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Significant Differences in Antigen-induced Transendothelial Migration of Human CD8 and CD4 T Effector Memory Cells

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Abstract

Objective—Circulating human T effector memory cell (T_{EM}) recognition of non-self MHC molecules on allograft endothelial cells (EC) can initiate graft rejection despite elimination of professional antigen presenting cells necessary for naïve T cell activation. Our prior studies of CD4 T_{EM} have established that engagement of the T cell receptor (TCR) not only activates T cells but also triggers transendothelial migration (TEM) by a process that is distinct from that induced by activating chemokine receptors (CR) on T cells, being slower, requiring microtubule organizing center (MTOC)-directed cytolytic granule polarization to and release from the leading edge of the T cell, and requiring engagement of proteins of the EC lateral border recycling compartment (LBRC). While CD4 T_{EM} may contribute to acute allograft rejection, the primary effectors are alloreactive CD8 T_{EM}. Whether and how TCR engagement affects TEM of human CD8 T_{EM} is unknown.

Approach and Results—We modeled TEM of CD8 T_{EM} across cultured human microvascular EC engineered to present superantigen under conditions of venular shear stress *in vitro* in a flow chamber. Here we report that TCR engagement can also induce TEM of this population that similarly differs from CR-driven TEM with regard to kinetics, morphological manifestations, and MTOC dynamics as with CD4 T_{EM}. However, CD8 T_{EM} do not require either cytolytic granule release or interactions with proteins of the LBRC.

Conclusions—These results imply that therapeutic strategies designed to inhibit TCR-driven recruitment based on targeting granule release or components of the LBRC will not affect CD8 T_{EM} and are unlikely to block acute rejection in the clinic.

Introduction

Allogeneic transplantation is the most effective treatment for many end-stage organ diseases. Facilitated by modern immunosuppressive regimens, acute allograft rejection rates have fallen dramatically, but have not been completely eliminated. Unlike typical laboratory rodents, adult humans have a high frequency of alloreactive T effector memory cells (T_{EM}) in their circulation and the pre-transplant frequency of donor-specific memory T cells correlates with risk of acute rejection episodes [1,2]. Allograft rejection by memory T cells can

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occur despite depletion of professional antigen presenting cells (APC) from the graft [3] or a need to prime the host immune response in secondary lymphoid organs [4,5]. Moreover, T_{EM} are more difficult to suppress than naïve T cells [6,7]. Thus, it is important to understand how human T_{EM} sense and are recruited to an allograft to further reduce rejection rates.

We have previously shown that human CD4 and CD8 T_{EM} can be activated by direct recognition of allogeneic class II and class I MHC molecule presentation, respectively, by cultured human endothelial cells (EC) [8] and that human EC may be rejected by adoptively transferred allogeneic T cells *in vivo* in immunodeficient mouse hosts [9,10]. In vitro, EC presentation of antigen to CD4 T_{EM} under conditions of flow not only causes T cell activation, but also induces transendothelial migration (TEM), a model of T cell recruitment [11]. Remarkably, this process shares many more features of the interactions of a T cell with an APC than it does with conventional chemotaxis or haptotaxis. Specifically, in response to TCR engagement, CD4 T_{EM} round up instead of flattening out and move their microtubule organizing center (MTOC) and cytosolic granules to the region of contact with the EC rather than into a trailing uropod. Unexpectedly, degranulation proved to be a necessary step in the TEM process, apparently requiring extracellular granzyme A activity to successfully cross the EC monolayer [12]. TEM itself begins by pushing a thick cytoplasmic foot-like process, that we have called a transendothelial protrusion (TEP), between adjacent EC. The nucleus then follows the MTOC into the TEP as TEM proceeds [13]. The transmigrating T cell engages the EC via LFA-1 binding to endothelial ICAM-1 as well as interactions with EC proteins associated with the lateral border recycling compartment (LBRC) such as PECAM-1 (CD31), CD99, CD112 and CD155 [11,14,15]. In contrast, chemokine-stimulated TEM of CD4 T_{EM} may use either endothelial ICAM-1 or VCAM-1 and does not require interactions with proteins of the LBRC, degranulation or extracellular granzyme A activity. While CD4 T_{EM} may contribute to rejection, the rejection process appears to correlate with the presence of CD8 cytotoxic T cells [10] which may arise from CD8 T_{EM} [16]. Recently, it has been demonstrated in mice that CD8 T cells can be recruited by antigen recognition on EC, triggering rejection independent of professional APC [17]. Little is known about TEM by human CD8 T_{EM}.

