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TCR Complex-Activated CD8 Adhesion Function by Human T Cells[1]

Jay C. Varghese and Kevin P. Kane[2]

The CD8 receptor plays a central role in the recognition and elimination of virally infected and malignant cells by cytolytic CD8$^+$ T cells. In conjunction with the TCR, the CD8 coreceptor binds Ag-specific class I MHC (MHC-I) molecules expressed by target cells, initiating signaling events that result in T cell activation. Whether CD8 can further function as an adhesion molecule for non-Ag MHC-I is currently unclear in humans. In this study, we show that in human CD8$^+$ T cells, TCR complex signaling activates CD8 adhesion molecule function, resulting in a CD8 interaction with MHC-I that is sufficient to maintain firm T cell adhesion under shear conditions. Secondly, we found that while CD8 adhesive function was triggered by TCR complex activation in differentiated cells, including in vitro generated CTL and ex vivo effector/memory phenotype CD8$^+$ T cells, naive CD8$^+$ T cells were incapable of activated CD8 adhesion. Lastly, we examine the kinetics of, and signaling for, activated CD8 adhesion in humans and identify notable differences from the equivalent CD8 function in mouse. Activated CD8 adhesion induced by TCR signaling may contribute to the more rapid and robust elimination of pathogen-infected cells by differentiated CD8$^+$ T cells. ***The Journal of Immunology,*** **2008, 181: 6002–6009.**

The elimination of virally infected and malignant cells by CTL involves Ag recognition, adhesion, and induction of target cell death. Recognition of antigenic peptide-MHC class I (MHC-I)[3] on the target cell (referred to as cognate recognition) is mediated by the TCR complex, with the aid of the CD8 coreceptor. As a coreceptor, CD8 binds to nonpolymorphic regions of the same MHC-I molecule bound by the TCR and provides costimulatory signals (1, 2). In mouse, following initial TCR activation, CD8 acquires the ability to adhere to any MHC-I on the target cell surface, including non-Ag MHC molecules not recognized by the TCR (1, 3, 4). In this second capacity—variously referred to as non-Ag, noncognate, enhanced, or activated adhesion function—CD8 behaves as an adhesion molecule, contributing to CTL-target cell conjugate formation and to the generation of activation signals (2, 5–7).

Adhesion molecules on T cells play a central role in many contact-dependent functions, ranging from cell conjugate formation to lymphocyte migration. Inside-out signaling, resulting from TCR engagement, activates adhesion receptors on the surface of T cells (8) and in this respect the mouse CD8 adhesion molecule is included (3, 9). TCR initiated signaling via PI3K and cytoskeletal reorganization has been shown to activate CD8 adhesion function, while phorbol ester stimulation of the protein kinase C (PKC) pathway promotes the cessation of CD8 adhesion (9, 10). These findings suggest that CD8 adhesion function is a regulated phenomenon rather than a constitutively active process.

Naive, effector, and memory T cell subsets manifest distinct functional capacities. In contrast to their naive cell counterparts, effector and memory T cells exhibit a more rapid and robust response to Ag, which is essential for the control and elimination of pathogens. This augmented response has been attributed to the increased frequency of responding cells for a given Ag, as well as to changes within individual cells including the constitutive transcription of genes associated with inflammation and CTL lytic capacity, and changes in the expression and use of adhesion molecules (11, 12). It is entirely unknown whether CD8 binding to non-Ag MHC-I is differentially regulated among subsets of CD8$^+$ T cells. Although CD8 expression levels are not augmented in effector or memory vs naive cells, additional mechanisms such as lower activation thresholds, enhanced TCR signaling, and improved CD8 mobility and clustering may result in differences in CD8 adhesion function between T cell subsets.

Apart from surface plasmon resonance and other biochemical reports, very few cellular studies have examined the function of CD8 as an adhesion molecule in humans. Furthermore, the cellular studies that did demonstrate that CD8 could adhere to non-Ag MHC-I typically involved expression of CD8 on cell types other than T cells and did not examine the influence of TCR engagement on this type of CD8 interaction with MHC-I (13–15).

In this report, we sought to identify whether CD8 on human T cells can function as an adhesion molecule for non-Ag MHC-I and whether this function was constitutive or induced upon TCR complex activation. We demonstrate that human T cells undergo CD8 adhesion only when triggered via their TCR/CD3 complex. We proceed to characterize CD8 adhesion molecule function with respect to kinetics and signaling requirements and demonstrate that this function is an attribute of differentiated but not naive CD8$^+$ T cells. Enhanced CD8 adhesiveness upon TCR engagement may contribute to the more rapid and robust elimination of pathogen-infected cells by differentiated CD8$^+$ T cells.

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[3] Abbreviations used in this paper: MHC-I, MHC class I; RCF, relative centrifugal force; PKC, protein kinase C.

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Materials and Methods

Hybridomas, Abs, and flow cytometric analysis

The anti-CD8β monoclonal 5F2 (16) (mouse IgG1) was purchased from Serotec and the anti-CD8α monoclonal 3B5 (mouse IgG2a) from Caltag Laboratories. Isotype control Abs A1 (17) (anti-mouse Ly49A, IgG2a) and Cwy3 (18) (mouse Ly49G2, IgG1) were generated from hybridomas in our laboratory. The anti-CD8α (OKT8 (19), mouse IgG2a), anti-HLA-A2 (MA2.1 (20), mouse IgG1), anti-HLA-B7 (BB7.1 (21), mouse IgG1), anti-HLA-B27 (ME1 (22), mouse IgG1), and anti-pan HLA (W6/32, mouse IgG2a) were prepared from American Type Culture Collection (ATCC) hybridomas. Abs prepared from hybridomas were purified using a protein G column. mAb UCHT1 (anti-CD3ε (23), mouse IgG1) was purchased from eBioscience, as were fluorochrome-conjugated mouse Abs CD45RA-PE (HI100, IgG2b), CD28-PE-Cy7 (CD28.2, IgG1), CD27-allophycocyanin (Clone LG.7F9, Armenian hamster IgG), CD62L-allophycocyanin-Cy7 (DREG-56, IgG1), CD8α-FITC (RPA-T8, IgG1), and CD3ε-allophycocyanin (UCHT1, IgG1). Anti-CCR7-FITC (Clone 150503, IgG2a) was purchased from R&D Systems. For flow cytometric analysis, cells were incubated with the indicated combinations of fluorochrome-conjugated Abs for 15 min at 4°C, followed by extensive washing and fixation (2% formaldehyde/PBS). Multicolor flow cytometry and cell sorting were performed using FACSCanto and FACSAria flow cytometers, respectively, and data was analyzed using either FACSDiva (BD Biosciences) or FCS Express 3 (De Novo Software) software.

Protein purification and ELISA

HLA molecules were purified from RPMI 8866 cells (for HLA-A2) or HMy2.C1R HLA-B27 transfectants by immunoaffinity chromatography. Between 0.5 and 1×10^{10} cells were lysed for 10 min on ice in 100–200 ml lysis buffer (15 mM sodium phosphate, 0.5% Nonidet P-40, and 1 mM phenylmethanesulfonyl fluoride (pH 7.3)). The lysate was cleared by centrifugation at 10,000 relative centrifugal force (RCF) for 20 min and again at 100,000 RCF for 45 min followed by 0.45 μm filtration. The filtered lysate was run at ~0.1 ml/min through a Sepharose 4B (Sigma-Aldrich) precolumn followed by a Sepharose 4B Ab-conjugated (MA2.1 for A2, or ME1 for B27) immunoaffinity column. The immunoaffinity column was washed extensively with 10 mM sodium phosphate, and 0.5% Triton X-100 (pH 7.3), and 10 mM Tris, 150 mM NaCl, and 0.5% Triton X-100 (pH 8.3). The HLA protein was then eluted in 25 mM diethylamine, 650 mM NaCl, and 0.5% octyl-β-glucoside (pH 11.5), and neutralized immediately with 2 M Tris (pH 8). HLA protein concentration was determined by MicroBCA (Pierce). Protein purity was monitored using Western blot and GelCode Blue protein gel stain (Pierce). Conformational integrity was assessed using W6/32 and MA2.1 or ME1 Abs by solid-phase ELISA as described previously (24). Peak fractions showing strong ELISA reactivity were pooled and used in adhesion assays.

