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Role of the Actin Cytoskeleton in T Cell Absorption and Internalization of Ligands from APC

Inkyu Hwang and Jonathan Sprent

A feature of T-APC interaction is that, via either TCR or CD28, T cells can absorb molecules from APC on to the cell surface and then internalize these molecules. Here, using both normal and TCR-transgenic T cells, we investigated the mechanism of T cell absorption of molecules from APC and the role of the cytoskeleton. The results show that although activated T cells could absorb APC molecules in the form of cell fragments, uptake of molecules by resting T cells required direct T-APC interaction. Based on studies with latrunculin B, surface absorption of molecules by resting T cells was crucially dependent upon the actin cytoskeleton for both CD28- and TCR-mediated absorption. Significantly, however, TCR-mediated absorption became strongly resistant to latrunculin B when the concentration of MHC-bound peptide on APC was raised to a high level, implying that the actin cytoskeleton is only important for absorption when the density of receptor/ligand interaction is low. By contrast, in all situations tested, the actin cytoskeleton played a decisive role in controlling T cell internalization of ligands from the cell surface. The Journal of Immunology, 2001, 166: 5099–5107.

T cell recognition of Ag on APC rapidly leads to tight conjugate formation and clustering of TCR and other molecules at the site of T-APC interaction (1–4). At this “synapse,” TCR interaction with MHC-peptide complexes on APC causes T cell activation and differentiation into effector cells; such activation generally requires costimulation of T cells via interaction of CD28 with B7 (B7-1, B7-2) on APC (5, 6).

Especially with high-affinity peptides, TCR-MHC-peptide interaction is often followed by TCR down-regulation and internalization (7–9). Until recently, TCR down-regulation was presumed to depend on TCR dissociation from MHC-peptide complexes. However, it is well established that TCR-MHC-peptide interaction can cause T cells to rapidly absorb MHC and other molecules, including B7, from APC (10–16). More recently, it has been shown that T cell absorption of molecules from APC is followed by internalization of these molecules (15, 16). Hence, TCR down-regulation may reflect internalization of intact TCR-MHC-peptide complexes. Surprisingly, T cell absorption and internalization of molecules from APC can also occur via CD28-B7 interaction, although not via LFA-1-ICAM-1 interaction (16).

The significance of T cell absorption and internalization of ligands derived from APC is uncertain. With high concentrations of specific peptide, T cell absorption of MHC-peptide complexes from APC renders T cells sensitive to fratricidal lysis by neighboring CTL (15). This mechanism could explain the exhaustion of T cells responding to high concentrations of viruses (17, 18) and may serve to guard against immunopathology resulting from overexuberant immune responses. Alternatively, T cell internalization of APC-derived ligands could be important for allowing T/APC dissociation, thus permitting effector T cells to leave lymphoid tissues and enter the circulation (16).

The form in which APC-derived molecules are absorbed by T cells is still unclear. In this respect it is notable that absorption includes bystander molecules on APC (16). Thus, when CD28−/− TCR-transgenic T cells are cultured with APC, addition of specific peptide causes the T cells to absorb (and internalize) not only the specific ligand recognized, i.e., MHC molecules, but also adjacent molecules on the APC, e.g., B7. Likewise, CD28-mediated uptake of B7 from APC (which is TCR independent) causes T cell uptake of both B7 and MHC molecules. It is likely, therefore, that T cells absorb APC-derived molecules as membrane fragments or vesicles. Whether these fragments-vesicles then fuse with the T cell membrane has yet to be studied.

In this paper, we investigated whether T cell absorption of molecules from APC requires direct T-APC interaction or can occur via uptake of cell fragments. Using latrunculin B (Lat B)3 (19, 20), we also examined the role of the cytoskeleton in T cell absorption and ligand internalization. The results show that the requirements for T cell absorption to the cell surface are highly complex and depend on the status of the T cells (resting vs activated), the T cell molecules involved (TCR vs CD28), and, for TCR-mediated absorption, the concentration of peptide on the APC. Likewise, T cell absorption requires the cytoskeleton in some situations but not in others. By contrast, the cytoskeleton plays a crucial role in ligand internalization.