In the present study, we applied our *in vitro* flow chamber model that we previously used to analyze TEM by CD4 T_{EM} to study the cell biology of human CD8 T_{EM} recruitment in response to antigen and compare this both to chemokine responses of CD8 T_{EM} and to our prior findings with CD4 T_{EM}. Unexpectedly, we find significant differences between the TCR-mediated responses of these two populations.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

We modeled TEM of CD8 T_{EM} across microvascular EC under conditions of venular shear stress *in vitro* in a flow chamber using TNF-activated, CIITA-transduced monolayers of untransformed HDMEC to present toxic shock syndrome toxin-1 (TSST-1), a superantigen,

to allogeneic T_{EM} isolated from PBMC. TSST-1 activates about 5-10% of the T cells, namely those clones which formed their TCR using a Vβ2 gene segment. This increases the frequency of responsive T cells to a level well above that activated by alloantigen (closer to 0.1%), significantly increasing the number of TCR-activated T cells available for analysis and allowing simultaneous analysis of both TCR-triggered Vβ2+ T_{EM} and Vβ2− T_{EM} responding to TNF-induced chemokines via chemokine receptors (CR) in the same field [11]. CIITA transduction is used to restore MHC class II expression lost by EC when placed in culture. While MHC class II molecules are not required for allogeneic responses of CD8 T cells, they are necessary for the presentation of superantigen to both CD4 and CD8 T cells. Shear stress is required to stimulate the rapid TEM of T cells, whether the signal is antigen or chemokine [11,18,19].

Using this assay for CD8 T_{EM}, we found this population to share many of the characteristics of CD4 T_{EM} undergoing TEM. Shortly after encounter with superantigen, ZAP70 is phosphorylated at levels well above that induced by chemokines, indicative of TCR signaling. The extent of the phosphorylation varies from cell to cell, but the ranges completely overlap between the two populations, suggesting that signal strengths within these T cell subsets are comparable (Figure 1A). In both cases, only the antigen-activated T_{EM} become circular and rounded-up rather than spread and crawling. In Vβ2+ T cells, the MTOC then locates to a position between the T cell nucleus and EC apical surface rather than trailing in the uropod, the latter being a shared feature of both CD4 and CD8 T_{EM} in response to chemokine (Figure 1B, Supplemental Figure I, and ref. [12]). By 15 minutes, TCR-activated cells start inserting transendothelial protrusions (TEPs) between and under the EC. This is followed by transit of the nucleus through the monolayer, thereby completing TCR-driven TEM. As in CD4 T_{EM}, TCR-driven TEM of CD8 T_{EM} is delayed compared to CR-driven TEM (Figure 1C and ref. [11]). Treatment of T cells with blebbistatin, an inhibitor of myosin IIA, allows TEP formation but prevents cell body transit across the monolayer during TCR-driven TEM; blebbistatin has no effect on CR-driven TEM (Figure 1D and ref. [13]). As previously observed for CD4 T_{EM}[12], the MTOC, identified by staining for γ-tubulin, precedes the nucleus across the EC monolayer during CD8 TCR-driven TEM in stark contrast to CR-driven TEM, where the MTOC trails the nucleus (Supplemental Figure 1A-C). Using NFAT translocation to the nucleus as a marker for T_{EM} activated by alloantigen, we find that the MTOC similarly precedes the nucleus in alloantigen-driven TEM of both CD4 and CD8 T_{EM} (Figure 1E).

Despite these similarities between the T cell subsets, we also observed important differences between CD4 and CD8 T_{EM}. Unlike CD4 T_{EM}, the cytosolic granules of many (but not all) CD8 T_{EM}, contain granzyme B as well as granzyme A and that when granzyme B is present, it co-localizes to the same granules as granzyme A ([20-22] and Supplemental Figure 1D). CD8 T_{EM} granules also contain perforin, a necessary component for cytolysis, which is lacking in CD4 T_{EM} ([23] and data not shown). As we had seen with CD4 T_{EM}, TCR signaling causes both the MTOC and cytosolic granules to be relocated to a region in proximity to the T cell-EC apical surface (Supplemental Figure 1). Unlike CD4 T_{EM}, however, treatment with concanamycin A, an inhibitor of vesicular H^+-ATPase on granules that thereby prevents acidification and renders them nonfunctional, failed to inhibit TCR-driven TEM of CD8 T_{EM} Figure 2A). In the presence of the serine protease inhibitor AEBSF, neither CR- nor

