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Ligand Binding but Undetected Functional Response of FcR after Their Capture by T Cells via Trogocytosis

Denis Hudrisier,*2‡ Béatrice Clemenceau,‡ Stéphanie Balor,† Sandrine Daubeuf,*2‡ Eddy Magdeleine,*2‡ Marc Daéron,§ Pierre Bruhns,§ and Henri Vie‡

Intercellular transfer of cell surface proteins by trogocytosis is common and can affect T cell responses. Yet, the role of trogocytosis in T cell function is still elusive, and it is unknown whether a molecule, once captured by T cells, harbors the same biological properties as in donor APC. In this study, we showed that FcγR as well as the associated FcγRγ subunit could be detected at high levels on murine and human T cells after their intercellular transfer from FcγR-expressing APC. Capture of FcγR occurred during coculture of T cells with FcγR-expressing APC upon Ab- or Ag-mediated T cell stimulation. Once captured by T cells, FcγR were expressed in a conformation compatible with physiological function and conferred upon T cells the ability to bind immune complexes and to provide B cells with this source of Ag. However, we were unable to detect downstream signal or signaling-dependent function following the stimulation of FcγR captured by T cells, and biochemical studies suggested the improper integration of FcγR in the recipient T cell membrane. Thus, our study demonstrates that T cells capture FcγR that can efficiently exert ligand-binding activity, which, per se, could have functional consequences in T cell-B cell cooperation. The Journal of Immunology, 2009, 183: 6102–6113.

Recognition by T cells of Ag at the surface of APC is a critical event for subsequent T cell activation and acquisition of effector functions. A well-recognized event associated with Ag recognition is the conformationalization of the TCR together with its Ag, which is formed by the peptide-MHC complex present at the membrane of APC (1–3). Membrane-bound peptide-MHC complexes are captured by T cells via membrane fragments containing both lipids and a large panel of membrane-bound proteins in a process termed trogocytosis (4, 5). Consequently, T cells harbor on their surface not only peptide-MHC complexes, but also a series of molecules that they do not synthesize themselves. Many different roles have been hypothesized for trogocytosis in T cell biology, but their physiological relevance and underlying mechanisms have not been demonstrated (1, 5–7). Some proposed functions of trogocytosis solely require that molecules captured by T cells interact with their ligands on the same or different cells without the need for intracellular signaling events. For instance, capture by T cells of the peptide-MHC complex and of costimulatory molecules, followed by interaction with the TCR and CD28 on other T cells, results in T cell-T cell interactions leading to activation, tolerance, or exhaustion (1). In contrast, it is expected that a proper integration of the captured molecule would be required for downstream intracellular signals to be triggered (5).

The transfer of receptors by trogocytosis has been reported (8, 9), but it is unclear whether they could transduce biochemical signals after transfer. This question is critical because it could greatly affect the functional outcome of receptor capture through trogocytosis by cell subsets that intrinsically do not express these given receptors.

The uptake by T cells of FcR released from macrophages was among the first descriptions of intercellular transfer of surface molecules (10). However, no mechanism was reported for this transfer, and the type of FcγR taken up by T cells as well as potential function after uptake have yet to be defined. FcγR play important roles in immune responses by coupling innate and adaptive immunity through their ability to bind Ab-Ag complexes and Ab-opsonized target cells, and thus, their role in phagocytosis and Ab-dependent cell cytotoxicity (ADCC).3 The function of FcγR depends on their type (activatory or inhibitory, FcγR subunit dependent or independent, high or low affinity) and on the cells expressing the FcγR (11, 12).

In this study, we thus examined the mechanisms of FcγR capture by T cells through trogocytosis and documented the capture of various types of FcγR by CD4+ and CD8+ murine and human T cells. Furthermore, we explored whether, once captured by T cells, FcγR could act as a receptor both in terms of ligand-binding and functional (signaling-based) properties.

Materials and Methods

Cell lines and mice

The plasmacytoma cell line P815 or HEK 293 cells and their stable transfectants expressing FcγR were used as target cells. Dendritic cells (DC) generated from B6 bone marrow (BM-DC) (13) or peritoneal B6 macrophages were used as APC. The OT-I CD8+ T cells specific for OVA peptide 257–264 (SIINFEKL) presented by the H-2Kd MHC class I molecules and the OT-II CD4+ T cells specific for the OVA peptide 322–332 were used as APC.

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3 Abbreviations used in this paper: ADCC, Ab-dependent cell cytotoxicity; β2m, β2-microglobulin; BM-DC, dendritic cells generated from B6 bone marrow; DC, dendritic cell; HEL, hen egg lysozyme; IC, immune complex; mFcγ, murine Fcγ.

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presented by the MHC class II I-A^b were obtained from the corresponding TCR-transgenic mice (Charles River Laboratories) (14, 15). Total splenocytes from TCR-transgenic animals were cultured in the presence of 0.1 mM appropriate antigenic peptide and used in trogocytosis or functional assays 4–6 days after stimulation. B cells specific for HEL were from BCR-transgenic MD4 mice (H-2d background) (16). The 1H11-34 cell line is a T cell hybridoma specific for HEL 107–116 presented by I-E^d (17). Activated human T cells transduced with the retroviral vector encoding human FcRIIIA-FcR\alpha chimeras were generated, as described previously (18).