Materials and Methods

Animals

C57BL/6j (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). CD28−/− mice (21) (originally purchased from The Jackson Laboratory), 2C αβ TCR-transgenic mice (22), and 2C.CD28−/− mice were bred at The Scripps Research Institute (La Jolla, CA). B7-2−/− mice were provided by Arlene Sharp (Harvard Medical School, Boston, MA) (23) and maintained at The Scripps Research Institute. B7-2−/−CD28−/− double knockout mice were bred at The Scripps Research Institute. All mice were on a B6 background except for B7-2−/− mice, which were on a 129 background.

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3 Abbreviations used in this paper: Lat B, latrunculin B; DC, dendritic cells; CD, cytochalasin D.
Cell lines and media

*Drosophila* APC were generated and maintained as described previously (24). RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM glutamine, 5 × 10^{-5} M 2-ME, and antibiotics was used for T cell culture.

Peptides

QL9 (QLSPFPFFDL) and P1A (LPYLGWLVF) peptides were synthesized and purified as described previously (24). L_{2}^{+}-expressing APC were loaded with the peptides for 2 h at room temperature before use. For measuring CD28-mediated uptake of L_{2}^{+}, L_{2}^{+} expression on APC was stabilized by addition of 10 μM P1A peptide; this peptide binds strongly to L_{2}^{+}, but is not recognized by 2C TCR molecules.

Cell purification

T cells were purified by sequential mAb plus C killing as described previously (25). Briefly, total lymph node cells were treated with anti-HSA (J11D) and anti-IAα (28-16-8s) mAbs plus a combination of guinea pig and rabbit complement for 40 min at 37°C followed by Ficoll gradient centrifugation. For the purification of 2C CD8^{+} T cells, anti-CD4 (RL174) mAb was included in the killing process along with J11D and 28-16-8s mAbs. Preactivated T cells were prepared by culturing purified T cells overnight with PMA (10 nM; Sigma) and ionomycin (370 ng/ml; Sigma) in a 37°C humidified CO_{2} incubator. Dendritic cells (DC) were prepared as described previously (16, 26).

Preparation of cell sonicates

Sonicates of APC were prepared by using a Misonix sonicator (Misonix, Farmingdale, NY). *Drosophila* APC (5 × 10^{6}ml) were subjected four times to a 5-s pulse of sonication (power 2, 15% input). Complete disruption of the cells was monitored by observing the sonicated cell suspension under the microscope.

Culture conditions

For the experiments using intact APC, 100 μl of purified resting T cells (8 × 10^{6}ml) or preactivated T cells (5 × 10^{6}ml) was incubated with 100 μl of APC (5 × 10^{6}ml) in a 96-well plate for the indicated period of time. To ensure immediate contact among T cells and APC, the plates were briefly spun at low rpm (200 × g for 15 s). For the experiments using sonicates, 100 μl of resting (8 × 10^{6}ml) or activated (5 × 10^{6}ml) T cells was incubated in a 96-well plastic tissue culture plate with 100 μl of sonicates prepared from the same number of APC as when intact APC were used. For the experiments using Transwells (Costar, Corning, NY), 200 μl of resting (8 × 10^{6}ml) or preactivated (5 × 10^{6}ml) T cells was added to a 24-well plate. Then, a Transwell (3-μm pore size, 6.5-mm diameter; Costar) was inserted, inside of which 200 μl of APC (1.5 × 10^{6}ml) was added. For the experiments using Lat B (Calbiochem, CA), T cells were pretreated with the indicated dose of Lat B for 1 h at 37°C before use. Lat B was also kept in the culture during incubation of T cells with APC.