Cells

Blood was collected from multiple HLA-A2 positive donors with informed consent in accordance with the University of Alberta Health Research Ethics Board approved protocol and the Helsinki Declaration as revised in 2005. Donor cell DNA was HLA typed at the University of Alberta Hospital Histocompatibility Laboratory. Human CD8$^+$ T cells were isolated by negative selection of PBMC (obtained from venous blood) using the EasySep CD8$^+$ T cell enrichment kit (StemCell Technologies). CD8$^+$ T cell purity was greater than 98% by flow cytometry. Purified CD8$^+$ T cells were either used immediately in assays or after weekly stimulation by coculture with irradiated human B lymphoblastoid RPMI 8866 cells (25) (gift from Dr. R. C. Bleackley, University of Alberta, Edmonton, Canada) and 80 U/ml IL-2, as described (26). Cell lines generated in this manner were named hCD8T-D0 through D7 (where "D" indicates anonymous donor number). The human B lymphoblastoid cell line HMy2.CIR (27) (ATCC), was stably transfected with genes encoding HLA-A2, HLA-B7, or HLA-B27 using Lipofectamine 2000 (Invitrogen).

Cytotoxicity assays

The HMy2.CIR and HMy2.CIR cells transfected with HLA-A2 or HLA-B7 were labeled with 100 μCi $Na^{51}CrO_4$ (^{51}Cr; PerkinElmer Life and Analytical Sciences) for 1 h at 37°C, followed by extensive washing. Target cells were then incubated in 96-well V-bottom microtiter plates for 4 h at 37°C along with human CD8$^+$ T cell lines. Following incubation, plates were centrifuged at 300 RCF for 5 min, after which 25 μl of culture supernatant was removed, mixed with scintillation mixture (OptiPhase SuperMix; PerkinElmer), and counted in a Wallac MicroBeta counter (PerkinElmer). Percent specific lysis was calculated as follows: 100 × [(experimental release) − (spontaneous release)]/[(maximal release) − (spontaneous release)]. Cytotoxicity assays were performed in triplicate wells and each experiment performed three separate times. Error bars indicate SE.

Adhesion assays

Adhesion assays were performed as previously described (10), with modifications. The MHC-I protein was diluted in PBS and immobilized on 96-well microplates (BD Biosciences) by incubation at 4°C for 4–16 h. Microplates were washed then blocked at room temperature with 1% BSA/PBS (Sigma-Aldrich). Human CD8$^+$ T cells labeled with Calcein AM fluorescent dye (Invitrogen) were treated with or without 0.5 μg/ml UCHT1 anti-CD3ε fluid phase Ab immediately before plating at $1–2 \times 10^5$ cells/well in 100 μl of assay medium (2% FBS, 1% glutamine, and RPMI 8866). Cells were gently pelleted by centrifugation at 20 RCF for 3 min followed by incubation at 37°C for 30 min unless otherwise indicated. For CD8 blocking, cells were pretreated for 30 min at room temperature with the indicated CD8-specific Abs before addition of UCHT1. To remove nonspecifically adhered cells after incubation, 150 μl of 4°C assay medium was gently added, microplates were placed on ice for 5 min, and a shear force was then applied using an electronic multichannel pipet (Eppendorf) set to aspirate/dispense 100 μl of assay medium at 50% of the maximal speed setting. Aspiration/dispensation was repeated six consecutive times, after which the medium was removed. Microtiter plates were then analyzed in a Wallac Victor2 1420 Multilabel Counter (PerkinElmer) using a fluorescein filter. Percent specific cell binding was calculated as 100 × [(experimental binding fluorescence) − (nonspecific binding fluorescence)]/[total cell fluorescence]. Nonspecific binding to 1% BSA-coated wells was generally between 10 and 20% and was subtracted in the calculation of percent adhesion, as indicated above. All determinations were done in triplicate. Error bars indicate SE. Three independent experiments were performed for each data presentation, yielding similar results.

Results

Human CD8$^+$ T cells undergo anti-CD3-triggered CD8 adhesion to non-Ag HLA

Although CD8 is commonly regarded as a coreceptor as it binds to cognate MHC-I simultaneously with the TCR, it is not known whether on humans T cells, CD8 can also function as an adhesion molecule by binding to non-Ag MHC-I (i.e., MHC-I not bound with peptide Ag and not recognized by the TCR of the CD8$^+$ T cell in question), and whether this function is constitutive or induced by TCR signals. To address this issue, we generated effector CD8$^+$ T cell lines by coculturing purified human CD8$^+$ T cells with RPMI 8866 lymphoblastoid cells (HLA-A2, HLA-A3, HLA-B7, HLA-Cw7 (28), as described (26). A number of these T cell lines, including hCD8T-D2 (HLA-A2, HLA-A26, HLA-B35, HLA-B40, HLA-Cw3, and HLA-Cw4), were allo-specific for HLA-B7 but not HLA-A2 since HLA-B7, but not A2-expressing target cells were lysed (Fig. 1).

Although HLA-A2 is a self MHC-I for the hCD8T-D2 cell line, in the absence of peptide Ag it is not a cognate ligand for the TCR (Fig. 1). Consequently, we were able to use purified HLA-A2 as an exclusive ligand for CD8 adhesion. We used solid-phase adhesion assays to examine CD8 adhesion to HLA to circumvent confounding effects by other cell surface receptor/ligand pairs such as LFA-1/ICAM-1 (1). Purified HLA-A2 protein was immobilized on microtiter wells at varying densities and native protein structure was confirmed by ELISA using the conformation-specific HLA Abs W6/32 (Fig. 2*A*) and MA2.1 (data not shown). The W6/32-specific ELISA reactivity as a function of HLA-A2 amount immobilized in microplate wells was linear up to ~0.4 μg/well HLA-A2, at which point a plateau was reached where no further HLA-A2 could be immobilized (Fig. 2*A*). In the absence of a TCR stimulus, hCD8T-D2 cells showed little or no adhesion to HLA-A2 across the linear range of immobilized HLA-A2 (Fig. 2*B*). However, upon CD3/TCR complex triggering, hCD8T-D2 cells were capable of adhering to non-Ag HLA-A2, suggesting that human CD8 can function as an adhesion molecule, but only subsequent to TCR

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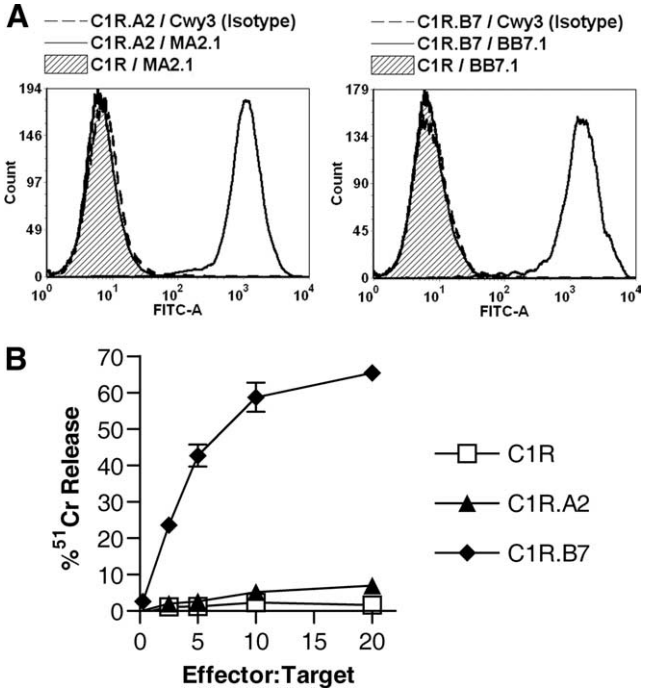