**Reagents, Abs, and molecular biology**

Peptides were synthesized in our laboratory and HPLC purified (>98%), and their identity was confirmed by mass spectrometry. The fluorescent lipid DiO, Indo-1, hen egg lysozyme (HEL), anti-Flag, and anti-OVA Abs were from Sigma-Aldrich. HEL-specific mAb F9 and F10 and their Fab were a gift of J.-C. Gueyr (INSERM U563, Toulouse, France). Fluorescently labeled mAb against mouse CD8^b (53.6.7.2), CD3 (2C11), CD4 (RM4-4 or RM4-5), CD8\beta (H35), CD27 (LG3A10), CD71 (C2F2), H-2K^b (Y3), or β2-microglobulin (β2m; S-19) were from BD Biosciences. Abs to GFP were from Abcam, and anti-rabbit IgG coupled to Alexa647 were from Invitrogen/Molecular Probes. Anti-human CD3 (OKT-3) and MHC class I (W6/32) were obtained from culture supernatant of the corresponding hybridoma. Rituximab was from Roche. Unlabeled rabbit polyclonal Ab to the FcR\alpha subunit was from Upstate Biotechnology/Euromedex. Construction of a vector encoding FcR\alpha GFP was done, as described previously (19). Vectors encoding murine flagged FcRIIB or FcRIIIA and the FcR\alpha subunit have been described previously (20). Retroviral vector encoding the human FcRIIIA-FcR\alpha chimera was previously reported (18).

**Trogocytosis**

Trogocytosis was performed, as previously described (21). Target cells labeled or not with the lipophilic probe DiO were incubated in U-bottom 96-well plates (0.5 × 10^6 cells/well in 100 μl final volume) with T cells.
cells (0.2 \times 10^6 cells/well in 100 \mu l final volume) for 1 h at 37°C. For redirected trogocytosis experiments, T cells were previously incubated with 5 \mu g/ml different unlabeled mAbs for 30 min at 4°C. For Ag-dependent trogocytosis experiments, the corresponding peptide Ag (final, saturating concentration = 1 \mu M) was incubated with target cells or target cells were transfected with a construct encoding the OVA-H-2Kb subunit or left untransfected (mock) were incubated with OT-I T cells coated or not with the anti-H-2Kb mAb. A. Cells were analyzed by flow cytometry using anti-CD8 mAb and a biotinylated mAb against Flag, followed by fluorescent streptavidin. Histograms show FcyR expression on gated OT-I T cells exposed to the indicated target cells in the absence (gray histograms) or presence (white histograms) of the anti-H-2Kb mAb. B. As in A, except that flow cytometry was performed using anti-CD8 and anti-FcyRIIB/RIIIA mAbs. C. HEK cells transfected with a vector encoding murine flagging FcyRIIB or FcyRIIIA plus the FcY subunit or left untransfected (mock) were incubated with OT-II T cells coated or not with the indicated mAbs. Cells were analyzed by flow cytometry using anti-CD4 mAb and anti-FcyRIIB/RIIIA mAbs. Numbers represent the median fluorescence intensity of anti-FcyRIIB/RIIIA staining on gated CD4^+ T cells. Note that HEK cells that were mock transfected do not allow triggering of trogocytosis by OT-I or OT-II T cells (data not shown).

**FIGURE 2.** Both FcyRIIB and FcyRIIIA trigger trogocytosis and are captured by OT-I and OT-II T cells. HEK cells transfected with a vector encoding murine flagging FcyRIIB or FcyRIIIA plus the FcY subunit or left untransfected (mock) were incubated with OT-I T cells coated or not with the anti-H-2Kb mAb. A. Cells were analyzed by flow cytometry using anti-CD8 mAb and a biotinylated mAb against Flag, followed by fluorescent streptavidin. Histograms show FcyR expression on gated OT-I T cells exposed to the indicated target cells in the absence (gray histograms) or presence (white histograms) of the anti-H-2Kb mAb. B. As in A, except that flow cytometry was performed using anti-CD8 and anti-FcyRIIB/RIIIA mAbs. C. HEK cells transfected with a vector encoding murine flagging FcyRIIB or FcyRIIIA plus the FcY subunit were incubated with OT-II T cells coated or not with the indicated mAbs. Cells were analyzed by flow cytometry using anti-CD4 mAb and anti-FcyRIIB/RIIIA mAbs. Numbers represent the median fluorescence intensity of anti-FcyRIIB/RIIIA staining on gated CD4^+ T cells. Note that HEK cells that were mock transfected do not allow triggering of trogocytosis by OT-I or OT-II T cells (data not shown).

**Binding of immune complexes**

Cells were incubated for 1 h at 4°C with immune complexes (IC) previously formed by incubating for 30 min at 37°C an equimolar mixture of HEL-specific F9 and F10 mAbs (murine IgG1, \(\kappa\)) with 10-fold lower molar equivalent of biotinylated HEL. Binding of IC was analyzed by flow cytometry (FACScalicibur; BD Biosciences) after staining with anti-CD8 or anti-CD4 mAb and fluorescent streptavidin.

**Measurement of intracellular Ca^{2+} flux**

To assess intracellular calcium (Ca^{2+} mobilization), T cells were separated from target cells at the end of trogocytosis experiments and incubated with Indo-1 (2 mM, 1 \times 10^6 cells/ml) at 37°C for 1 h. After washing (three times) and staining with anti-CD4 or anti-CD8 mAb, T cells were resuspended at 1 \times 10^6 cells/ml and the calcium-dependent fluorescence of Indo-1 in T cells was assessed by flow cytometry on a LSRII cytometerometer (BD Biosciences). The total recording time was 10 min. At different time points, IC or PMA/ionomycin was added.