Abs and flow cytometric analysis

Anti-L_{d}^{+} (30-5-7) (27), anti-CD8 (YTS-169), and anti-MHC class II (M5/114) mAbs were prepared in ascites form in our laboratory. FITC-conjugated anti-CD4 (L3T4), anti-B7-1 (16-10A1), anti-B7-2 (GL1), anti-TCR γδ (H57-597), PE-conjugated anti-B7-1 (16-10A1), anti-B7-2 (GL1), anti-IAα (AF6-120.1), and anti-CD28-2 (37.51) mAbs were purchased from PharMingen (San Diego, CA). PE-conjugated donkey anti-mouse IgG(H+L) (catalogue no. 715-116-151) and Rhodamine Red-X-conjugated donkey anti-mouse IgG(H+L) (catalogue no. 715-295-151) were purchased from Jackson ImmunoResearch (West Grove, PA). Cy-5-conjugated anti-CD4 (L3T4), FITC-B7-1, FITC-B7-2, and Alexa 568-conjugated anti-MHC class II mAbs were prepared in our laboratory using by using Cy-5 (Amersham, Arlington Heights, IL) and Alexa 568 protein labeling kit (Molecular Probes, Eugene, OR), respectively. For staining of Lat B-treated cells, Lat B was included during the staining procedure and was also present in the wash buffer. A FACS Calibur (Becton Dickinson, Mountain View, CA) was used for flow cytometry, and CellQuest (Becton Dickinson) was used for data analysis.

Laser scanning confocal microscopy

Specimens for confocal microscopy were prepared as described previously (16). Briefly, 100 μl of Lat B-treated and nontreated or preactivated T cells (8 × 10^{6}ml) was incubated with 100 μl of *Drosophila* APC (8 × 10^{6}ml) or DC (8 × 10^{6}ml) on poly-l-lysine-coated coverslips in a 37°C humidified CO_{2} incubator for the indicated period of time. After incubation, cells on the coverslips were sequentially fixed with freshly prepared paraformaldehyde (2% in PBS) for 30 min at room temperature and per-

meabilized with saponin (0.05% in PBS) for 10 min. The fixed cells were treated with a blocking solution (1% BSA and 5% horse serum in PBS) for 1 h at room temperature and stained with mAbs for 30 min at room temperature. For staining for CD8, B7-1, B7-2, and IAα, directly conjugated mAbs (Cy5-CD8, FITC-B7-1, FITC-B7-2, and Alexa 568-MHC class II mAbs) were used. For L_{d}^{+} staining, Rhodamine Red X-conjugated donkey anti-mouse polyclonal Ab was used as a secondary Ab. The stained cells were observed using an Axiosvert S100 TU inverted microscope (Zeiss, New York, NY). LaserSharp (Bio-Rad, Hercules, CA) software was used for creation and analysis of the images.

Results

To examine the requirements for T cells to absorb molecules from APC, purified lymph node T cells were cultured with intact APC, either in direct contact with APC or separated from APC in Transwells; in some experiments T cells were cultured with APC sonicates. After culture, T cells were stained for APC-derived molecules and then analyzed by or examined by confocal microscopy. For most experiments, transfected *Drosophila* cells expressing B7-1, ICAM-1, and MHC class I L_{d}^{+} molecules (B7-1-ICAM-1-L_{d}^{+} APC) were used as a source of APC. T cells were prepared from normal B6 mice or TCR-transgenic mice on a B6 background. Since B6 (H2^{D}) T cells are B7-1^{+} ICAM-1^{+} L_{d}^{+}, we examined T cell absorption of B7-1 and L_{d}^{+} molecules from APC; previous work with

![FIGURE 1](http://www.jimmunol.org/)
T cells from ICAM-1−/− mice showed that in addition to B7 and MHC molecules, T cells can absorb ICAM-1 from APC (16).

**CD28-mediated absorption**

As documented previously, T cell absorption of molecules from APC involves at least two receptor-ligand interactions, i.e., CD28-B7 and TCR-MHC-peptide interactions (15, 16). With culture of polyclonal normal B6 T cells, the absorption of molecules from APC is largely a reflection of CD28-B7 interaction, with little or no involvement of TCR molecules.

