic some aspects of AgR-mediated activation of T or B cell. As shown in Fig. 5, the 145-2C11 anti-CD3 or the OVA peptide recognized by the OT-I TCR triggered OT-I cell degranulation (as measured by CD107 surface expression; Fig. 5A), IFN-γ production (Fig. 5B), as well as CD69 up-regulation (Fig. 5C) in redirected activation assays. Similar results were obtained with the 17A2 and 500A2 mAb to CD3ε and by the H57 anti-TCR mAb, although more weakly (not shown). In marked contrast, and with the exception of the anti-CD27 mAb which triggered a weak but reproducible CD107 up-regulation, none of the other mAbs tested triggered signs of OT-I cell activation, independent of the fact that they could trigger trogocytosis (CD2 and CD28) or not (CD18). On DO11.10 T cells, the anti-CD3 and anti-TCR mAbs but none of the other mAbs tested triggered CD69 up-regulation (Fig. 5D and data not shown). Surface expression of CD154, a recently described test for CD4⁺ T cell activation (31), was also triggered very efficiently by the anti-CD3ε and anti-TCR mAbs but was also triggered, although more weakly, by several other mAbs such as anti-CD27 and anti-CD28 (Fig. 5E). Other mAbs (for instance the anti-CD2 mAb) did not trigger CD154 expression (Fig. 5E). Finally, when B cells were used, soluble HEL and the anti-BCR mAbs (markedly for the anti-κ and modestly for the anti-µ) but not the anti-MHC class II mAb triggered CD69 up-regulation (Fig. 5F) or BCR internalization (not shown). Remarkably, the mAb we used against CD40 did not trigger trogocytosis or BCR internalization but did up-regulate CD69 expression (not shown). Therefore, our results, summarized in Fig. 5, G–I, show that engagement of AgRs triggered T or B cell activation, as well as trogocytosis, but that targeting molecules other than the AgRs can lead to a variety of
responses, and that triggering of trogocytosis is not necessarily linked to the induction of activation markers.

Identification of surface receptors triggering trogocytosis on heterogeneous populations of naive C57BL/6 (B6) and BALB/c splenocytes

Next, we tested freshly isolated splenocytes from B6 or BALB/c mice in redirected experiments. As shown in Fig. 6, we found that essentially the same receptors triggered trogocytosis in naive T and B cells as compared with Ag-specific activated T and naive B cells (see the effect of anti-CD2, anti-CD3, anti-CD4/8, anti-CD27, anti-CD28, and anti-MHC I on B6 and BALB/c T cells and of the anti-κ and of anti-MHC class I and class II on B6 and BALB/c B cells). Several different anti-CD4 and anti-CD8 mAb of the IgG isotype but not of the IgM isotype triggered trogocytosis on CD4+ T and CD8+ T cells (data not shown). This experimental system was better suited to illustrate the specific effect of anti-MHC class I mAb than the transgenic lines used in the previous experiments. For example, the anti-β2-microglobulin (β2m), which reacts only with cells of the H-2b haplotype and triggered trogocytosis only on OT-I cells, also triggered trogocytosis by CD4+, CD8+, and B cells from B6 but not BALB/c mice (Fig. 6). Similar results were obtained with the Y3 anti-H-2Kb mAb. Finally, the H97 mAb which reacts with H-2Kb and H-Dd, and not with H-2b molecules, triggered trogocytosis by CD4+, CD8+, and B cells of the H-2d haplotype only.

Trogocytosis performed by T cells, but not by B cells, requires actin cytoskeleton