Intracellular cytokine staining was performed, as described previously (24). OT-I T cells (0.5 \times 10^6 cells in 200 \mu l) separated from target cells at the end of trogocytosis experiments were incubated with IC or the OVA peptide SIINFEKL for 2 h at 37°C in U-bottom 96-well plates. Brefeldin A was then added, and incubation was extended for additional 2 h. Cells were then stained with anti-CD8 mAb, fixed with 2% p-formaldehyde, permeabilized with a permeabilization buffer (PBS, 0.5% saponin, 1% BSA), and labeled with anti-TNF-\(\alpha\) mAb. Cells were then analyzed by flow cytometry.

**ADCC assays**

Raji cells were labeled with 100 \mu Ci (3.7 MBq) of ^51Cr (PerkinElmer) for 1 h at 37°C and washed three times with culture medium. Cells were incubated with the humanized anti-CD20 mAb rituximab (5 \mu g/ml) or with medium as control, and 5000 cells were placed in a 96-well U-bottom plate. Effector T cells (previously incubated with the indicated FcyR-expressing target during trogocytosis experiments) were added at the indicated E:T ratio to Raji cells (final volume: 200 \mu l) for a 5-h incubation period at 37°C. A total of 100 \mu l of the supernatant was then harvested and counted in a gamma counter (Packard Instrument).

**T cell-B cell cooperation assays**

OT-I T cells (having captured or not FcyRIIB or FcyRIIIA through previous incubation with HEK-murine Fcy (mFcY)RIIB or HEK-mFcYRIIIA
cells coexpressing or not the construct encoding H-2Kb-OVA-β2m, as described above) were loaded and then washed with IC formed with anti-OVA Ab and the OVA-HEL Ag, prepared as described previously (26). Then, 10^7 MD4 B cells, expressing a HEL-specific IgM BCR, were coinubated at 37°C for 24 h with 2 × 10^5 of these OT-I T cells and 10^6 1H11-34 cells. The levels of IL-2 in the harvested culture supernatants were measured using an ELISA kit (BD Biosciences). In some assays, CD69 up-regulation or BCR down-modulation on MD4 B cells was analyzed 2 h after their incubation with OT-I T cells having acquired FcRγ. The fluorescent lipophilic dye DiO was also included in the set of molecules captured by T cells during redirected trogocytosis. For these experiments, we generated HEK cells expressing mFcγRIIB or with cDNA encoding murine FcγRIIB/IIIA mAb. The fact that we could detect FcγR using anti-Flag mAb demonstrates that the expression of FcγR by T cells resulted from capture from transfected HEK cells and not from rapid de novo synthesis or mobilization of intracellular stores, as described previously (27). Taken together, our results indicate that both FcγRIIB and FcγRIIIA can be captured by T cells during redirected trogocytosis.

**Results**

**CD4**^+ **and CD8**^+ **T cells acquire murine FcγR upon coculture with FcγR-expressing cells**

We recently showed that trogocytosis can be triggered in T cells in a so-called redirected manner (21). This is observed when T cells are cocultured with FcγR-expressing target cells in the presence of Abs directed against certain molecules of the surface of the T lymphocytes (such as CD3, CD2, or MHC class I/β2m complex for instance) (21). For the current study, we determined whether the FcγR itself was included in the set of molecules captured by T cells during this process. We found that OT-I CD8^+ T cells captured the fluorescent lipophilic dye DiO as well as FcγR when exposed to P815 cells in the presence of various mAbs triggering trogocytosis, but not in control conditions with no mAb (Fig. 1, A and B), with isotype controls or mAb not triggering trogocytosis (data not shown) (21). For any given stimulatory mAb, the intensity of FcγR capture was proportional to that of fluorescent lipophilic dye (Fig. 1B). Similar results were obtained with OT-II CD4^+ T cells (Fig. 1C), and a similar correlation between the capture of a lipophilic dye and that of FcγR was observed (data not shown). Captured FcγR were still detectable on T cells following an overnight culture in the absence of P815 cells (data not shown). Capture of FcγR by T cells was also observed with other FcγR^+ tumor cell lines such as the B lymphomas A20 or LB27.4 (data not shown). Furthermore, using HEK cells transfected with cDNA encoding murine FcγRIIB or with cDNA encoding murine FcγRIIIA plus the FcγRI subunit (also called FceRIγ or γ) necessary for FcγRIII expression (20), we found that the expression of FcγRIIB or FcγRIIIA allowed trogocytosis triggered by stimulatory mAb to occur. Indeed, FcγRIIB or FcγRIIIA was captured by OT-I (Fig. 2, A and B) or OT-II T cells (Fig. 2C), as detected by using either anti-Flag or anti-FcγRIIB/IIIA mAb. The fact that we could detect FcγR using anti-Flag mAb demonstrates that the expression of FcγR by T cells resulted from capture from transfected HEK cells and not from rapid de novo synthesis or mobilization of intracellular stores, as described previously (27). Taken together, our results indicate that both FcγRIIB and FcγRIIIA can be captured by T cells during redirected trogocytosis.