In confirmation of previous findings (16), short-term (60-min) culture of normal B6 T cells with B7-1,ICAM-1.Ld Drosophila APC led to prominent absorption of both B7-1 and Ld molecules from the APC as detected by FACS analysis (Fig. 1). Such absorption applied to both CD4+ cells (Fig. 1) and CD8+ cells (data not shown) and affected resting T cells (Fig. 1A) as well as pre-activated T cells (T cells exposed to PMA and ionomycin; Fig. 1B). Confirming the crucial role of CD28 in absorption, T cell uptake of both B7-1 and Ld molecules from APC was almost undetectable with CD28−/− T cells (Fig. 1B). This finding implies that for absorption, CD28 binds B7-1 molecules that are physically linked to Ld molecules. It should be noted that uptake of B7-1 and Ld molecules from APC was substantially higher with activated T cells than with naive T cells (compare Fig. 1, A vs B) As discussed previously (16, 28, 29), this finding correlates with the much higher expression of CD28 on activated T cells.

A priori, T cell absorption of molecules from APC could reflect either direct T-APC interaction or uptake of small membrane fragments released from APC. To examine this second possibility, T cells were separated from APC in Transwell plates or cultured with APC sonicates. With resting T cells, absorption of B7-1 and Ld through the Transwell membrane was almost undetectable, even with a relatively large pore size of 3 μm (Fig. 1A). Likewise, uptake of these molecules was very low following T cell culture with APC sonicates (Fig. 1A); for Transwells, absorption required a high density of APC, i.e., 3-fold higher than for direct T-APC interaction. In both situations, absorption was low or undetectable with CD28−/− T cells.

To examine the role of the cytoskeleton in T cell absorption of APC-derived molecules, T cells were cultured with APC in the presence of Lat B, an inhibitor of actin polymerization (19, 20). For resting T cells, addition of Lat B at 20 μg/ml during direct T-APC interaction almost totally inhibited T cell binding of Ld and B7-1 (Figs. 2A and 3A); this finding applied to binding measured at 10–60 min of culture (Fig. 3A) and was observed even with quite low doses of Lat B, i.e., 0.25 μg/ml (Fig. 2B). Different
A. Intact T-APC Interaction

B. Activated CD4+ with APC in Transwells

FIGURE 3. Influence of culture time on the capacity of Lat B to inhibit CD28-mediated T cell absorption of B7-1 and Ld molecules from Drosophila APC. Normal resting or activated B6 T cells were cultured with or without Lat B (20 µg/ml) with Drosophila APC either directly (A) or in Transwells (B), as described in the legend to Fig. 1, and then stained at 10, 30, or 60 min (A) or after 60 min (B). The data show the change in mean fluorescence intensity (Δ MFI) for staining of CD4+ cells for B7-1 and Ld.

findings occurred with preactivated T cells (Figs. 2C and 3A). With these T cells, even high doses of Lat B (20 µg/ml) caused only partial inhibition of T cell binding following direct T-APC interaction. By contrast, Lat B strongly inhibited T cell binding in Transwell cultures (Figs. 2C and 3B).

The above findings make two main points. First, whereas both resting and activated T cells can absorb B7-1 and Ld molecules through direct contact with intact APC, only activated T cells absorb these molecules when cultured with cell fragments. Second, based on the effects of adding Lat B, both resting and activated T cells require actin polymerization for CD28-mediated binding of APC-derived molecules; for activated T cells, however, the requirement for actin polymerization is much more stringent for absorption of cell fragments than for absorption following direct T-APC interaction.