TCR-driven TEM of CD8 T_{EM} was inhibited, in contrast to TCR-driven TEM of CD4 T_{EM} (Figure 2B). Furthermore, again in contrast to our prior studies of CD4 T_{EM} [12] and experiments repeated here in parallel, quantification of granzyme A+ granules in T cells that receive TCR signals indicates that degranulation does not occur during TCR-driven TEM of CD8 T_{EM} (Figure 2C). Interestingly, concanamycin A did inhibit CR-driven TEM of CD8, but not CD4 T_{EM}, to a limited extent (Figure 2A).

We next interrogated whether components of the LBRC were involved in TEM of CD8 T_{EM}. Such interactions have been shown to be essential for human neutrophil and monocyte TEM [24]. While our prior studies showed that CD4 T_{EM} did not utilize LBRC proteins in response to chemokines, they did require their use when TEM was induced by TCR signals [14,15]. Using approaches documented previously and repeated in parallel here, knockdown of PECAM-1 (CD31, knockdown confirmed by immunofluorescence staining) or blocking antibodies to CD112 and CD155 (on EC) or CD96 (on T cells) affected CD4 T_{EM} TCR-driven TEM, but had no effect on CD8 T_{EM} TEM in response to chemokine or antigen (Figure 3A-C). Furthermore, treatment of EC with an inhibitor of soluble adenylyl cyclase, an intracellular enzyme that facilitates leukocyte TEM by mobilizing vesicles of the LBRC to the plasma membrane [25], selectively inhibited TCR-driven TEM of CD4 T_{EM} (Figure 3D).

We next examined the roles of other EC adhesion molecules in TEM, namely ICAM-1, VCAM-1, JAM-A, and JAM-B. TCR-driven TEM of both CD4 and CD8 T_{EM}, as well as CR-driven TEM of CD8 T_{EM}, showed a dependence on EC ICAM-1, as determined in experiments using ICAM-1 blocking mAb on the EC (Figure 4A) as well as knockdown of EC ICAM-1 by siRNA (Figure 4B, Supplemental Figure II). Blocking mAbs to or siRNA knockdown of VCAM-1 reduced CD4 T_{EM} TCR-driven TEM, and knockdown of JAM-A had a small but significant effect on CD4 T_{EM} CR-driven TEM, as reported previously [14], but none of these treatments affected CD8 T_{EM} TEM (Figure 4C-E, Supplemental Figure II). JAM-B knockdown had no effects on TEM (Figure 4F, Supplemental Figure II).

Treatment with a blocking mAb to integrin VLA-4 (α_4 subunit, CD49d), the known receptor for VCAM-1, selectively reduced CD4 T_{EM} TCR-driven TEM, while blocking mAb to integrin LFA-1 (α_L subunit, CD11a) and mAb to the common LFA-1 and Mac-1 beta subunit (β_2, CD18) effectively diminished both CD4 and CD8 T_{EM} TCR-driven TEM (Figure 5A-C). Since the mAb to LFA-1/Mac-1 appeared to be more potent than the mAb to LFA-1 in affecting CD8 T_{EM} TCR-driven TEM, we also tested a blocking mAb to CD11b, the Mac-1 alpha subunit (α_M), but found no significant effect (Figure 5D). The blocking mAbs to LFA-1 and VLA-4 had similar effects on total T_{EM} adhesion (Figure 5E). However, in contrast to CD4, CD8 T_{EM} did not show an antigen-induced increase in binding, unless treated with an integrin blocking mAb (Figure 5F).