**T cells capture the signal-transducing FcγR subunit along with FcγR during trogocytosis**

Because the FcγR subunit is necessary for signal transduction after FcγR stimulation, we analyzed whether T cells captured the FcγR subunit together with FcγR during trogocytosis. For these experiments, we generated HEK cells expressing mFcγRIIB or
mFcγRIIIA and a fusion protein in which FcγR is fused to GFP. Using these target cells in redirected trogocytosis experiments, we found that the FcγRIIIA subunit was captured efficiently by OT-I T cells when the co-culture was performed in the presence, but not in the absence of stimulatory mAb (Fig. 3A). T cells co-cultured with HEK expressing both FcγRIIB and the FcγRIIIA subunit in the presence of a stimulatory mAb captured the FcγRIIIA subunit (Fig. 3A) together with FcγRIIB (data not shown), but no capture occurred when we used HEK cells expressing the FcγRIIIA subunit in the absence of FcγR (Fig. 3A). In all cases, when the FcγR subunit was captured by T cells, its detection using anti-GFP Ab required permeabilization of the cells (data not shown). Capture of the FcγR subunit was confirmed using OT-II T cells that were co-cultured with P815 cells in the presence or absence of stimulatory mAb before analysis by flow cytometry. As shown in Fig. 3B, the FcγR subunit was strongly detected in OT-II cells exposed to P815 cells in the presence of a mAb triggering trogocytosis, and provided that staining with anti-FcγR mAb was performed in permabilized cells. In the absence of permabilization, the FcγR subunit was only barely detectable (Fig. 3B). Taken together, our results indicate that the FcγR subunit is captured by CD4+ T cells together with FcγR and appears to be mostly expressed in a correct orientation after capture.

Capture of FcγR is triggered by Ag recognition both on immortalized cell lines and primary APC.

In the previous experiments, FcγR capture was triggered by stimulatory mAb. To determine whether FcγR were captured upon physiological stimulation of T cells with Ag, we first used immortalized cell lines expressing the Ag. As shown in Fig. 4A, OT-I T cells captured FcγRIIIA when cultured with HEK cells cotransfected with a vector encoding the covalent H-2Kb-SIINFEKL peptide complex (23), but not in the absence of the latter construct. As expected, no capture was detected when HEK cells did not express FcγRIIIA (Fig. 4A). We next examined whether FcγR capture by T cells would occur in the presence of primary APC. For these experiments, peritoneal macrophages or BM-DC generated from B6 mice were used as APC and cocultured with OT-I T cells in the presence of OVA. As shown in Fig. 4B, OT-I T cells readily captured FcγR following stimulation with BM-DC or peritoneal macrophages in the presence of the OVA peptide. The efficiency of FcγR capture triggered by the Ag was comparable to that observed in redirected trogocytosis experiments with anti-CD2 mAb (Fig. 4B). Similar results were obtained with OT-II T cells (data not shown). Thus, Ag recognition by T cells on artificial as well as primary APC-expressing FcγR results in the capture of FcγR by T cells.

T cells bind IC after FcγR acquisition

An important question regarding receptors captured by trogocytosis is whether they remain functional. To determine whether FcγR retained their ability to bind IC once captured by OT-I CTL, T cells were first subjected to trogocytosis in the presence of FcγR-expressing APC and then incubated with IC formed by an equilibrium mixture of the F9 and F10 anti-HEL mAbs complexed with biotinylated HEL. As shown in the top panels of Fig. 5A, OT-I cells that had captured FcγR (i.e., cocultured with P815 in the presence of mAb) strongly bound IC, but their FcγR-negative counterparts (i.e., cocultured with P815 in the absence of mAb) did not. No staining was detected when OT-I cells bearing FcγR were incubated with HEL alone or with a mixture of biotinylated F9 and F10 mAb alone (Fig. 5B). In contrast, P815 cells (lower right quadrants) bound IC both in the presence and absence of mAb. Furthermore, as shown in the lower panels of Fig. 5A, neither OT-I cells nor P815 cells were stained with IC formed with Fab’ fragments of F9 and F10 instead of whole mAb molecule, confirming that binding of IC by captured as well as native FcγR occurred through interactions with the Fe fragment of mAb. Similarly, OT-II T cells could bind IC after capture of FcγR from P815 or L27 cells (data not shown). Thus, FcγR captured by T cells conferred onto these cells the ability to efficiently bind IC.

Failure to detect biochemical or functional signal from the stimulation of captured FcγR

To determine whether FcγR could transduce intracellular signals after their capture by trogocytosis, we stimulated FcγR on T cells having acquired FcγR (FcγRIIIA+) and, as a control, on T cells

FIGURE 4. Capture of FcγR by OT-I T cells from primary DC or macrophages occurs during mAb- or Ag-mediated trogocytosis. A, HEK cells (left panel) or a stable transfectant expressing FcγRIIIA/FcγR (right panel) were used as target cells and were incubated with OT-I T cells in the absence (gray histograms) or presence (dotted line) of the anti-CD2 mAb. For Ag-mediated trogocytosis, HEK cells and HEK-FcγRIIIA/FcγR were transiently transfected with a vector encoding a covalent H-2Kb-OVA complex 48 h before incubation with OT-I T cells (solid line). Transfection efficiency was >80% (data not shown). At the end of the incubation period, cells were labeled with anti-CD8 and anti-FcγRIIB/RIIIA mAbs. Histograms show FcγRIIB/IIIA staining on gated CD8+ T cells. B. As in A, except that BM-DC (left panels) or peritoneal macrophages (right panels) from B6 mice were pulsed (OVA) or not (medium) with the SIINFEKL peptide and then incubated with OT-I T cells. OT-I T cells were coated (anti-CD2) or not with the anti-CD2 mAb in control experiments. At the end of the 1-h incubation period, cells were analyzed by flow cytometry using anti-CD8 and anti-FcγRIIB/RIIIA mAbs. Numbers in bold indicate the median fluorescence intensity of anti-FcγR staining on CD8+ T cells. The other numbers represent the percentage of cells in each quadrant. Similar results were obtained in a second independent experiment.
cocultured with P815 cell in the absence of mAb (FcR<sup>IIIC</sup>-), and we measured various biochemical and functional responses. Upon stimulation with IC, we were unable to detect the production of cytokines such as TNF-α by FcR<sup>IIIC</sup>- or FcR<sup>IIIB</sup>- OT-I T cells (Fig. 6A), whereas TNF-α was produced in response to antigenic stimulation (Fig. 6B). Similarly, we could not detect Ca<sup>2+</sup> flux upon IC stimulation of OT-I FcR<sup>IIIC</sup>- T cells above levels observed for control FcR<sup>IIIC</sup>- OT-I T cells. In contrast, both types of T cells strongly mobilized their Ca<sup>2+</sup> stores upon treatment with PMA and ionomycin (Fig. 6, C and D). Similar results were obtained with OT-II T cells (data not shown). As a control, we found that P815 target cells displayed Ca<sup>2+</sup> mobilization upon stimulation with IC (data not shown). No ADCC activity against Raji cells was detected with P815 cells that were incubated with HEK cells in the presence or absence of the anti-CD2 mAb (Fig. 6A). Cells were then analyzed using anti-CD8 mAb and fluorescent streptavidin. Numbers represent the percentage of cells in each quadrant.