FIGURE 1. HLA-B7 but not HLA-A2 is a cognate allo-Ag for the human CD8$^+$ T cell line hCD8T-D2. *A*, Flow cytometry measuring surface expression of HLA-A2 and HLA-B7 on HMy2.C1R or HMy2.C1R stably transfected to express HLA-A2 or HLA-B7. Cells were stained with MA2.1 (*left panel*) or BB7.1 (*right panel*), and their respective isotype control Abs. *B*, ^{51}Cr release assay using hCD8T-D2 effector cells and target cells described in *A*.

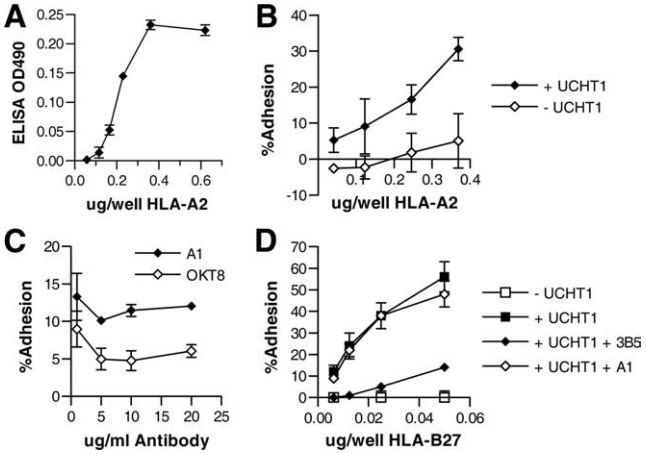

FIGURE 2. Human CD8$^+$ T cells undergo CD3-triggered CD8 adhesion to immobilized non-Ag HLA. *A*, ELISA using pan-HLA class I-specific Ab (W6/32) detects various densities of purified HLA-A2 immobilized on microtiter plates. *B*, Adhesion of hCD8T-D2 CTL to immobilized non-Ag HLA-A2 in the presence, but not absence, of soluble anti-CD3 (UCHT1). *C*, Assessment of soluble UCHT1 Ab-triggered CD8 adhesion by hCD8T-D2 to immobilized HLA-A2 (0.12 μg/well) with, or without, CD8 blocking. Cells were pretreated for 30 min at room temperature with 1–20 μg/ml anti-CD8α (OKT8) or isotype control (A1) Abs before UCHT1 Ab-triggering and plating on HLA-A2. *D*, Measurement of hCD8T-D0 CTL adhesion to immobilized purified non-Ag HLA-B27 with or without UCHT1 triggering, and with or without CD8 blocking. Cells were pretreated for 20 min at room temperature with 5 μg/ml anti-CD8α (3B5) or isotype control (A1) Ab before UCHT1 Ab-triggering and plating on HLA-B27.

activation (Fig. 2*B*). Triggered binding was HLA density-dependent, increasing with the amount of HLA-A2 immobilized and correlating with the HLA-A2 density determined by ELISA (Fig. 2, *A* and *B*). To confirm that adhesion to non-Ag HLA-A2 was specifically mediated by CD8, hCD8T-D2 cells were pretreated with the known CD8-blocking Ab, OKT8 (13, 14), before triggering with anti-CD3. Blocking CD8 resulted in a substantial reduction in hCD8T-D2 triggered adhesion to HLA-A2 (Fig. 2*C*). The finding that human CD8$^+$ T cells undergo enhanced CD8 binding was not limited to hCD8T-D2 cells but was observed in CD8$^+$ T cell lines derived from multiple donors (data not shown). To assess whether HLA molecules other than A2 could serve as a ligand for TCR-activated CD8 adhesion, we used purified HLA-B27 as a non-Ag, non-self HLA ligand for the hCD8T-D0 T cell line (HLA-A2, HLA-A26, HLA-B15, HLA-B51, HLA-Cw1, and HLA-Cw4). The hCD8T-D0 cells underwent CD3-triggered CD8 adhesion to HLA-B27 in a density-dependent manner, which was blocked by the CD8 Ab, 3B5 (Fig. 2*D*). No adhesion to HLA-B27 was observed in the absence of anti-CD3 treatment, indicating that activated CD8 adhesion to HLA-B27 is not due to TCR allo-reactivity (Fig. 2*D*). Collectively, these results demonstrate that triggering of the CD3 complex in human CD8$^+$ T cells activates CD8 adhesion function for both self and non-self, non-Ag class I HLA molecules.

Differentiated, but not naïve, CD8$^+$ T cells undergo CD3-triggered CD8 adhesion

Individual subsets of CD8$^+$ T cells express specific constellations of receptors on their cell surface and each population exhibits functionally distinct characteristics. For example, naive human CD8$^+$ T cells that have not encountered Ag are characterized by the expression of CD45RA, the lymph node homing receptors CCR7 and CD62L, and the costimulatory receptors CD28 and CD27 (29). Effector and memory CD8$^+$ T cells, in contrast, express a complex and variable array of the above markers, most of which are typically reduced or absent from the cell surface. Functionally, differentiated cells have an enhanced ability to control pathogens upon secondary exposure owing to their increased frequency, rapid acquisition of effector function, and localization to peripheral sites of infection (11).

The preceding experiments demonstrated that effector CD8$^+$ T cell lines have the ability to undergo activated CD8 adhesion. However, an important question is whether naive human CD8$^+$ T cells are capable of TCR-triggered CD8 adhesion to non-Ag HLA, or whether this capacity is a unique property of CD8$^+$ T cells that have differentiated. To address this issue, we began by comparing fresh ex vivo CD8$^+$ T cells with in vitro generated effector CD8$^+$ T cell lines obtained from the same donor. Purified resting CD8$^+$ T cells from donor 2 were predominantly naive (>80%), appearing as a distinct population of CCR7^{high}CD45RA^{high} cells expressing the characteristic uniform high levels of CD27, CD28, and CD62L (L-selectin) (Fig. 3*A*). Conversely, 35-day hCD8T-D2 cytotoxic cells (generated by weekly stimulation with Ag), showed the characteristic effector cell staining profile that was negative/low for CCR7, CD45RA, CD62L, and CD28, and generally reduced in CD27 expression, compared with naive CD8$^+$ T cells (Fig. 3*A*). Moreover, hCD8T-D2 behaved functionally as bona fide effector T cells since they demonstrated a robust Ag-specific cytolytic capacity (Fig. 1*B*). We found that fresh ex vivo (day 0) CD8$^+$ T cells—the majority (83%) of which were naive—showed minimal CD3-triggered CD8 adhesion to HLA-A2 in comparison to the day 35 hCD8T cells (Fig. 3*B*). Naive and effector/memory