To examine whether the above findings with Drosophila APC apply to normal APC, T cells were cultured with syngeneic B6 DC as APC; the T cells were then stained for three molecules on the APC, i.e., B7-1, B7-2, and IAβ. Since T cells are B7-1- B7-2- IAβ-, the T cells were prepared from B7-2-/- mice; these mice were on a 129 (H2b) background. B7-2/- CD28-/- T cells were used as a control. For resting CD4+ cells, absorption of B7-1, B7-2, and IAβ after short-term, direct culture with B6 DC was strong for B7-2-/- CD28- T cells, but weak, although significant, for B7-2-/- CD28-/- T cells (Fig. 4A), indicating a marked, although not exclusive, role for CD28 in absorption from DC. Significantly, as with Drosophila APC, T cell uptake of molecules from DC was almost totally inhibited by Lat B (Fig. 4A). This finding refers to resting T cells. With activated T cells (Fig. 4B), Lat B caused only partial inhibition of absorption, i.e., as for activated T cells and Drosophila APC. When activated T cells and DC were separated in Transwells, by contrast, T cell absorption of molecules from DC (which was lower than with Drosophila APC; see Discussion) was totally inhibited by Lat B (Fig. 4C). Collectively, these data with DC as APC were thus in close accord with the data with Drosophila APC.

TCR-mediated absorption

Because of prominent absorption of APC-derived molecules via CD28-B7 interaction, examining the role of TCR-MHC-peptide interaction in absorption is best studied with TCR transgenic T cells on a CD28-/- background. Confirming previous findings (16), direct culture of resting CD28-/- 2C-transgenic CD8+ cells (H2b) with Ld1.B7-1.1CAM-1 Drosophila APC in the presence of the highly immunogenic Ld-associated QL9 peptide (10 µM) led to strong uptake of both Ld and B7-1 molecules from the APC. Data for uptake of B7-1 are shown in Fig. 5A; no uptake occurred with nonimmunogenic P1A peptide (data not shown). As for CD28-mediated absorption by resting normal B6 T cells (Fig. 1), uptake of Ld and B7-1 molecules by resting CD28-/- 2C CD8+ cells required direct T-APC interaction, i.e., only very minimal uptake occurred when T cells and APC were separated in Transwell plates (see below). In marked contrast to CD28-mediated absorption by normal resting B6 T cells (Figs. 2 and 3), however, peptide-dependent absorption by resting CD28-/- 2C CD8+ cells was only partly inhibited by Lat B (Fig. 5A).

These data refer to addition of QL9 peptide at a high concentration, i.e., 10 µM. The effects of titrating the concentration of peptide on uptake of B7-1 by resting CD28-/- 2C cells following direct T-APC interaction are shown in Fig. 5B. As expected, uptake of B7-1 was proportional to the concentration of QL9 peptide added (reflecting that B7-1 uptake depends on TCR-Ld-QL9 interaction). With a high concentration of peptide (10 µM), inhibition of B7-1 absorption by Lat B was mild, i.e., ~50%. With a low concentration of peptide (0.001 µM), by contrast, Lat B totally blocked B7-1 absorption. Similar results were obtained with preactivated CD28-/- 2C cells (Fig. 5, D and E). Note that, as for CD28-mediated absorption (Fig. 1), peptide-dependent absorption by CD28-/- 2C cells was higher for activated than resting T cells.

The above findings indicated that TCR-mediated uptake of molecules from APC became relatively resistant to Lat B when the concentration of peptide was raised to a high level. These findings applied to both resting and activated CD28-/- 2C CD8+ cells (Fig. 5, C and F); for resting T cells, absorption via Transwells was very weak and apparent only with a high concentration of peptide.

In the above experiments, the use of CD28-/- T cells precluded a possible contribution of CD28 to TCR-mediated absorption. The effects of using normal CD28+ 2C cells are shown in Fig. 6. In this situation, 2C cell coabsorption of B7-1 and Ld from APC in the presence of QL9 peptide presumably involves both CD28-B7 and TCR-MHC-peptide interaction; each of these interactions causes direct binding of the specific ligand and, because of the physical association between B7-1 and Ld, indirect binding of the other ligand.
As expected, with CD28+ 2C cells direct T-APC interaction without peptide led to appreciable absorption of B7-1 (Fig. 6A) and Ld (data not shown), reflecting CD28-B7-mediated absorption; with activated CD8+ 2C cells (Fig. 6D), absorption of B7-1 (and Ld) was higher than with resting 2C cells (Fig. 6A), reflecting the much higher density of CD28 on activated T cells. With addition of QL9 peptide, the absorption of B7-1 (and Ld) increased considerably, paralleling the concentration of peptide added (Fig. 6, A and D); this applied to both resting and activated 2C cells. Confirming the results with normal T cells (Figs. 2 and 3), addition of Lat B abolished TCR-independent, CD28/B7-dependent uptake, i.e., the uptake observed in the absence of QL9 peptide (Fig. 6, A and D). With addition of QL9 peptide, uptake became Lat B resistant with high concentrations of peptide (Fig. 6, A and D). This resistance to Lat B was most apparent when mean fluorescence index values for cells cultured in the absence of peptide were subtracted, i.e., the data were expressed as the change in the mean fluorescence index induced by addition of peptide (Fig. 6, B and E). By contrast, as for CD28−/− cells, absorption by CD28+ 2C cells via Transwells was abolished by Lat B (data not shown).