The experiment shown is representative of three independent experiments. B, As in A, except that OT-I T cells having captured FcγRIIIA-FcR<sup>IIIC</sup> during coculture with P815 cells in the presence or absence of the anti-CD2 mAb were then incubated at 4°C for 1 h with biotinylated HEL (left two panels) or biotinylated anti-HEL F9 and F10 IgG (right two panels).

**FIGURE 5.** OT-I bind IC after FcγR acquisition. A, OT-I T cells (appearing in the upper quadrants) were incubated with P815 (appearing in the lower quadrants) in the presence of the indicated mAb, as described in Fig. 1. At the end of the incubation period, cells were incubated at 4°C for 1 h with IC formed between biotinylated HEL and either whole anti-HEL F9 and F10 IgG (top panels) or F(ab')<sub>2</sub> of the same mAb (bottom panels). Cells were then analyzed using anti-CD8 mAb and fluorescent streptavidin. Numbers represent the percentage of cells in each quadrant.

The experiment shown is representative of three independent experiments. B, As in A, except that OT-I T cells having captured FcγRIIIA-FcR<sup>IIIC</sup> during coculture with P815 cells in the presence or absence of the anti-CD2 mAb were then incubated at 4°C for 1 h with biotinylated HEL (left two panels) or biotinylated anti-HEL F9 and F10 IgG (right two panels).

we failed in transducing OT-I and OT-II T cells with a sufficient efficiency using the viral vector encoding FcγRIIIA-FcRγ chimera (data not shown). Therefore, we transposed our findings to a human system in which PBMC transfected with FcγRIIIA-FcRγ could be readily generated (18) (see also Fig. 7A). Similar to our observations made in the murine model (21), we found that addition of anti-CD3 OKT3 mAb or W6/32 anti-MHC class I mAb in cocultures of HEK cells expressing FcγRIIIA-FcRγ chimera and human CD4<sup>+</sup> and CD8<sup>+</sup> T cells led to the capture of the FcγRIIIA-FcRγ chimera by T cells (data not shown and Fig. 7B). Interestingly, T cells having captured FcγRIIIA-FcRγ chimera (FcγRIIIA-FcRγ<sup>IIIC</sup>- T cells) did not lyse Raji cells coated with rituximab better than control T cells incubated with HEK cells in the absence of any mAb (FcγRIIIA-FcRγ<sup>IIIC</sup>- T cells) (Fig. 7C) or not incubated with HEK cells (data not shown). In contrast, T cells transduced with the retroviral vector expressing FcγRIIIA-FcRγ efficiently lysed Raji cells coated with rituximab, showing that T cells do possess a functional machinery able to translate FcγR stimulation into a functional response. Taken together, although T cells can efficiently capture FcγR, we were unable to detect functional signal upon stimulation, whereas we could do so when FcγR were endogenously expressed by T cells.

**FcγR captured by T cells exhibit different features as compared with FcγR endogenously expressed by target cells**

The discrepancy between the strong ligand-binding ability and the undetected function of FcγR captured by T cells could be due to the fact that these FcγR may not be properly integrated in the T cell membrane, and consequently, could not properly connect to the signaling machinery. Therefore, we examined whether evidence of improper integration of FcγR could be found. First, as shown in Fig. 8, we found that upon noncytolytic acid treatment, a large proportion of FcγR acquired by T

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cells was removed from the T cell surface (76%, loss as calculated using median fluorescence intensities), whereas this treatment had little or no effect on FcγRIIA expressed by P815 cells (11% loss). Addition of polybrene, known to favor fusion, during or after acid treatment, did not lead to increased acid resistance of FcγRIIA captured by T cells (data not shown). Second, we found that FcγRIIA captured by T cells were not down-modulated upon treatment with the C2F2 Ab against CD71, whereas this treatment induced a massive down-modulation of FcγRIIA endogenously expressed by P815 cells (97% loss, as calculated using median fluorescence intensities), further strengthening the hypothesis of a distinct molecular layout of captured vs endogenous FcγRIIA in the membrane (Fig. 8). By using these two different approaches, we could thus show that the majority of FcγR molecules captured by T cells had features suggesting their improper insertion in the T cell membrane.