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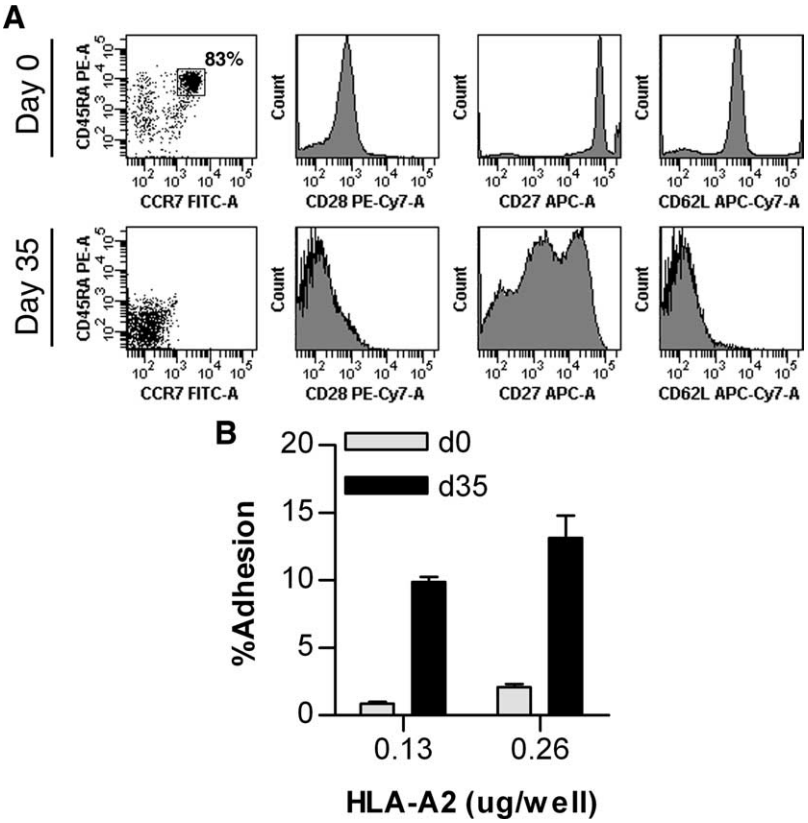

FIGURE 3. Human effector CD8$^+$ T cells undergo CD3-triggered CD8-mediated adhesion, whereas this function is minimally detected with bulk ex vivo CD8$^+$ T cells. *A*, 5-color flow cytometry of donor 2 CD8$^+$ T cells immediately after purification (day 0) or 35 days post-Ag/IL-2-stimulated culture (cell line hCD8T-D2). Cells were stained with fluorochrome-conjugated Abs CD45RA-PE, CCR7-FITC, CD28-PE-Cy7, CD27-allophycocyanin, and CD62L-allophycocyanin-Cy7. Soluble UCHT1 anti-CD3 triggered adhesion to immobilized HLA-A2 (*B*) using days 0 and 35 CD8$^+$ T cells characterized in *A*.

CD8$^+$ T cell subsets were then freshly isolated from donor blood to directly compare these subsets for CD3-triggered non-Ag HLA binding by CD8. Two distinct ex vivo subsets were identified and sorted based on surface marker staining: a naive subset expressing uniform and high levels of CCR7, CD45RA, CD27, CD28, and CD62L, and a subset primarily comprised of CD8$^+$ T memory and/or effector cells (Fig. 4*A* and data not shown). Both sorted CD8$^+$ T cell populations were between 94 and 98% pure. We found that the naive CD8$^+$ T cell population was unable to adhere to non-Ag HLA-A2 in response to a soluble anti-CD3 stimulus, whereas the population containing memory/effector cells underwent adhesion to HLA-A2 (Fig. 4*B*). The CD3-triggered non-Ag HLA binding by sorted effector/memory cells (Fig. 4*B*) contrasts with minimal binding by bulk CD8$^+$ T cells (Fig. 3*B*) and reflects the low frequency of memory/effector cells in the bulk CD8$^+$ T cell population (Fig. 3*A*). The CD3-stimulated HLA binding by the ex vivo effector/memory subset are consistent with that found with the Ag-specific in vitro-stimulated CD8$^+$ T cell lines (Fig. 3, *A* and *B*). We found that even with Ab cross-linked anti-CD3, sorted naive CD8$^+$ T cells were unable to adhere to HLA-A2 (Fig. 4*C*). Conversely, upon cross-linked anti-CD3 treatment, the effector and memory CD8$^+$ T cell population showed at least a 2-fold increase in adhesion to HLA-A2 (Fig. 4*C*), beyond their already significant adhesion when triggered with soluble anti-CD3 alone (Fig. 4*B*). Although the naive CD8$^+$ T cell sorted subset was incapable of CD3-triggered CD8 adhesion, these cells acquired enhanced CD8 adhesion function after undergoing in vitro differentiation by weekly stimulation with APCs and IL-2 (data not shown), further indicating that the Ag-driven maturation state of a CD8$^+$ T cell determines its capacity for CD3-triggered CD8 adhesion. Collectively, these findings demonstrate that ex vivo differentiated CD8$^+$ T cells have the ability to undergo CD3-triggered adhesion to non-Ag HLA class I, unlike their naive CD8$^+$ T cell counterparts. Furthermore, Ab-induced CD3 clustering augments CD3-triggered non-Ag HLA binding by the effector/memory CD8$^+$ subset.

CD8 adhesion to non-Ag HLA following CD3-triggering of human CD8$^+$ T cells is transient

We have previously shown that in mice, CD3-triggered CD8 adhesion to non-Ag MHC-I increases with time after stimulus, reaching a maximum at 60 min, which is sustained for over 5 h (4, 30). To determine whether these kinetics also held for human T cells, we performed the anti-CD3-triggered non-Ag HLA adhesion assay while varying the amount of time for which cells were allowed to bind before adhesion was measured. We used hCD8T-D2 effector CTL in these assays as they exhibited CD3-induced CD8 binding as observed with fresh ex vivo effector/memory CD8$^+$ T cells; thus, they are likely functionally equivalent in this regard, and moreover, they could be produced in significant numbers. The hCD8T-D2 CTL showed increasing levels of adhesion to immobilized HLA-A2 in response to CD3 triggering, with maximal adhesion observed in multiple assays in a range spanning 30 to 60 min poststimulus (Fig. 5 and data not shown). However, in marked contrast to mouse CD3-triggered CD8 binding to non-Ag MHC-I (30), the equivalent in human was seen to decrease by 90 min and was nearly nonexistent by 4 h (Fig. 5). These observations indicate that CD3-activated CD8 binding by human T cells is transient, in comparison to mouse.

PI3K, PKC, and the actin cytoskeleton in the regulation of CD3-triggered CD8 binding to non-Ag HLA

The transient nature of CD3-triggered CD8 adhesion by human CD8$^+$ T cells suggests that it may be regulated by a signaling mechanism similar to CD3-triggered LFA-1 adhesion to ICAM-1, which is also transient (31). To approach this issue, we began by focusing on phorbol ester stimulation, which is known to activate