Upon Ag recognition on APC, T cells perform trogocytosis in a manner that requires signaling as shown by its blocking or inhibition with various mAbs and/or pharmacological inhibitors such as PP2, an src kinase inhibitor, or latrunculin B (8, 14, 36). To document further the nature of the mechanisms triggered during redirected trogocytosis by T or B cells, we next used latrunculin B, an inhibitor of the actin cytoskeleton. As shown in Fig. 7A, trogocytosis performed by OT-I (upper panel) or DO11.10 (lower panel) was substantially decreased in the presence of latrunculin B; this occurred for all the mAbs tested. In marked contrast, trogocytosis triggered by mAbs to IgM (not shown), κ-chain, or MHC-II on MD4 B cells was insensitive to latrunculin B (Fig. 7B). On naive CD4+ T cells, trogocytosis triggered by anti-CD3 or...
anti-CD4 was almost completely inhibited by latrunculin B (Fig. 7C). Similar results were obtained with anti-CD3 or anti-CD8 mAb on naive CD8+ T cells (Fig. 7C). For MD4 B cells, we found no inhibitory effect of latrunculin B on trogocytosis triggered by anti-κ or anti-MHC class II mAb on B6 B cells (Fig. 7C). Because anti-MHC class I mAbs are the only ones we identified that can trigger trogocytosis on both T and B cells, we compared the effect of latrunculin B in redirected trogocytosis assays in T and B cells from B6 splenocytes (Fig. 7C). Trogocytosis mediated by anti-β2 m or anti-H-2Kb mAb was blocked by latrunculin B on both CD4+ and CD8+ T cells but not on B cells, although the nature and affinity of the receptor-ligand interaction was strictly identical in the two experimental setups. When cytochalasin D was used instead of latrunculin B to inhibit the actin cytoskeleton, the same differential effect on trogocytosis performed by T cell and B cells was obtained (Fig. 7D for primary splenocytes; data not shown for OT-I T cells and MD4 B cells). By performing time course experiments, we have found that, under the conditions used for this study, the t1/2 for redirected trogocytosis was ~30 min for both B and T cells (not shown), which is very similar to the rates recorded for Ag-driven trogocytosis in T cells (8). This result rules out the possibility that the different requirements of trogocytosis in T and B cells for an active actin cytoskeleton could be explained by a difference in the kinetics of acquisition of material. Altogether, our results strongly suggest that trogocytosis by B and T cells relies, at least partly, on distinct cellular mechanisms.

Discussion

Using a method based on bridging mAb-coated lymphocytes to their targets via the FcR of the target, we have been able to explore the ability of a large panel of proteins found at the surface of T and/or B cells to trigger trogocytosis. We report that trogocytosis can be triggered by the engagement of a set of lymphocyte-specific surface receptors either individually or in combination. In addition, based on their sensitivity to the presence of a functional actin cytoskeleton, we have found that trogocytosis can have different functional requirements depending on the cell type used as effector cell rather than on what receptor is involved.

Trogocytosis was previously reported as a process whereby CD4+ T, CD8+ T, and B cells capture their Ag anchored in the membrane of APC via the transfer of membrane fragments (5). Because this process requires the presence of Ag, it was assumed that AgR were central in this process. This assumption received more direct experimental support when blocking mAbs to the AgR or coreceptors expressed by T cells were reported to inhibit Ag capture (4, 8, 37) or when thymocytes from TCRα-deficient mice exhibited substantially decreased MHC capture as compared with wild-type thymocytes (38). In addition, it was reported that CD28, a costimulatory receptor expressed by T cells, mediated capture of CD80 by T cells, suggesting that non-AgRs could participate in trogocytosis (33, 36). Using a large panel of anti-receptor mAbs in a redirected trogocytosis assay, i.e., trogocytosis initiated by FcR-expressing target cells, we tested the ability of individual cell surface molecules to trigger trogocytosis upon engagement by mAbs. We have found that this assay indeed allows identification of capacity to trigger trogocytosis and for those that do not (S. Daubeuf, manuscript in preparation). At this stage, we therefore believe that the situations in which a surface molecule can trigger trogocytosis with certain Abs and not with others are more unusual than the norm.