FcγRIIB/IIIA-expressing OT-I T cells present IC to B cells, allowing T cell help

Having shown that FcR capture allows T cells to bind IC in the absence of detected signaling-dependent function, we wondered whether immune functions involving solely IC binding could be bestowed on T cells by FcγRIIB/IIIA acquisition. For that, we determined whether FcγRIIB/IIIA capture by T cells allowed them to present IC to B cells in order for B cells to benefit from T cell help. To address this question, we used the experimental system presented in Fig. 9A, in which OT-I T cells having captured FcγRIIB or not in a previous culture with HEK-FcγRIIB were incubated with OVA-HEL IC. Then after extensive washing, these cells were incubated with MD4 B cells and the activation of the B cells by CD69 up-regulation (presented in Fig. 9B). Note that, in this case, we used an OVA-HEL Ag complexed with anti-OVA Ab.
to allow recognition of HEL by the MD4 BCR. To determine whether IC presented by OT-I T cells were captured and processed by the B cells, we then added the 1H11-34 T cell hybridoma specific for the HEL Ag presented by the MHC II I-E\(^d\) molecule and measured IL-2 production by ELISA (presented in Fig. 9, C and D). Importantly, the source of IL-2 in this system can only be identified as the 1H11-34 T cell hybridoma and not the OT-I T cells because OT-I T cells are of the H-2\(^b\) haplotype, whereas MD4 B cells (and the 1H11-34 T cell hybridoma) are of the H-2\(^d\) haplotype. As shown in Fig. 9B, CD69 was up-regulated at the surface of MD4 B cells exposed to OT-I T cells having acquired FcR when the latter cells were coated with IC, but not when left uncoated. No detectable CD69 up-regulation was noticed in the presence of OT-I T cells that had not captured FcR. As a control, CD69 was markedly up-regulated on B cells exposed to HEL-OVA and slightly up-regulated when exposed to IC. Each time we could detect CD69 up-regulation, we noticed a correlated BCR \(\kappa\)-chain internalization (data not shown). Thus, T cells having captured FcR may play a functional role in vivo by provisioning B cells with immobilized Ag and permitting T cell help.

Discussion

Although the transfer of proteins between immune cells has been anecdotally reported for years, the widespread nature of this phenomenon and its potential biological significance have only been described recently. Indeed, protein transfer, and in particular trogocytosis, has been proposed to play a role in fundamental processes such as the initiation and regulation of immune responses (5, 6). Thus, it is critical to identify molecules that can be captured by immune cells, understand the molecular basis of the transfer, and, most importantly, define the function of captured molecules on recipient cells. In this study, we showed that T cells efficiently acquired FcR from APC during mAb- or Ag-mediated stimulation and expressed it in a correct orientation and topology on their surface. After capture of FcR, T cells could bind FcR ligands

FIGURE 7. Human CD4\(^+\) and CD8\(^+\) T cells perform detectable ADCC after endogenous expression, but not capture of human FcγRIIIA–γ. Activated PBMC were transduced or not with a retroviral vector encoding the chimeric FcγRIIIA-FcR\(\gamma\) molecule. Nontransduced PBMC were incubated with HEK cells expressing FcγRIIIA-Fc\(\gamma\) in the presence or absence of the OKT-3 anti-CD3 mAb found to trigger the capture of FcγRIIIA by T cells. Then T cells were separated from adherent HEK cells and used in a 5-h chromium release assay against Raji cells, coated or not with the anti-CD20 mAb rituximab. A, Flow cytometry analysis showing Fc\(\gamma\)RIIIA expression of transduced (right panel) or nontransduced PBMC (left panel). Numbers represent the percentage of cells in each quadrant. B, Analysis by flow cytometry of the capture of Fc\(\gamma\)RIIIA-Fc\(\gamma\) by nontransduced PBMC (as shown in A) incubated with HEK expressing FcγRIIIA-Fc\(\gamma\) in the presence (open histogram) or absence (closed histograms) of the OKT3 anti-CD3 mAb. Top panel, Represents Fc\(\gamma\)R staining on gated CD8\(^+\) T cells; bottom panel, on gated CD4\(^+\) T cells. C, Percent specific lysis of Raji cells coated with rituximab in the presence of PBMC exposed to HEK cells expressing FcγRIIIA-Fc\(\gamma\) in the presence of OKT3 (Fc\(\gamma\)RIIIA–\(\gamma\)– , gray symbols) or not (Fc\(\gamma\)RIIIA–\(\gamma\)– , white symbols) or PBMC endogenously expressing FcγRIIIA-Fc\(\gamma\) (Fc\(\gamma\)RIIIA– , black symbols) after retroviral transduction. Note that killing of Raji cells not coated with rituximab was comparable to killing obtained with Fc\(\gamma\)RIIIA–\(\gamma\)– and Fc\(\gamma\)RIIIA–\(\gamma\)– (data not shown).
FUNCTIONALITY OF FcγR CAPTURED BY T CELLS

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**FIGURE 8.** FcγR captured by T cells display different stability and internalization as compared with FcγR expressed by target cells. OT-I T cells were incubated with P815 cells in the absence (left panels) or presence (right panels) of the anti-CD2 mAb. At the end of the incubation period, cells were left untreated (top panels), incubated 1 min in a noncytolytic (pH 3.0) buffer, and then extensively washed (middle panels) or incubated 1 h at 37°C with anti-CD71 mAb. Cells were then analyzed by flow cytometry using anti-CD8 and anti-FcγRIIB/IIIa mAb. Quadrants were placed such that T cells appear in the upper quadrants and target cells in the lower quadrants. Percentages represent the percentage of cells in each quadrant. Numbers represent the percentage of cells in each quadrant. Note that we do not want to mean that anti-CD71-mediated FcγRIIB/IIIa internalization has any physiological relevance, but is simply yet another way to show that FcγR do not behave in recipient T cells as in donor P815 cells.

very efficiently, but we were unable to detect biochemical or functional response resulting from this binding. Furthermore, we found evidence for a different behavior of FcγR captured by T cells as compared with receptors endogenously expressed by donor cells that was most likely secondary to the incomplete integration of captured FcγR into the T cell membrane. Based on our results, we propose that the capture of FcγR by T cells could provide them novel opportunities to interact with ligands such as IC or IgG-opsonized cells. This may possibly occur without triggering signaling-dependent responses in these recipient T cells because we were unable to detect such signals.