Discussion

The fundamental points made by this study is that human CD8 T_{EM}, like CD4 T_{EM}, can be triggered to undergo a kinetically and morphologically distinct TEM process characterized by dramatic rearrangement of organelles to the leading edge of the cell, rather than to a

trailing uropod, and an invasion between adjacent ECs led by a blunt TEP. Despite these similarities, CD4 and CD8 T_{EM} TCR-driven TEM do differ in several important ways. Specifically, TCR-driven TEM of CD4 T_{EM} requires granzyme A stored in cytolytic granules to be exocytosed to traverse the endothelium in a process that requires interactions with proteins of the lateral border recycling compartment (LBRC), including PECAM-1, CD99, CD112, and CD155, with their receptors on the T cells [12,14,15]. Extracellular enzymatic activity of granzyme A is then required to permit TEM. The target of this serine protease is unknown, but it is possible that it may be needed to mobilize the LBRC to the plasma membrane. As shown here, CD8 T_{EM} do not require either degranulation or engagement of endothelial proteins of the LBRC in order to transmigrate in response to TCR signals, although there is a small but significant effect of concanamycin A on CR-driven TEM of CD8 T_{EM}. The limited effect of concanamycin on human CD8 T_{EM} appears to contradict a prior report showing that granzyme B contributed to antigen-independent transmigration of differentiated CD8 CTL [26]. These differences likely reflect a difference between CD8 T_{EM}, which are poised to become CTL, and functionally mature CTL. Only the latter degranulate in response to TCR signals. The modest effect of concanamycin A on CR-driven TEM of CD8 T that we observed could be explained by the presence of a relatively small subset of mature CTL in our freshly isolated peripheral blood human T_{EM} subsets. Moreover, analysis of freshly isolated human CD8 T is likely to be complicated by the heterogeneity of subsets within this population; recent multiparameter phenotyping by CyTOF indicates that there are at least 4 subtypes of human Tc cells, and all lack expression of CCR7, like the T_{EM} used here [27]. Nevertheless, the key point is that TCR-driven TEM of CD8 T_{EM} does not appear to be affected by reagents that affect degranulation or TEM via the LBRC, as the same reagents effectively inhibit CD4 T_{EM} in experiments performed in parallel.

The exocytosis of granules appears to be a key difference between TCR-driven TEM of CD4 and CD8 T_{EM}. It was recently shown that human memory CD4 T activated by antibodies for 24 h will actually secrete more granzyme B than CD8 T cells, even though the percentage of granzyme B-containing CD4 T cells is much lower and CD8 T cells contain more intracellular granzyme B per cell [28]. While this study compared freshly synthesized granzyme B in CD4 T cells to predominantly pre-made granzyme B in CD8 T cells, it nevertheless indicates that exocytosis of granules is under stricter control in memory CD8 T cells. With regard to transmigrating T cells, strict control of exocytosis in CD8 T_{EM} makes sense teleologically, since a relatively large number of CD8 T_{EM} contain both granzymes A and B as well as perforin and would likely kill the EC if they were to degranulate during TEM. This would be a potentially disastrous consequence if CD8 T_{EM} were being recruited to defend against a reinfection by an intracellular pathogen, the physiological role of this cell population. Interestingly, mature CTL will degranulate when encountering allogeneic EC, causing cell lysis [16,29] highlighting an important difference between CD8 CTL and CD8 T_{EM}. The molecular explanation(s) for the differences in degranulation by CD4 and CD8 T_{EM} are unknown but experiments to determine this difference are an area of active investigation. One potential explanation could be a difference in the strength and/or duration of TCR signaling; it was recently shown that TCR-induced P-ZAP70 in mouse CD8 T cells is completely dephosphorylated within 30 minutes [30]. However, our assessment of the extent

of ZAP70 phosphorylation suggests that the difference in degranulation between human CD4 and CD8 T_{EM} interacting with antigen presented by EC cannot be explained by differences in the quantitative strength or duration of signaling.

It is interesting to speculate that the lack of degranulation of CD8 T_{EM} during TCR-driven TEM and the lack of the need for interactions with proteins of the LBRC may be related. Other leukocyte cell types that utilize the LBRC for TEM are also dependent on serine proteases similar to granzyme A, e.g., elastase [31] or proteinase 3 [32]. Perhaps cleavage of an endothelial receptor by a serine protease is required to activate mobilization of the LBRC. With the exception of TCR-driven TEM by CD4 T_{EM}, T cells appear to be independent of this mechanism. Perhaps LFA-1 engagement of EC ICAM-1 provides an alternative signal.

In contrast to our earlier results [11], we now saw that VCAM-1 blocking Abs can selectively inhibit TCR-driven TEM of CD4 T_{EM}. We attribute this discrepancy to the differences among anti-VCAM-1 antibodies. The mAb used in our prior report had a lower affinity than the two mAbs used here. We believe the new finding to be correct both because we had similar effects by siRNA knock down of VCAM-1 in EC and with use of a VLA-4 blocking mAb.