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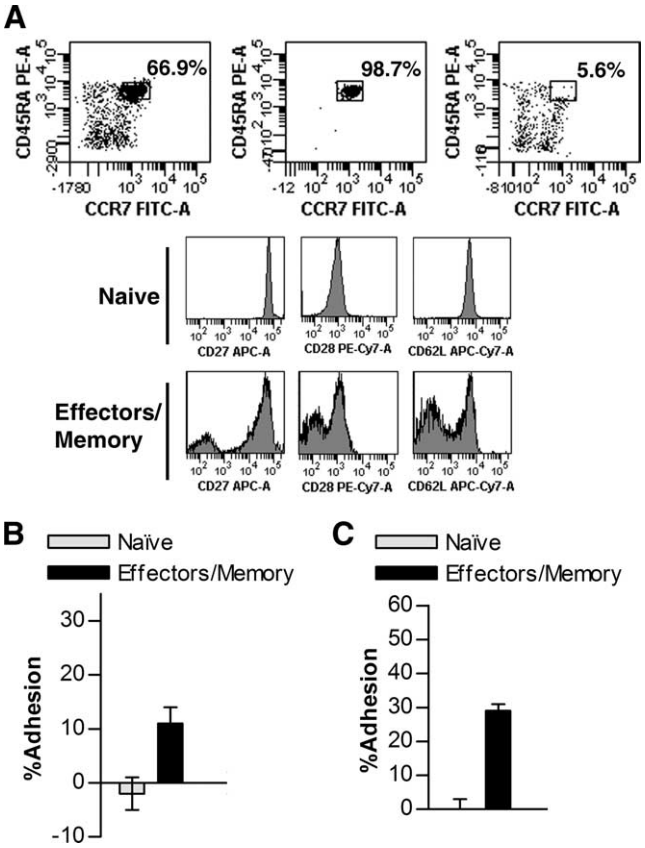

FIGURE 4. Fresh ex vivo differentiated but not naive CD8$^+$ T cells undergo CD3-triggered CD8-mediated adhesion. *A*, Dot plots (*top row panels*) show CD8$^+$ T cells from donor 7 stained with fluorochrome-conjugated Abs recognizing CCR7 and CD45RA. The CD8$^+$ T cells were sorted for the naive cell gated population, which comprised 66.9% of total cells (*left dot plot*). Postsort analysis on the gated naive cell population showed 98.7% sorting efficiency (*middle dot plot*), while the remaining nongated effector/memory cells showed 94.4% sorting efficiency (*right dot plot*). Histograms (*below*) show CD27-allophycocyanin, CD28-PE-Cy7, and CD62L-allophycocyanin-Cy7 staining corresponding to gated (naive) and all remaining (effector/memory) CD8$^+$ sorted populations as indicated. *B*, Soluble UCHT1 anti-CD3-triggered adhesion by sorted naive and effector/memory cells to HLA-A2 immobilized at 0.26 μg/well. Cells were rested postsorting for 6 h at 37°C before commencing adhesion assays. *C*, Identical adhesion assay as in *B*, except that in addition to soluble UCHT1, cells were simultaneously treated with 1 μg/ml rat anti-mouse IgG.

TCR/CD3 downstream targets including PKC. Phorbol ester stimulation has been shown to activate T cell adhesion by adhesion molecules such as LFA-1 (31); however, phorbol ester treatment is inhibitory for mouse activated CD8 adhesion, promoting de-adhesion (9). We treated hCD8T-D2 CTL with the phorbol ester PMA as a potential triggering stimulus for CD8 adhesion in substitution of CD3 engagement. We found that treatment with PMA at the low-dose range of 1–30 nM resulted in adhesion of hCD8T-D2 CTL to HLA-A2 (Fig. 6*A*). Adhesion was maximal at 10 nM PMA and remained at this level as higher concentrations of phorbol ester were used. To determine whether PMA specifically activated CD8 to undergo adhesion to HLA-A2, we performed the same adhesion assay but with anti-CD8 blocking Abs (14, 32) (Fig. 6*B*). Blocking CD8 ablated PMA-induced hCD8-D2 adhesion to HLA-A2 (Fig. 6*B*), indicating that phorbol ester is stimulatory for enhanced CD8 adhesion by human T cells.

PI3K is a signaling molecule downstream of the TCR/CD3 complex that is involved in TCR-activated adhesion by mouse CD8$^+$

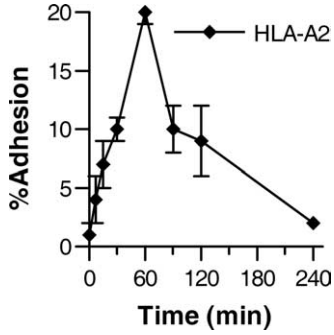

FIGURE 5. Kinetics of CD3-triggered CD8 adhesion to HLA-A2. Adhesion of hCD8T-D2 CTL to 0.12 μg/well of immobilized HLA-A2 in the presence of soluble anti-CD3 (UCHT1) assayed at various time points and plotted as a function of time.

T cells (9, 33). To examine the role of PI3K in human CD3-triggered CD8 adhesion, we used the irreversible PI3K-selective inhibitor Wortmannin. Treatment of hCD8T-D0 cells with 1–100 nM Wortmannin immediately before triggering with anti-CD3 resulted in a decrease of between 40 and 70% in CD8-mediated T cell adhesion to immobilized HLA-A2 (Fig. 7*A*). This result is consistent with previous studies implicating a role for PI3K in TCR/CD3-triggered cell adhesion pathways (9, 33, 34).

The actin cytoskeleton has an essential function in the formation of stable T cell conjugates and cytoskeletal rearrangements are important for T cell activation (35). We found that CD3-activated CD8 adhesion by hCD8T-D0 CTL was exquisitely sensitive to treatment with Cytochalasin D, an actin filament disrupter/polymerization inhibitor, as a low dose of 0.5 μM Cytochalasin D was sufficient to nearly ablate adhesion to HLA-A2, while higher doses completely prevented hCD8T-D0 from adhering to non-Ag HLA (Fig. 7*B*). These results indicate a critical role for the actin cytoskeleton in CD3-activated CD8 adhesion to non-Ag HLA by human CTL.

As a whole, these findings demonstrate that TCR/CD3 activation of CD8 adhesion to non-Ag HLA is regulated in a manner similar to that of other adhesion molecules such as LFA-1 by the involvement of the actin cytoskeleton and downstream signaling molecules from TCR engagement including PI3K and possibly PKC. Furthermore, the stimulatory role of PMA in human TCR-triggered CD8 adhesion is in direct contrast to mouse, where PMA induces the de-adhesion of CD8 from non-Ag MHC-I (9).

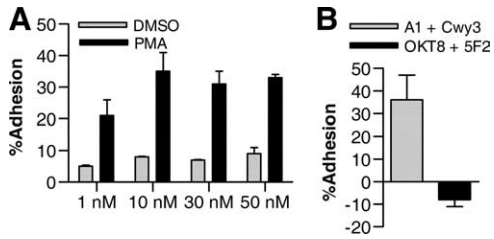

FIGURE 6. PMA treatment of human CD8$^+$ T cells induces CD8 adhesion to non-Ag HLA-A2. *A*, hCD8T-D2 CTL were stimulated with various concentrations of PMA in DMSO (black bars) or solvent alone (gray bars), immediately before plating on wells containing 0.12 μg/well immobilized HLA-A2. *B*, hCD8T-D2 were pretreated for 30 min with either anti-CD8α (OKT8) plus anti-CD8β (5F2) (black bar), or isotype controls A1 plus Cwy3 (gray bar). Cells were then stimulated with 10 nM PMA immediately before plating on wells containing 0.24 μg/well immobilized HLA-A2.

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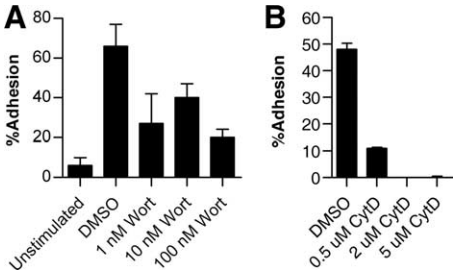

FIGURE 7. PI3K and the actin cytoskeleton are involved in CD3-triggered CD8 adhesion of human $CD8^+$ T cells to non-Ag HLA molecules. *A*, Activated CD8 adhesion by hCD8T-D0 CTL pretreated with Wortmannin in DMSO or solvent alone, immediately before UCHT1 anti-CD3 triggering and plating onto wells immobilized with 0.17 μg/well HLA-A2. *B*, Treatment of hCD8T-D0 CTL with Cytochalasin D in DMSO or solvent alone, simultaneously with UCHT-1 Ab, immediately before plating on wells immobilized with 0.16 μg/well HLA-A2.