FcγR are not generally considered as typical molecules expressed by conventional T cells (28). However, it is now clear that subpopulations of conventional T cells both in the mouse (10, 29–35) and in humans (36–39) do endogenously express FcγR or subunits of the FcγR complex. Apart from γδT cells and NK T cells, this is for instance the case of double-negative T cells (32), some conventional CD4+ or CD8+ αβT cells exhibiting an activated phenotype (18, 36, 39), and some tumor-infiltrating lymphocytes (40). Expression of FcγR by these cells was shown to confer onto them novel biological functions, such as the ability to perform ADCC (18, 35, 36, 39), to produce lymphokines (35, 41, 42) and, in the case of the FcγRIIB subunit, to functionally replace the ζ-chain in the TCR complex (37, 43, 44). Besides endogenous expression, it was suspected years ago that T cells could uptake FcγR released from macrophages (10). However, it was not known whether a proteolytic cleavage or other mechanisms more closely related to tropocytosis were involved in this protein transfer. In this study, we demonstrated that the expression of FcγR by T cells shortly after coculture with FcγR-expressing target cells reflected capture by tropocytosis. T cells could capture both FcγRIIB/FcγRIIIA from immortalized cell lines, but also from primary, bona fide APC upon stimulation with the Ag or with defined stimulatory mAb. Furthermore, FcγR capture was observed for both CD4+ and CD8+ T cells and for murine as well as human T cells, suggesting that this process commonly occurs in all T cells. Thus, FcγR extends the list of molecules captured by T cells upon productive recognition of APC.

We found that FcγR and the FcγRII subunit were expressed in a correct conformation and topology after their capture by tropocytosis. This conclusion is based on the facts that extracellular epitopes were detected with conformational mAb, intracellular epitopes were detected mainly after cell permeabilization, and acquired FcγR could efficiently bind IC. Our data are reminiscent of those obtained by Davis and colleagues (24, 25) regarding the capture of Cw6-GFP molecules by NK cells, and support the notion that both the conformation and orientation of molecules captured by tropocytosis are correct. In the case of FcγR, this result bears the additional significance that the ability of captured FcγR to bind IC could endow T cells with novel and unexpected functional properties.

Multiple hypotheses have been proposed regarding the role played by molecules captured by T cells. Usually, these roles were inferred from those played by the same molecules when expressed on the donor cell, assuming that the same roles would be played on recipient T cells. However, little data support this notion in many cases. The most convincing function demonstrated for tropocytosis concerns the impact of captured molecules (such as peptide-MHC complexes) on immune regulation through T-T cell interactions (1, 6). Noticeably, these novel functions depend on the T cell ability to present the ligands they acquired to receptors present on their own membrane or on surrounding cells. Similar to these reports, we found that FcγR was perfectly able to bind physiological ligands such as IC after capture by T cells. As suggested by our results presented in Fig. 9, this could affect the immune response by providing T cells with the ability to bind and present IC to neighboring cells expressing FcγR or BCR. By engaging FcγRIIB, the inhibitory FcγR expressed by B cells, soluble Ag-Ab complexes inhibit B cell activation (43). In contrast, as suggested by our results, Ag-Ab complexes bound to FcR captured by T cells could activate rather than inhibit B cells most likely because Fc fragments engage FcR on T cells and would not be available for FcγRIIB recognition on B cells. Furthermore, acquired FcγR could function similarly to another member of the FcγR family whose function does not depend on intracellular signaling, i.e., the glycosphosphatidylinositol-anchored FcγRIIB, resulting, for example, in recruitment of cells to IC (12, 46). Nevertheless, additional studies are clearly required to understand whether any roles could be played by T cells having acquired FcγR by tropocytosis in vivo.

Although the capture of several receptors by T and NK cells via tropocytosis has already been documented, their functionality on recipient cells was always examined in terms of ligand-binding, but not signaling properties (6, 8, 47, 48). We thus examined whether FcγR captured by T cells could induce intracellular signals and/or signaling-dependent functions. Studies of FcγR function in T cell subpopulations endogenously expressing FcγR have
established that the cellular machinery necessary for FcγR signaling is usually present in T cells (18, 36, 39, 41). However, we were unable to detect any Ca\(^{2+}\) flux or cytokine production upon IC stimulation of FcγR captured by T cells. Furthermore, T cells having acquired FcγR did not induce detectable ADCC even though they expressed high levels of FcγR after capture and could efficiently bind IC. In contrast, T cells endogenously expressing FcγR after transduction are quite efficient at performing ADCC, which is a very sensitive assay. These results show that, at least in this case, the fact that we could not detect ADCC with captured FcγR is not due to an improper association with the FcγR subunit. Note that we previously reported that ADCC could easily be detected in the case of some memory T cells naturally expressing FcγRIIIA in humans, at levels lower than those observed in this study after capture (39) (our unpublished data), indicating that detectable function could be expected from the high levels of FcγR captured.