Although not the main focus of this study, we also found that, unlike CD8 T_{EM}, CR-driven TEM of CD4 T_{EM} cells is not inhibited by ICAM-1 blocking antibodies. The lack of effect of ICAM-1 blockade could imply either that ICAM-1 plays no role or that it is redundant in TEM of CR-driven CD4 T_{EM}. Curiously, LFA-1 blocking Abs did have a small inhibitory effect on CR-driven TEM by CD4 T_{EM} cells. This effect of LFA-1 blocking Abs is consistent with the inhibitory effects of JAM-A knockdown, since JAM-A is an alternative ligand for LFA-1 [33]. Others have reported strong to modest effects of LFA-1 blocking Abs on TEM of freshly isolated human peripheral blood lymphocytes across cytokine-activated human umbilical vein endothelial cells in static assays, with or without a chemotactic gradient [34-36] and approximately 33% inhibition in flow assays with apically presented SDF-1α [18]; in all cases, VLA-4 blocking Abs had no effect on their own, but the combination of VLA-4 and LFA-1 blocking Abs was consistently potent. However, SDF-1α recruitment is not restricted to the T_{EM} subset and other T cell subsets may be more dependent upon LFA-1 interactions [19]. Interestingly, effector T cells generated by TCR activation and prolonged culture in IL-2 are especially dependent upon LFA-1 for TEM [37].

Not surprisingly, both LFA-1 and VLA-4 blocking Abs inhibited adhesion of CD4 and CD8 T_{EM} cells; a limited number of experiments indicated that using both LFA-1 and VLA-4 antibodies have an additive effect. A curious observation is that the contribution of TCR signals to adhesion is different between CD4 and CD8 T_{EM}. As noted previously [38] and replicated here, TCR signaling enhances adhesion of CD4 T_{EM}, i.e., the proportion of adherent T cells that are antigen specific is higher than the starting population. Such seems not to be the case for adhesion of CD8 T_{EM} to EC, although an effect could be observed in the presence of integrin blocking mAbs.

The differences between CD4 and CD8 T_{EM} detailed here has significant clinical implications, particularly with regard to T cell-mediated graft rejection, a process dependent

on the response of host T cells to the vascular endothelium of the allograft [39]. Blocking interactions of circulating T_{EM} with proteins of the LBRC that might be effective in blocking recruitment of CD4 T_{EM} may fail due to the independence of CD8 T_{EM} recruitment of these target proteins. In contrast, agents that target common features, such as antibodies to LFA-1 or ICAM-1 can reduce TCR-driven TEM of both CD4 and CD8 T_{EM} and, hence, inhibit graft rejection, but redundancy with VLA-4 (CD 49d/CD29) with VCAM-1 may still permit CR-induced recruitment. Experiments using humanized mice may help to address these questions as a complement to clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations List

CMA	concanamycin A
CR	chemokine receptor
EC	endothelial cell
LBRC	lateral border recycling compartment
MTOC	microtubule organizing center
TCR	T cell receptor
T_{EM}	effector memory T cells
TEM	transendothelial migration
TEP	transendothelial protrusion
TSST-1	toxic shock syndrome toxin 1

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Highlights

TCR engagement of alloantigen presented by human EC triggers transendothelial migration (TEM) of effector memory CD8 T cells by a process that differs from TEM triggered by chemokine receptors.

While TCR-triggered TEM of human effector memory CD8 T cells resembles that occurring in human effector memory CD4 T cells in several respects, it differs in that CD8 T cells do not require granule exocytosis nor engagement of components of the lateral border recycling compartment to undergo TEM.

Differences in effector memory T cell between TEM triggered by antigen recognition or by chemokines and between CD8 and CD4 T cells suggest that multiple therapeutic approaches will be required to effectively inhibit T cell recruitment to human allografts.