Discussion

It was not known whether the TCR can activate CD8 adhesion molecule function in human T cells and, moreover, which $CD8^+$ T cell populations may have this capacity. In this report, we show that human $CD8^+$ T cells undergo TCR/CD3-activated CD8 adhesion to non-Ag MHC-I. Furthermore, we found that while CD8 adhesion was enhanced by CD3 triggering in differentiated cells, including in vitro-generated CTL and ex vivo effector/memory phenotype $CD8^+$ T cells, naive $CD8^+$ T cells were incapable of CD3-enhanced CD8 adhesion. Lastly, we report that CD8 adhesion function in humans is inducible by PMA stimulus and is kinetically transient, both of which are in contrast to mouse CD8 adhesion function (9, 30).

Freshly isolated effector/memory phenotype $CD8^+$ T cells were capable of CD8 adhesion to non-Ag MHC-I, as were naive $CD8^+$ T cells that had undergone Ag-mediated T cell differentiation. The acquisition of enhanced CD8 adhesion functions became apparent as early as after two rounds of stimulation of naive $CD8^+$ T cells by APCs (e.g., day 10 in vitro) and correlated with the emergence of an effector cell population exhibiting the loss of CD45RA, CCR7, CD62L, and CD28 cell surface markers (data not shown). These results reinforce our finding that naive cells are incapable of TCR-enhanced CD8 adhesion but must undergo differentiation into an effector or memory cell before acquiring this function. The mechanism(s) underlying this difference in CD8 function between subsets is unclear but may involve physical properties of the CD8 molecule itself and/or differences in signaling between $CD8^+$ T cell subsets. Human memory phenotype $CD8^+$ T cells do not express greater levels of cell surface CD8α and β-chains than naive $CD8^+$ T cells (data not shown), thereby excluding CD8 expression levels as an explanation for differential CD8 adhesion by T cell subsets. Differential glycosylation by the sialyltransferase ST3Gal-I has been proposed to regulate the affinity of CD8 for noncognate MHC-I during thymocyte development (36, 37); however, the postulated role of ST3Gal-I in regulating CD8 affinity for MHC-I is controversial and likely more complex than predicted (38). Nevertheless, differential glycosylation of CD8 on naive and effector/memory peripheral T cell subsets could be a possible explanation for the different functional capacities of CD8 we observed between naive and differentiated T cells (39, 40). Aside from changes in CD8 itself, inherent signaling differences between naive and effector/memory cells in response to TCR induction may account for CD8 adhesion function in the latter T cell subset. For example, PKC activity has been shown to be greater in $CD45RO^+$ differentiated human T cells compared with $CD45RA^+$ naive cells, in response to an anti-CD3 stimulus (41). Enhanced PKC activity in differentiated T cells may provide sufficient signaling to mobilize CD8 adhesion function, and such a mechanism would be in keeping with our finding that a PMA stimulus can activate CD8 adhesion function. Irrespective of mechanism, TCR-regulated CD8 adhesion in differentiated T cell subsets may contribute to the rapid and robust memory recall and effector cytolytic response to Ag.

We found that TCR-induced regulation of CD8 function in human T cells differs from that of mouse with respect to the transient nature of adhesion and the stimulatory effect of PMA. Nevertheless, human $CD8^+$ T cells also share similarities with their mouse counterparts (9, 10) as both use the PI3K pathway and require the actin cytoskeleton to mediate TCR-activated enhanced CD8 adhesion. We found Wortmannin inhibition of enhanced CD8 adhesion to be significant, although incomplete, suggesting that CD8 adhesion is likely regulated by PI3K and additional pathways. In marked contrast to our findings in human, mouse TCR-activated CD8 adhesion is inhibited by PMA and is not transient but rather stable for over 3 h (9, 30). It is not readily apparent why the kinetics of TCR-activated CD8 adhesion would differ between mouse and human. The discrepancy may simply be a reflection of differential regulation by signaling intermediates. Additionally, the murine CD8 interaction with MHC-I is higher affinity than that of human due to a substantially lower dissociation constant (42); this may result in a more stable, and consequently longer-lived, adhesion response as seen in the mouse. Whether the kinetic difference observed between species serves functionally different roles or is merely incidental is not clear. It may be that transient TCR-activated CD8 adhesion in humans preferentially contributes to conjugate formation during CTL lytic function, whereas TCR-activated CD8 adhesion in mouse may be involved not only in CTL lytic function but also more sustained interactions observed between CTL and dendritic cells (43). However, it is also likely that de-adhesion signals (9), from extracellular stimuli present in vivo may alter the kinetics of activated CD8 adhesion to suit the desired function. Additional studies correlating the duration of cellular contact by memory and effector $CD8^+$ T cells with short- and long-term functional outcomes will be required to determine whether TCR-activated CD8 adhesion fulfills different functional roles in humans vs mice.

The mechanism by which TCR stimulation enhances CD8 binding to MHC-I is not known. Although modification of CD8 glycosylation can alter CD8 affinity for MHC-I, these changes are unlikely to account for the rapid kinetics of TCR-activated CD8 adhesion indicated by our data. It is possible that enhanced CD8 adhesion involves multiple mechanisms, perhaps including CD8 conformational changes resulting in higher binding affinity (44, 45), and localization in membrane microdomains such as lipid rafts (46, 47).

The coupling of CD8 with the TCR has been shown to occur via CD3δ and the TCR α-chain connecting peptide motif, with the latter required for optimal MHC-I binding and functional responses including thymocyte positive selection (48, 49). The interaction between CD8 and non-Ag MHC-I may also enhance association between the CD8 cytoplasmic domain and the CD3 complex when local TCR are also engaged, thereby allowing the CD8-associated kinase Lck to phosphorylate ITAM motifs on the CD3ζ-chain for efficient T cell signaling and activation (42, 50). Recent studies have shown that CD8 interaction with non-Ag MHC-I augment T cell-APC conjugate formation, as well as the kinetics and magnitude of intracellular Ca^{2+} mobilization during T cell activation (50, 51). Although the CD8 receptor was shown to bind non-Ag MHC-I in the absence of TCR engagement, cell conjugate formation and activation required the presence of cognate

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MHC-I Ag (50). Our data show that TCR engagement is essential for CD8-mediated adhesion by human T cells, since we observed little or no adhesion to non-Ag MHC-I in the absence of triggering of the TCR complex. Collectively, these results suggest that while CD8 can interact weakly with MHC-I in a constitutive TCR-independent manner at the synapses of cell-cell contact (50, 52), TCR-induced signals are required for optimal CD8 orientation and/or cell morphological changes necessary for tight adhesion between cells. In further support of this model is our finding that cytoskeletal disruption abrogates CD8-mediated human T cell adhesion to non-Ag MHC-I.

The density of cognate MHC-I is typically low in comparison to non-Ag MHC-I on virally infected and malignant cells (53, 54); however, the $CD8^+$ T cell response exhibits remarkable sensitivity and specificity in that as few as 1–3 cognate MHC-I are sufficient to elicit activation (55, 56). Indeed, studies have shown that when cognate MHC-I Ag is limiting, CD8 interactions with non-Ag MHC-I are essential for T cell conjugate formation and activation, thereby supporting a model in which CD8 binding to non-Ag MHC-I contributes to the sensitivity of the T cell response (7, 50, 51). Our data with human T cells also support this model as we found that a low dose of soluble anti-CD3, known to be substimulatory (7), was sufficient to trigger CD8-mediated T cell adhesion to non-Ag MHC-I. In addition to likely increasing the sensitivity of the T cell response, we show that CD8 binding to non-Ag MHC-I contributes to the adhesive aspect of T cell function. The adhesion of human CD8 induced by TCR engagement was seen not only to non-Ag self MHC-I but also to non-Ag allogeneic MHC-I (Fig. 2*D*). The latter CD8 interaction may contribute to allogeneic $CD8^+$ T cell responses in transplant rejection by CTL.