Altogether, our results yield a picture that is different for T and for B cells. For T cells, it appears that a coordinated set of receptors known to play a critical role in T cell activation (39, 40) have the ability to trigger trogocytosis and might therefore cooperate in...
promoting the acquisition of various materials by T cells. Given the varied roles proposed for trogocytosis in T cell activation, it is therefore possible that the presence or absence of these molecules on T cells and of their ligands on APC could determine the positive (activation) or negative (induction of anergy) roles the captured material will play in subsequent T-T interactions (11). For B cells, capture is mainly due to the BCR and poorly or not at all to the BCR-associated costimulatory molecules, suggesting that BCR signaling is not important for Ag capture. Interestingly, our results that trogocytosis by T cells is very sensitive to latrunculin B or cytochalasin D but remains insensitive to high doses of these drugs on B cells (this study) and to other inhibitors (A. Aucher, submitted for publication) also goes in this direction. Furthermore, it has recently been shown that BCR signaling and internalization are mutually exclusive events (41). In addition it was previously reported that several initial steps of Ag recognition by B cells do not require signaling (42). The fact that mAbs to MHC class I and MHC class II can trigger trogocytosis on T and B cells was somewhat unexpected but could be reminiscent of previously published observations that these molecules participate in cell adhesion together with their respective counterreceptors, CD8 and CD4 (43, 44) and that the engagement of MHC molecules either via mAbs or with physiological partners impact on both effector and target cells of the immune system (45, 46). Regarding the capacity of certain molecules that are not part of the Ag receptor complex to trigger trogocytosis by lymphocytes, our observation that engagement of either CD8 or CD28 can trigger trogocytosis in our non-physiological assay fits well with the reports of Pardigon et al. (10) that intraepithelial lymphocytes acquire TL, a nonclassical MHC class I molecule in a CD80α-mediated manner both in vitro and in vivo and that CD28 mediates the capture of CD80 (33). The events recorded in the somewhat artificial setup of a redirected trogocytosis assay are therefore apparently in good agreement with physiological trogocytosis processes.

An important future question will be to understand why it is only certain receptors that trigger trogocytosis, and not others, because our study has not allowed us to define a clear-cut criterion. The fact that mAbs of different isotypes can trigger trogocytosis rules out a restrictive role of a given isotype in triggering trogocytosis. Nevertheless, the relative affinities of the different mouse, rat, and hamster isotypes for the FcR expressed on P815 might have an impact on the intensity of trogocytosis making our measurement of trogocytosis semiquantitative. In this regard, our data with Abs of various isotypes recognizing the same molecules suggest that the rat IgG2a isotype could be more efficient than IgG2b. The expression level is clearly not a critical criterion because among the receptors that are expressed strongly, certain can trigger trogocytosis (MHC, CD2, CD4, CD8, BCR for instance), and other not (CD11a, CD18, CD43, CD45 for instance). Certain receptors expressed more modestly can also trigger trogocytosis (CD3, CD9, CD27, CD28) or not (CD117, CD71 for instance). The stimulatory or inhibitory nature of the mAbs used does also not seem to be a criterion. For instance, activatory mAbs such as those we used against the TCR/CD3 complex or inhibitory ones such as those against the CD4 or CD8 coreceptors all triggered trogocytosis. Although most receptors reported in this study as triggering trogocytosis are known to transduce signals, particularly via src kinase family members (CD2, CD3, CD4, CD5, CD8, CD27, CD28, MHC class I, and MHC class II), this is not the case for all of them (the CD9 and CD81 tetrascapins for instance) (47, 48). In addition, several cell surface determinants known to be associated with a signaling machinery did not trigger trogocytosis (adhesion molecules or CD45 for instance).

The assay described here could conceptually be used to identify other receptors involved in trogocytosis in B and T cells, but also by other types of immune cells. Regarding the relevance of our method for the study of human lymphocytes, preliminary data indicate that the same surface molecules also trigger trogocytosis by circulating human CD4 and CD8 T cells obtained from healthy blood donors (not shown).

Furthermore, this method may also prove to be useful to study the mechanisms of trogocytosis and cellular interactions within and outside of the immune system. One such system is that of reproduction where trogocytosis between the egg and the sperm is proposed as a preliminary step before fusion (49), and the use of an experimental setup based redirected trogocytosis could provide the means to identify the molecular partners involved in this process. Concerning immune cells, although the in vitro identification of molecular triggers of trogocytosis does not per se provide a proof of the in vivo physiological relevance of this process, the results reported here may provide the experimental means to trigger trogocytosis independently of T or B cells activation. In the newly and rapidly emerging field of cell-cell communication based on membrane exchange, the identification of receptors involved in trogocytosis processes will be central to the understanding of how these events are triggered and how they may impact on the ensuing biological processes.

Acknowledgments
We thank Florent Navarro and the staff of Becton Dickinson Europe for their help with mAbs. We thank the staff of our animal facility for the maintenance of the various strains of mice. We thank Isabelle Maridonneau-Parini for the gift of cytochalasin D.

Disclosures
The authors have no financial conflict of interest.

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