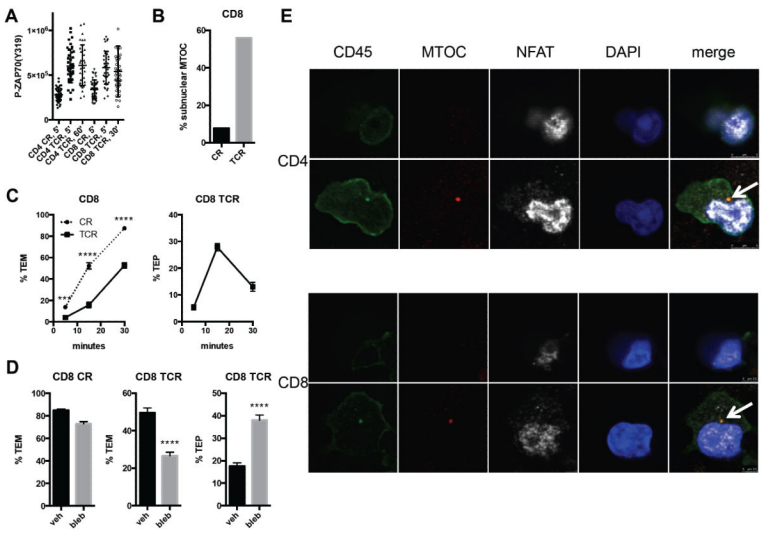

Figure 1.

Similarities between CD4 and CD8 T_{EM} TEM. **A**. ZAP70 activation during CR-driven and TCR-driven TEM of CD4 and CD8 T_{EM}. Graph shows quantification of staining for P-ZAP70(Y319) of individual CD4 T_{EM} and CD8 T_{EM} after the indicated durations of flow. CR and TCR denote chemokine- and TCR-driven TEM, respectively. P<0.0001 for all CR and TCR comparisons, and ns for all CR vs CR and TCR vs TCR comparisons. **B**. MTOC localization during CR- and TCR-driven TEM of CD8 T_{EM}. Graph shows % of MTOC localized between the T cell nucleus (sub-nuclear) and EC apical surface for CR-driven (CR) and TCR-driven (TCR) cells after 5 min flow. N=77 and 75 for CR and TCR, respectively, from 3 separate experiments with different donors. **C**. Kinetics of CR-driven and TCR-driven TEM. Left graph shows % CR-driven (circles connected by dotted lines) and TCR-driven (squares connected by solid lines) TEM of CD8 T_{EM} at 5, 15, and 30 min. Right graph shows % transendothelial protrusions (TEP) at 5, 15, and 30 min during TCR-driven TEM. Data combined from three experiments with different donors. **D**. Myosin IIA is required for TCR-driven TEM of CD8 T_{EM} at a step after TEP formation. TEM assays of CD8 T_{EM} treated with blebbistatin (bleb) or vehicle (veh). Left graph shows % TEM of CR-driven TEM, middle graph shows % TEM of TCR-driven TEM, and right graph shows % TEP of TCR-driven TEM. Data combined from three experiments with different donors. **E**. MTOC precedes the nucleus in alloantigen-driven TEM. Flow assay samples of CD4 and CD8 T_{EM} on HDMEC treated with interferon-γ 72h and TNF 20h stained for CD45 (green), γ-tubulin (MTOC), NFAT, and nuclei (DAPI). The bottom panels represent confocal slices beneath the EC monolayer, and the top panels are slices taken 1.44 μm (CD4) and 1.84 μm (CD8) above the lower panels. Note the nuclear localization of NFAT, indicative of TCR activation. The arrows indicate the MTOC positioned near the front of the nucleus; the nucleus is in the process of traversing the monolayer.

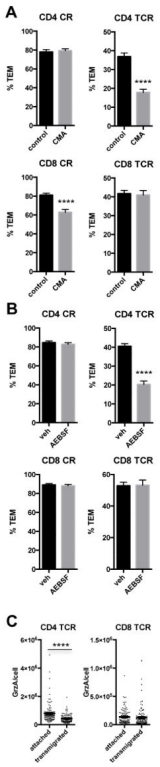

Figure 2.

Exocytosis of lytic granules is not necessary for TCR-driven TEM of CD8 T_{EM}. **A**. TEM assay of CD4 and CD8 T_{EM} treated with concanamycin A (CMA) or vehicle (veh). Upper graphs show CD4, and lower CD8. Left graphs show % TEM of CR-driven TEM, and right graphs show % TEM of TCR-driven TEM. Data combined from three experiments with different donors. **B.** Graphs show %TEM of assays performed in the presence of serine protease inhibitor AEBSF. **C**. Quantification of granzyme A in cells responding to antigen after 2 min flow (attached) and after transmigration. Graphs show granzyme A content/cell measured from pictures taken of stained cells; exposure times were 800 and 50 milliseconds for CD4 and CD8, respectively, due to stronger staining of CD8. Data combined from three experiments with different donors. Horizontal and vertical bars within the dots represent mean +/− SEM, respectively. P=0.44 for CD8.