Direct comparison of TCR-mediated uptake of B7-1 by CD28−/− vs CD28+ 2C cells following direct T-APC interaction is shown in Fig. 6, C and F. In the absence of Lat B, it can be seen that peptide-induced B7-1 absorption by CD28+ and CD28−/− 2C cells was virtually identical for both resting (Fig. 6C) and activated (Fig. 6F) 2C cells; similar findings applied to the absorption of Ld (data not shown). However, with addition of Lat B to activated T cells, absorption induced by a high concentration of peptide was appreciably (and reproducibly) higher with CD28+ than CD28−/− cells; this difference was not seen with resting T cells. Thus, CD28 had little, if any, effect to enhance TCR-mediated absorption, but, if only for activated T cells, was nevertheless able to promote resistance to Lat B.

**Ligand internalization**

In previous studies, uptake of APC-derived molecules onto the surface of T cells was shown to be followed by rapid internalization of
the ligands (15, 16). As for absorption to the cell surface, internalization reflected by either CD28-B7 or TCR-MHC-peptide interaction and applied to both the specific ligand recognized and adjacent ligands on APC. Using confocal microscopy, we examined whether T cell internalization of ligands from APC was Lat B sensitive.

In all situations examined, T cell internalization of ligands from APC proved to be highly sensitive to Lat B. The key finding was that Lat B blocked internalization in situations where Lat B only partly inhibited absorption on to the cell surface; this applied to both CD28-mediated and TCR-mediated uptake.

Data for CD28-mediated absorption of B7-1 from L^d,B7-1,ICAM-1 Drosophila APC by normal activated B6 CD8^+ cells are shown in Fig. 7A. In the absence of Lat B, fixation and permeabilization of nondissociated cultures of T cells and APC followed by staining for B7-1 (green) and CD8 (blue) revealed intracellular distribution of B7-1 in the T cells; cultures were examined after 60 min. In the presence of Lat B, by contrast, intracellular B7-1 staining of the T cells was undetectable. In these undisturbed cultures, conjugate formation between T cells and APC made it difficult to detect B7-1 staining on the T cell surface. When the cultures were dissociated before staining, B7-1 staining of T cells in the absence of Lat B was apparent both on the cell surface and intracellularly (Fig. 7B). With addition of Lat B during culture, however, B7-1 expression was limited to the cell surface; note that with Lat B, surface staining of B7-1 on T cells was blue-green, implying close association with the cell membrane. For CD28-mediated absorption of B7-1 via Transwells, B7-1 (pink) staining of T cells was apparent both intracellularly and on the cell surface when cells were cultured without Lat B (Fig. 7C). With addition of Lat B, B7-1 staining of T cells was undetectable on the cell surface (confirming the FACS data in Fig. 2) or intracellularly (Fig. 7C). Lat B also blocked internalization of B7-2 (Fig. 7D) and IA^b (Fig. 7E) when activated CD28^+ B7-2/-/- CD8^+ cells were cultured with B6 DC.