Taken together, although we were not able to detect whether very early activation events could be triggered by IC stimulation of T cells having acquired FcγRII/III, one possible interpretation of these results is that biochemical signals might not be transduced by FcγR captured by T cells or at levels too low to be detected. In agreement with our results, it was recently found that bystander B cells rapidly acquire Ag receptors from activated B cells by trogocytosis, and that they display Ca\(^{2+}\) flux upon stimulation of the endogenously expressed BCR, but not the acquired BCR (9). As a counterexample, the observations that CTL (2) or DC (49) having acquired peptide-MHC complexes are lysed by CTL specific for the acquired Ag could suggest that peptide-MHC complexes are well integrated in the recipient T cell membrane and functional as they sensitize T cells to fratricide killing. However, it is unknown whether peptide-MHC integration in the recipient T cell membrane is mandatory for the execution of perforin-mediated killing. Furthermore, the observation that acquired molecules such as peptide-MHC complexes can participate in signaling in the recipient T cells (3) is compatible with our data, because in this case, the acquired molecules interact with a functional, endogenously expressed receptor to maintain signal propagation in recipient T cells. The fact that acquired FcR may not transmit intracellular signal could be seen as a means to prevent T cells from responding to stimulation by promiscuous IC.

**FIGURE 9.** After FcγR acquisition, OT-I present IC to B cells to allow B cell-T cell cooperation. A, Cartoon showing the cell cultures and molecular signals allowing us to determine whether OT-I T cells having acquired FcγR could present IC to MD4 B cells and allow them to participate in a T cell-B cell cooperation. B, MD4 B cells were incubated with OT-I T cells, which, in a first coculture, had acquired FcγRIIB (OT-I-FcγRIIB\(^{acq}\)) or not (OT-I-FcγRIIB\(^{acq}\)) from HEK-FcγRIIB. OT-I cells were pulsed (open histograms) or not with HEL-OVA IC (gray histograms) before extensive washing and addition to MD4 B cells. After 2 h, cells were stained with mAbs directed against CD69 and B220 before analysis by flow cytometry. Shown are overlaid histograms of CD69 expression on gated B220\(^{-}\) cells in conditions in which IC were present or not on OT-I T cells. Controls show CD69 expression on MD4 B cells alone exposed or not to IC or, on the left panel, to HEL-OVA. C, OT-I T cells were incubated for 1 h at 37°C with HEK-FcγRIIB or FcγRIIIA FcγR coexpressing or not the OVA Ag through transfection by the construct encoding the covalent H-2K\(^{b}\)-OVA complex (to trigger Ag-mediated trogocytosis), or in the presence of the anti-CD2 mAb (to trigger mAb-mediated trogocytosis), or with no particular stimulus (none). As in B, except that the HEL-specific I-E\(^{a}\)-restricted 1H11-34 T hybridoma was added to the culture and that IL-2 production was measured by ELISA in duplicate wells in the experimental and control conditions indicated below the bars. ■ and □. Represent two independent experiments. D, As in C, except that this panel shows the controls performed as indicated below the graph for the experiment presented in ■ in C.
Several models could explain how captured FcγR may not trigger signaling or signaling-dependent function in T cells. It is possible that either receptors are not properly inserted within the plasma membrane of the recipient cell (Fig. 10A) or they are well integrated in the plasma membrane, but not properly connected to the signaling machinery (Fig. 10B). Although we do not exclude this last possibility, we found indications that FcγR captured by T cells exhibited features best compatible with the first hypothesis. First, a large proportion of FcγR captured by T cells was shed upon noncytolytic acid treatment (whereas this treatment had little effect on the expression of endogenous FcγR by donor cells). However, whether the acid-resistant molecules are integrated in the recipient cell membrane or are not integrated, but still resistant to release, remains unclear. Second, we showed that FcγR captured by T cells were not down-modulated upon treatment with an Ab against CD71, whereas this treatment induced a massive down-modulation of FcγR endogenously expressed by P815 cells (possibly reflecting comodulation of these two molecules in recycling compartments upon anti-CD71 mAb stimulation), further strengthening the hypothesis of a distinct molecular layout of captured vs endogenous FcγR in the membrane. Finally, reminiscent of the structures observed by Davis and colleagues (25) on NK cells, our preliminary electron microscopy analysis of FcγR-GFP expressed on T cells after capture supports the notion that FcγR are present in membranous structures loosely attached to the plasma membrane of T cells (data not shown). Finally, Patel et al. (50) observed that cells synthesizing, but not those having acquired a given molecule, were sensitive to complement lysis triggered by mAb against this molecule. Considering that the completion of fusion events is usually so fast that the different steps are very difficult to observe (51), we believe that membrane fragments containing FcγR could readily fuse with the T cell membrane in a proper way within the time frame of our coculture assays (up to 5 h). Note that the inhibition of functional properties of T cells having captured these molecules (6) could simply be due to masking of critical ligands or receptors by vesicles acquired from other cells rather than by signaling events transduced from captured receptors.

In conclusion, we propose that molecules captured by trogocytosis may perform functions that do not require their integration in the plasma membrane, such as interactions with ligands on the same or different cells. Whether this property could be generalized to other receptors and immune cells has yet to be determined because no other receptors were reported to date for their ability to transmit intracellular signals once captured by T cells.

Most of the experiments performed in this study used mAb to trigger trogocytosis. This choice was made because mAb often trigger trogocytosis more efficiently than Ag. Importantly, we found no qualitative differences in trogocytosis triggered by Ab or Ag stimulation with regard to the capture of FcγR by T cells, suggesting that both processes exhibit comparable features. Furthermore, although these studies involved other hematopoietic cells, trogocytosis triggered by therapeutic mAb such as rituximab has been reported both in vitro (52) and in vivo (53), suggesting that this process has biological relevance.

Our results have important implications for the consequences of trogocytosis in T cell biology. Indeed, not only did we show that T cells could capture FcγR that subsequently bound IC with great efficiency, but our data further advance the two major questions concerning the mechanisms of trogocytosis, e.g., the molecular basis of protein transfer and the extent of the function of captured molecules.

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**Disclosures**

The authors have no financial conflict of interest.

**References**