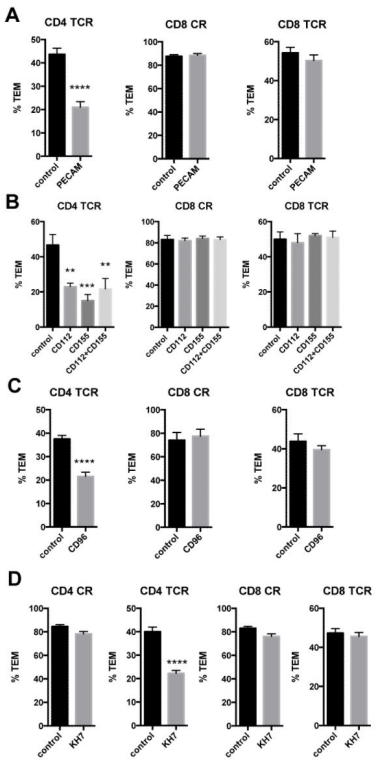

Figure 3.

Components of the lateral border recycling compartment are not necessary for TCR-driven TEM of CD8 T_{EM}. **A**. TEM assays on EC treated with control and PECAM-1 siRNA. Graphs show, from left to right, % TEM of CD4 TCR-driven TEM, CD8 CR-driven TEM and CD8 TCR-driven TEM. Data combined from three experiments with different donors. **B**. TEM assays on EC treated with blocking antibodies to CD112 and CD155. Left graph shows % TEM of CD4 TCR-driven TEM, middle graph shows % TEM of CD8 CR-driven TEM, and right graph shows % TEM of CD8 TCR-driven TEM. One representative experiment of three with different donors. **C**. TEM assays of T_{EM} treated with blocking antibody to CD96. Left graph shows % TEM of CD4 TCR-driven TEM, middle graph shows % TEM of CD8 CR-driven TEM, and right graph shows % TEM of CD8 TCR-driven TEM. Data combined from three experiments with different donors. **D**. TEM assays of T_{EM} on EC treated with soluble adenylyl cyclase inhibitor KH7. Graphs show, from left to right, % TEM of CD4 CR-driven, CD4 TCR-driven, CD8 CR-driven, and CD8 TCR driven TEM. Data combined from four experiments with different donors.

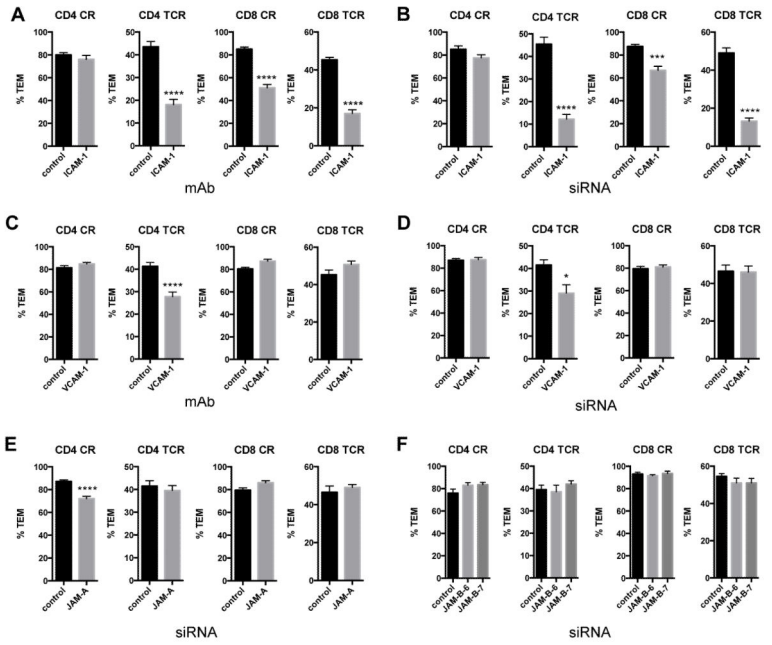

Figure 4.