In summary we have found that differentiated human $CD8^+$ T cells mediate CD8-dependent firm adhesion to non-Ag HLA when stimulated through the TCR/CD3 complex. Enhanced CD8 adhesion likely contributes to the sensitivity of Ag recognition and adhesive forces for T cell conjugate formation and activation.

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Disclosures

The authors have no financial conflict of interest.

References

1. Shen, L., T. A. Potter, and K. P. Kane. 1996. Glu227->Lys substitution in the acidic loop of major histocompatibility complex class I α 3 domain distinguishes low avidity CD8 coreceptor and avidity-enhanced CD8 accessory functions. *J. Exp. Med.* 184: 1671–1683.
2. O'Rourke, A. M., and M. F. Mescher. 1992. Cytotoxic T-lymphocyte activation involves a cascade of signaling and adhesion events. *Nature* 358: 253–255.
3. O'Rourke, A. M., J. Rogers, and M. F. Mescher. 1990. Activated CD8 binding to class I protein mediated by the T-cell receptor results in signaling. *Nature* 346: 187–189.
4. Durairaj, M., R. Sharma, J. C. Varghese, and K. P. Kane. 2003. Requirement for Q226, but not multiple charged residues, in the class I MHC CD loop/D strand for TCR-activated CD8 accessory function. *Eur. J. Immunol.* 33: 676–684.
5. Lustgarten, J., T. Waks, and Z. Eshhar. 1991. CD4 and CD8 accessory molecules function through interactions with major histocompatibility complex molecules which are not directly associated with the T cell receptor-antigen complex. *Eur. J. Immunol.* 21: 2507–2515.
6. Mescher, M. F. 1992. Surface contact requirements for activation of cytotoxic T lymphocytes. *J. Immunol.* 149: 2402–2405.
7. Kane, K. P., and M. F. Mescher. 1993. Activation of CD8-dependent cytotoxic T lymphocyte adhesion and degranulation by peptide class I antigen complexes. *J. Immunol.* 150: 4788–4797.
8. Hogg, N., R. Henderson, B. Leitinger, A. McDowall, J. Porter, and P. Stanley. 2002. Mechanisms contributing to the activity of integrins on leukocytes. *Immunol. Rev.* 186: 164–171.
9. Jensen, P. L., and M. F. Mescher. 2001. Role of phosphoinositide 3-kinase in TCR-signaled regulation of CD8-mediated adhesion to class I MHC protein. *Eur. J. Immunol.* 31: 3612–3621.
10. O'Rourke, A. M., J. R. Apgar, K. P. Kane, E. Martz, and M. F. Mescher. 1991. Cytoskeletal function in CD8- and T cell receptor-mediated interaction of cytotoxic T lymphocytes with class I protein. *J. Exp. Med.* 173: 241–249.
11. Kaech, S. M., E. J. Wherry, and R. Ahmed. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nat. Rev.* 2: 251–262.
12. Shimizu, Y., W. Newman, T. V. Gopal, K. J. Horgan, N. Graber, L. D. Beall, G. A. van Seventer, and S. Shaw. 1991. Four molecular pathways of T cell adhesion to endothelial cells: roles of LFA-1, VCAM-1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. *J. Cell Biol.* 113: 1203–1212.
13. Norment, A. M., R. D. Salter, P. Parham, V. H. Engelhard, and D. R. Littman. 1988. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature* 336: 79–81.
14. Rosenstein, Y., S. Ratnofsky, S. J. Burakoff, and S. H. Herrmann. 1989. Direct evidence for binding of CD8 to HLA class I antigens. *J. Exp. Med.* 169: 149–160.
15. Sun, J., D. J. Leahy, and P. B. Kavathas. 1995. Interaction between CD8 and major histocompatibility complex (MHC) class I mediated by multiple contact surfaces that include the α 2 and α 3 domains of MHC class I. *J. Exp. Med.* 182: 1275–1280.
16. DiSanto, J. P., L. A. Terry, and N. Flomenberg. 1991. Generation of anti-human CD8 β-specific antibodies using transfectants expressing mixed-species CD8 heterodimers. *J. Immunol. Methods* 141: 123–131.
17. Nagasawa, R., J. Gross, O. Kanagawa, K. Townsend, L. L. Lanier, J. Chiller, and J. P. Allison. 1987. Identification of a novel T cell surface disulfide-bonded dimer distinct from the α/β antigen receptor. *J. Immunol.* 138: 815–824.
18. Chang, C. S., E. T. Silver, and K. P. Kane. 1999. Generation of a monoclonal antibody that recognizes a polymorphic epitope of C57BL/6 Ly-49G2. *Hybridoma* 18: 423–429.
19. Kung, P., G. Goldstein, E. L. Reinherz, and S. F. Schlossman. 1979. Monoclonal antibodies defining distinctive human T cell surface antigens. *Science* 206: 347–349.
20. McMichael, A. J., P. Parham, N. Rust, and F. Brodsky. 1980. A monoclonal antibody that recognizes an antigenic determinant shared by HLA A2 and B17. *Hum. Immunol.* 1: 121–129.
21. Brodsky, F. M., P. Parham, C. J. Barnstable, M. J. Crumpton, and W. F. Bodmer. 1979. Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* 47: 3–61.
22. Ellis, S. A., C. Taylor, and A. McMichael. 1982. Recognition of HLA-B27 and related antigen by a monoclonal antibody. *Hum. Immunol.* 5: 49–59.
23. Burns, G. F., A. W. Boyd, and P. C. Beverley. 1982. Two monoclonal anti-human T lymphocyte antibodies have similar biologic effects and recognize the same cell surface antigen. *J. Immunol.* 129: 1451–1457.
24. Kane, K. P., P. Champoux, and M. F. Mescher. 1989. Solid-phase binding of class I and II MHC proteins: immunoassay and T cell recognition. *Mol. Immunol.* 26: 759–768.
25. Finegold, I., J. L. Fahey, and H. Granger. 1967. Synthesis of immunoglobulins by human cell lines in tissue culture. *J. Immunol.* 99: 839–848.
26. Heibein, J. A., M. Barry, B. Motyka, and R. C. Bleackley. 1999. Granzyme B-induced loss of mitochondrial inner membrane potential ($\delta\ \psi$ m) and cytochrome c release are caspase independent. *J. Immunol.* 163: 4683–4693.
27. Storkus, W. J., D. N. Howell, R. D. Salter, J. R. Dawson, and P. Cresswell. 1987. NK susceptibility varies inversely with target cell class I HLA antigen expression. *J. Immunol.* 138: 1657–1659.
28. Llano, M., M. Guma, M. Ortega, A. Angulo, and M. Lopez-Botet. 2003. Differential effects of US2, US6 and US11 human cytomegalovirus proteins on HLA class Ia and HLA-E expression: impact on target susceptibility to NK cell subsets. *Eur. J. Immunol.* 33: 2744–2754.
29. Appay, V., and S. L. Rowland-Jones. 2004. Lessons from the study of T-cell differentiation in persistent human virus infection. *Semin. Immunol.* 16: 205–212.
30. Mescher, M. F., A. M. O'Rourke, P. Champoux, and K. P. Kane. 1991. Equilibrium binding of cytotoxic T lymphocytes to class I antigen. *J. Immunol.* 147: 36–41.
31. Dustin, M. L., and T. A. Springer. 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 341: 619–624.
32. Devine, L., D. Thakral, S. Nag, J. Dobbins, M. E. Hodsdon, and P. B. Kavathas. 2006. Mapping the binding site on CD8 β for MHC class I reveals mutants with enhanced binding. *J. Immunol.* 177: 3930–3938.
33. Robertson, L. K., L. R. Mireau, and H. L. Ostergaard. 2005. A role for phosphatidylinositol 3-kinase in TCR-stimulated ERK activation leading to paxillin phosphorylation and CTL degranulation. *J. Immunol.* 175: 8138–8145.
34. O'Rourke, A. M., H. Shao, and J. Kaye. 1998. A role for p21ras/MAP kinase in TCR-mediated activation of LFA-1. *J. Immunol.* 161: 5800–5803.
35. Cannon, J. L., and J. K. Burkhardt. 2002. The regulation of actin remodeling during T-cell-APC conjugate formation. *Immunol. Rev.* 186: 90–99.
36. Moody, A. M., D. Chui, P. A. Reche, J. J. Priatel, J. D. Marth, and E. L. Reinherz. 2001. Developmentally regulated glycosylation of the CD8$\alpha\beta$ coreceptor stalk modulates ligand binding. *Cell* 107: 501–512.
37. Daniels, M. A., L. Devine, J. D. Miller, J. M. Moser, A. E. Lukacher, J. D. Altman, P. Kavathas, K. A. Hogquist, and S. C. Jameson. 2001. CD8 binding to MHC class I molecules is influenced by T cell maturation and glycosylation. *Immunity* 15: 1051–1061.
38. Kao, C., M. M. Sandau, M. A. Daniels, and S. C. Jameson. 2006. The sialyltransferase ST3Gal-I is not required for regulation of CD8-class I MHC binding during T cell development. *J. Immunol.* 176: 7421–7430.
39. Galvan, M., K. Murali-Krishna, L. L. Ming, L. Baum, and R. Ahmed. 1998. Alterations in cell surface carbohydrates on T cells from virally infected mice can