The above findings refer to CD28-mediated absorption. Comparable data applied to TCR-mediated absorption. This is illustrated in Fig. 7, F and G, where culturing either resting (Fig. 7F) or activated (Fig. 7G) CD28^-/- 2C CD8^+ cells with L^d,B7-1,ICAM-1 Drosophila APC plus a high concentration of QL9 peptide led to both surface and intracellular staining of T cells for L^d (pink) in the absence of Lat B, but only surface staining in the presence of Lat B. Similar findings applied to CD28^+ 2C cells (data not shown).

Discussion
As described here and previously, T cell absorption of molecules from APC is receptor (TCR or CD28) specific, evident with both transfected cell lines (Drosophila cells) and DC as APC, and occurs in vivo as well as in vitro (15, 16); for the molecules studied, transfer from APC to T cells is unidirectional. Since the molecules absorbed include not only the specific ligand recognized but also adjacent bystander molecules on the APC, the molecules are presumably absorbed in the form of cell fragments/vesicles.

As shown here, the absorption of molecules from APC proved to be most efficient when T cells and APC were in direct contact. This requirement for direct cell-cell contact was most stringent for resting T cells. Thus, for both CD28-mediated and TCR-mediated absorption, uptake of molecules from APC by resting T cells was clearly apparent after direct T-APC interaction, but was very low or undetectable when T cells and APC were separated in Transwells or T cells were incubated with APC sonicates. Interestingly, however, activated T cells behaved differently. Thus, unlike resting T cells, activated T cells were able to absorb molecules from APC via Transwells and also from APC sonicates; as for direct cell contact, T cell uptake of this shed material from APC was TCR or CD28 dependent.

With regard to the mechanism of absorption, in other studies we have found that absorption of shed material from APC by activated
The observation that T cell uptake of APC-derived molecules is followed by rapid internalization of these molecules raised the question of whether this sequence of events involves the actin cytoskeleton. Since the cytoskeleton controls receptor-mediated endocytosis of soluble molecules, e.g., transferrin (36), and is also important for T cell activation by peptide-pulsed APC (8, 37, 38), we expected the actin cytoskeleton to play a key role in both T cell absorption and internalization of molecules from APC. For internalization of ligands, this was indeed the case. Thus, in all situations tested Lat B totally inhibited internalization of ligands from APC. For absorption of APC molecules onto the surface of T cells, however, the cytoskeleton was required in some situations but not in others. Here, for direct T-APC interaction, two factors were important: 1) the activation status of the T cells, and 2) in the case of T cell absorption of shed material from APC, the concentration of peptide added to APC. To address these points, CD28- and TCR-mediated absorptions need to be considered separately.

For T cell uptake via CD28-B7 interaction, absorption was highly sensitive to Lat B for resting T cells, but was comparatively resistant for activated T cells. This difference could reflect greater mobility of CD28 in the membrane of activated than resting T cells. The possibility we favor, however, is that Lat B sensitivity is largely a reflection of enhanced LFA-1-ICAM-1 interaction by activated T cells compared to the pre-existing local density of CD28 at the contact site is sufficiently high to mediate B7 absorption; this model does not exclude the possibility that the CD28 molecules at the contact site form actin-independent microclusters (see below).

The effects of Lat B on absorption mediated by TCR-MHC-peptide interaction were unexpected. Here, the notable finding was that sensitivity of absorption to Lat B was inversely related to CD28 density on APC. For absorption from DC, the much stronger absorption by activated than resting T cells (5, 29). For resting cells, the low density of CD28 on these cells may severely limit contact with B7 on APC. For absorption of shed molecules from APC, the cytoskeleton was required in some situations but not in others. Here, for direct T-APC interaction, two factors were important: 1) the activation status of the T cells, and 2) in the case of TCR-mediated absorption, the concentration of peptide added to APC. To address these points, CD28- and TCR-mediated absorptions need to be considered separately.

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peptide concentration. Thus, absorption was strongly Lat B sensitive with low concentrations of peptide, but was relatively resistant with high concentrations. The implication, therefore, is that the actin cytoskeleton does control TCR-mediated absorption, but only when the concentration of MHC-bound peptide on APC is relatively low.