ICAM-1, but not VCAM-1, is necessary for CR- and TCR-driven TEM of CD8 T_{EM}. **A**. TEM assays of T_{EM} on EC treated with control IgG (control) and blocking antibodies to ICAM-1 (ICAM-1). From left to right, graphs show % TEM of CD4 CR-driven TEM, CD4 TCR-driven TEM, CD8 CR-driven TEM and CD8 TCR-driven TEM. Data combined from two (CD4) and three (CD8) experiments with different donors. **B**. TEM assays of T_{EM} on EC transfected with control and ICAM-1 siRNA. Graphs are in the same order as in panel A. Data combined from 3 experiments with different donors. **C**. TEM assays of T_{EM} on EC treated with control IgG (control) and blocking antibodies to VCAM-1. From left to right, graphs show % TEM of CD4 CR-driven TEM, CD4 TCR-driven TEM, CD8 CR-driven TEM and CD8 TCR-driven TEM. Data combined from three experiments with different donors. **D**. TEM assays of T_{EM} on EC transfected with control and VCAM-1 siRNA. Graphs are in the same order as in panel C. Data combined from two experiments with different donors. E. TEM assays of T_{EM} on EC transfected with control and JAM-A siRNA. Data combined from two experiments with different donors. F. TEM assays of T_{EM} on EC transfected with control and JAM-B siRNAs. Data combined from two experiments with different donors.

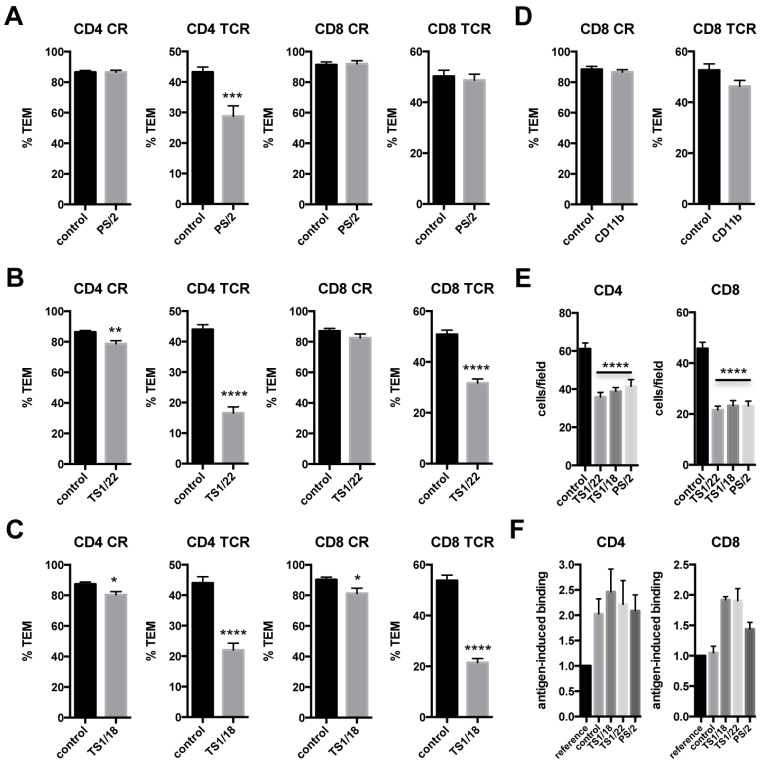

Figure 5.

Both CD4 and CD8 T_{EM} TCR-driven TEM are inhibited by LFA-1 blocking mAbs. A. TEM assays in the presence of VLA-4 blocking mAb clone PS/2. Graphs show % TEM of data combined from 3 experiments. B. TEM assays in the presence of LFA-1 blocking mAb clone TS1/22. Graphs show % TEM of data combined from 5 experiments with different donors. C. TEM assays in the presence of LFA-1/Mac-1 blocking mAb clone TS1/18. Graphs show % TEM of data combined from at least 3 experiments with different donors. D. TEM assays in the presence of Mac-1 (CD11b) blocking mAb. Graphs show % TEM of data combined from 3 experiments with different donors. E. Adhesion of CD4 and CD8 T_{EM}. Graphs show the combined raw data of 3 (CD4) and 2 (CD8) experiments with different donors. F. Antigen-induced binding of CD4 and CD8 T_{EM}. Graphs show fold enrichment of antigen-specific T cells attached compared to the input (reference) population, mean and sem from 3 experiments with different donors.