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distinguish effector/memory CD8$^+$ T cells from naive cells. *J. Immunol.* 161: 641–648.
40. Casabo, L. G., C. Mamalaki, D. Kioussis, and R. Zamoyska. 1994. T cell activation results in physical modification of the mouse CD8 β chain. *J. Immunol.* 152: 397–404.
41. Robinson, A. T., N. Miller, and D. R. Alexander. 1993. CD3 antigen-mediated calcium signals and protein kinase C activation are higher in CD45R0$^+$ than in CD45RA$^+$ human T lymphocyte subsets. *Eur. J. Immunol.* 23: 61–68.
42. Purbhoo, M. A., J. M. Boulter, D. A. Price, A. L. Vuidepot, C. S. Hourigan, P. R. Dunbar, K. Olson, S. J. Dawson, R. E. Phillips, B. K. Jakobsen, et al. 2001. The human CD8 coreceptor effects cytotoxic T cell activation and antigen sensitivity primarily by mediating complete phosphorylation of the T cell receptor ζ chain. *J. Biol. Chem.* 276: 32786–32792.
43. Guarda, G., M. Hons, S. F. Soriano, A. Y. Huang, R. Polley, A. Martin-Fontecha, J. V. Stein, R. N. Germain, A. Lanzavecchia, and F. Sallusto. 2007. L-selectin-negative CCR7$^-$ effector and memory CD8$^+$ T cells enter reactive lymph nodes and kill dendritic cells. *Nat. Immunol.* 8: 743–752.
44. Devine, L., M. E. Hodsdon, M. A. Daniels, S. C. Jameson, and P. B. Kavathas. 2004. Location of the epitope for an anti-CD8α antibody 53.6.7 which enhances CD8α-MHC class I interaction indicates antibody stabilization of a higher affinity CD8 conformation. *Immunol. Lett.* 93: 123–130.
45. Kern, P. S., M. K. Teng, A. Smolyar, J. H. Liu, J. Liu, R. E. Hussey, R. Spoerl, H. C. Chang, E. L. Reinherz, and J. H. Wang. 1998. Structural basis of CD8 coreceptor function revealed by crystallographic analysis of a murine CD8$\alpha\alpha$ ectodomain fragment in complex with H-2Kb. *Immunity* 9: 519–530.
46. Arcaro, A., C. Gregoire, T. R. Bakker, L. Baldi, M. Jordan, L. Goffin, N. Boucheron, F. Wurm, P. A. van der Merwe, B. Malissen, and I. F. Luescher. 2001. CD8β endows CD8 with efficient coreceptor function by coupling T cell receptor/CD3 to raft-associated CD8/p56(lck) complexes. *J. Exp. Med.* 194: 1485–1495.
47. Pang, D. J., A. C. Hayday, and M. J. Bijlmakers. 2007. CD8 Raft localization is induced by its assembly into CD8α β heterodimers, not CD8α α homodimers. *J. Biol. Chem.* 282: 13884–13894.
48. Naeher, D., I. F. Luescher, and E. Palmer. 2002. A role for the α-chain connecting peptide motif in mediating TCR-CD8 cooperation. *J. Immunol.* 169: 2964–2970.
49. Doucey, M. A., L. Goffin, D. Naeher, O. Michielin, P. Baumgartner, P. Guillaume, E. Palmer, and I. F. Luescher. 2003. CD3 δ establishes a functional link between the T cell receptor and CD8. *J. Biol. Chem.* 278: 3257–3264.
50. Yachi, P. P., J. Ampudia, N. R. Gascoigne, and T. Zal. 2005. Nonstimulatory peptides contribute to antigen-induced CD8-T cell receptor interaction at the immunological synapse. *Nat. Immunol.* 6: 785–792.
51. Anikeeva, N., T. Lebedeva, A. R. Clapp, E. R. Goldman, M. L. Dustin, H. Mattoussi, and Y. Sykulev. 2006. Quantum dot/peptide-MHC biosensors reveal strong CD8-dependent cooperation between self and viral antigens that augment the T cell response. *Proc. Natl. Acad. Sci. USA* 103: 16846–16851.
52. Wyer, J. R., B. E. Willcox, G. F. Gao, U. C. Gerth, S. J. Davis, J. I. Bell, P. A. van der Merwe, and B. K. Jakobsen. 1999. T cell receptor and coreceptor CD8 $\alpha\alpha$ bind peptide-MHC independently and with distinct kinetics. *Immunity* 10: 219–225.
53. Tsomides, T. J., A. Aldovini, R. P. Johnson, B. D. Walker, R. A. Young, and H. N. Eisen. 1994. Naturally processed viral peptides recognized by cytotoxic T lymphocytes on cells chronically infected by human immunodeficiency virus type 1. *J. Exp. Med.* 180: 1283–1293.
54. Cox, A. L., J. Skipper, Y. Chen, R. A. Henderson, T. L. Darrow, J. Shabanowitz, V. H. Engelhard, D. F. Hunt, and C. L. Slingluff, Jr. 1994. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science* 264: 716–719.
55. Purbhoo, M. A., D. J. Irvine, J. B. Huppa, and M. M. Davis. 2004. T cell killing does not require the formation of a stable mature immunological synapse. *Nat. Immunol.* 5: 524–530.
56. Sykulev, Y., M. Joo, I. Vturina, T. J. Tsomides, and H. N. Eisen. 1996. Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity* 4: 565–571.

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