On this point there are close similarities with the role of the actin cytoskeleton in T cell activation. Thus, for T cell clones cultured with peptide-pulsed APC, a rise in the intracellular Ca$^{2+}$ concentration and IFN-$\gamma$ production can be inhibited by cytochalasin D (CD), a drug that, like Lat B, disrupts the actin cytoskeleton (37). Significantly, however, the inhibitory effects of CD on T cell activation are much less apparent with high concentrations of peptide.

In considering these findings, it is notable that although CD inhibits stable “synapses” at the site of T/APC interaction, CD does not block the formation of small clusters of molecules (CD3$\zeta$) at the contact site (39). Hence, with a high concentration of peptide on APC, actin-independent microclustering of TCR molecules interacting with a high density of MHC/peptide at the T/APC contact site may be sufficient to induce T cell activation and also to allow TCR-dependent absorption from the APC. Thus, under these particular conditions the actin cytoskeleton is not required.

This scenario could also apply to CD28-B7 interaction mediated by activated T cells (see above). However, the situation may be different for LFA-1-ICAM-1 interaction. As discussed earlier, LFA-1-ICAM-1 interaction is largely unnecessary for absorption following direct T-APC interaction, but is crucial for absorption of shed material from APC. For absorption of shed material, there is thus an interesting correlation between the requirement for LFA-1-ICAM-1 interaction and the marked sensitivity to Lat B. To explain this correlation, one could postulate that LFA-1-ICAM-1 interaction is crucially dependent upon LFA-1 associating with the actin cytoskeleton. Indirect support for this idea is provided by the finding that treating T cells (but not APC) with CD prevents redistribution of ICAM-1 on APC during T-APC interaction (40).

As described above, there are striking parallels between the role of the actin cytoskeleton in T cell activation and TCR-mediated absorption to the cell surface. An obvious question here is whether T cell absorption (and internalization) of MHC and other molecules from APC plays a significant role in T cell triggering. This possibility seems unlikely in view of the evidence that T cells can be triggered in situations where T cell absorption of MHC/peptide presumably cannot occur, e.g., when T cells are stimulated by MHC/peptide conjugated to beads or plastic (38, 41). It is also notable that CD28 plays a crucial costimulatory role in T cell triggering, but does not seem to be required for TCR-mediated absorption. Thus, peptide-induced absorption of B7-1 and L$\alpha$ molecules from APC was not higher with CD28$^+$.
2C cells than with CD28−/− cells. In fact, for these cells the only difference observed was that for absorption by activated cells, CD28−/− cells showed greater Lat B resistance than CD28−/− cells (which is difficult to explain). Despite the lack of correlation between TCR-mediated absorption and T cell triggering, there is an interesting correlation between TCR-mediated absorption and TCR down-regulation (8). Thus, following direct contact with APC, both processes occur in parallel and are largely CD28 and LFA-1 independent. The implication, therefore, is that, like TCR down-regulation, the extent of TCR-mediated absorption may directly reflect the intensity of TCR-MHC-peptide interaction. Thus, rather than correlating to T cell stimulation (which requires a combination of signals 1 and 2), TCR-mediated absorption may be a good guide to the strength of signal 1.

With regard to biological significance, we view T cell absorption of molecules during peptide-specific T-APC interactions as a manifestation of strong receptor/ligand interactions at the T/APC synapse. As mentioned earlier, internalization of the absorbed molecules may serve to disrupt the synapse after T cell activation, thus allowing stimulated T cells to dissociate from APC and migrate elsewhere to mediate their effector functions. A similar scenario may apply to CD28-mediated absorption. Thus, movement of normal resting T cells through the lymphoid tissues could be impeded by continuous contact of CD28 with B7 on APC. T cell absorption and ingestion of B7 could resolve this problem. In favor of this idea, T cell uptake of molecules from host cells in vivo is apparent for cells harvested from spleen and LN, but not from blood, implying that the absorbed molecules are rapidly internalized (or shed) before the cells enter the circulation (our unpublished observations).

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References


