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Dynamic Differentiation of Activated Human Peripheral Blood CD8\(^+\) and CD4\(^+\) Effector Memory T Cells

Jochen Schwendemann, Carmen Choi, Volker Schirrmacher, and Philipp Beckhove

Two functionally different memory T cell subsets were originally defined based on their different CCR7 expression profile, but the lineage relationship between these subsets referred to as central memory T cells (T\(_{CM}\)) and effector memory T cells (T\(_{EM}\)) is not resolved. A prevalent model proposes a linear progressive differentiation from T\(_{CM}\) to T\(_{EM}\). Our results demonstrate that on activation, human CCR7\(^-\)CD62L\(^-\) peripheral blood CD8\(^+\) and CD4\(^+\) T\(_{EM}\) cells exhibit a dynamic differentiation, involving transient as well as stable changes to T\(_{CM}\) phenotype and properties. Whereas the larger fraction of T\(_{EM}\) cells increases expression of effector molecules, such as perforin or IFN-\(\gamma\), a smaller fraction first acquires CCR7 expression. We demonstrate that this acquisition of lymph node homing potential is associated with strong proliferation similar to that of activated T\(_{CM}\) cells. After proliferation, most of these cells lose CCR7 expression again and acquire effector functions (e.g., perforin production). A small proportion (\(\sim 6\%\)), however, maintain phenotypic and functional T\(_{CM}\) properties over a long time interval. These results suggest that T\(_{EM}\) cells provide immediate effector function by a fraction of cells as well as self-renewal by others through up-regulation of CCR7 followed by either secondary peripheral effector function or long term maintenance of T\(_{CM}\)-like properties. The Journal of Immunology, 2005, 175: 1433–1439.

Since the identification of memory T cell subsets, referred to as central memory T cells (T\(_{CM}\)),\(^2\) expressing the chemokine receptor CCR7, and effector memory T cells (T\(_{EM}\)), which lack the expression of this chemokine receptor (1), the question of their lineage relationship is not resolved. CD4\(^+\) and CD8\(^+\) T cells apparently manifest a signal strength-dependent differentiation program (2–7), in which the strength of signals delivered by TCR and cytokine receptors drives T cells through hierarchical thresholds of differentiation (8).

According to the progressive linear differentiation hypothesis (8), differentiation involves a phase of proliferation preceding the acquisition of fitness and effector function. Because T cell stimulation is a stochastic event, not all T cells receive the identical strength of signals. Therefore, primed T cells reach a variety of differentiation stages that contain effector cells as well as cells that have been arrested at intermediate levels of differentiation. These intermediates retain expression of lymph node homing receptors such as CCR7 and CD62L and have initiated but not completed the remodeling events of genes involved in effector function (e.g., IL-4, IL-5, IFN-\(\gamma\), perforin). They thus retain a flexible gene imprinting. Cells that may survive after the retraction phase of an immune response can be resolved into distinct subsets of either T\(_{EM}\) representing cells at intermediate levels of differentiation or fully differentiated memory T cells with effector capacity (T\(_{EM}\)). Reactivation of cells arrested at intermediate stages should lead to a progression of gene remodeling and imprinting. Consequently, differentiation of stimulated memory T cells was proposed to follow a linear process from T\(_{CM}\) to T\(_{EM}\) (1, 8–10).

There is, however, growing evidence that a differentiation pathway from T\(_{EM}\) to T\(_{CM}\) may also occur, given that stimulation of sorted HIV-specific CD8\(^+\) T\(_{EM}\) cells induced their CCR7 expression (11). Similarly, stimulation of CD4\(^+\) T\(_{EM}\) cells from healthy individuals induced a transient expression of CCR7 (12). This phenotypic change suggests a T\(_{CM}\) conversion associated with migration to lymph nodes, but data resolving the functional implications of this change are still missing. Investigations performed in a mouse model analyzing the differentiation process of Ag-specific CD8\(^+\) T\(_{EM}\) and T\(_{CM}\) cells in vivo suggest a differentiation from T\(_{EM}\) to T\(_{CM}\) cells after adoptive transfer (13).

We decided to isolate memory subsets based on two markers, so that CCR7\(^-\)CD62L\(^-\) double-negative cells represent T\(_{EM}\) and CCR7\(^+\)CD62L\(^+\) double-positive T\(_{CM}\).

We undertook this study to investigate the potential functional implications of CCR7 up-regulation in T\(_{EM}\) cell subsets. We show that human peripheral blood CD8\(^+\) and CD4\(^+\) T\(_{EM}\) cells activated by high signal strength through anti-CD3/CD28 stimulation exhibit a dynamic differentiation. Whereas a majority of the cells immediately produced perforin or IFN-\(\gamma\) but showed little proliferation, a smaller subset acquired phenotypic and functional characteristics of T\(_{CM}\) cells characterized by expression of CCR7 and associated with strong proliferation and little effector potential. After proliferation, most of these cells lost CCR7 expression again and reacquired a T\(_{EM}\)-like functional capacity characterized by low proliferation but strong perforin production. A proportion of \(\sim 6\%\) of the CCR7\(^+\) T\(_{EM}\), however, maintained T\(_{CM}\) properties (CCR7 expression and high proliferative potential) for the long term.

These data suggest a flexible differentiation of activated T\(_{EM}\) cells that in parallel allows execution of immediate effector function, extensive expansion followed by a secondary effector phase, as well as stable maintenance of self-renewing capacity.

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Materials and Methods

Donors

Peripheral blood of healthy normal donors was used after informed consent.
Monoclonal Abs

The following mouse anti-human mAbs were used: aliphosphocyanin-Cy7- or PE-Cy5-CD8 (clone 3B5; Caltag; clone HIT8a, BD Biosciences); allophycocyanin-Cy7-CD4 (clone S3.5; Caltag Laboratories); allophycocyanin-CD45RA (clone MEM-56; Caltag Laboratories); FITC-CD62L (clone Dreg56; BD Biosciences); IFN-γ-FITC (clone 4S.B3; BD Biosciences); biotin-CCR7 (clone 3D12; BD Biosciences) together with streptavidin-PE (BD Biosciences) or streptavidin-PE-Cy7 (Caltag Laboratories); FITC- or PE-Ki67 (clone B6; BD Biosciences); and FITC- or PE-perforin (clone 6G9; BD Biosciences).

Isolation of $T_{EM}$ and $T_{CM}$ cells

Mononuclear cells from peripheral blood of healthy donors were separated by Ficoll gradient (Biochrom) centrifugation. Endobulin (Baxter)-treated PBMCs were then stained with mouse anti-human mAbs against CD8, CD4, CD45RA, CD62L, and CCR7. $T_{EM}$ cells (CD8$^+$CD4$^+$CD45RA$^-$CD62L$^-$CCR7$^-$) and $T_{CM}$ cells (CD8$^+$CD4$^+$CD45RA$^+$CD62L$^+$CCR7$^+$) were isolated using a FACSVantage SE (BD Biosciences) with CellQuest Pro software (version 4.0.2; BD Biosciences). Sorted memory T cell subsets were subsequently analyzed for CCR7 and CD62L expression without restaining using the same FACS and settings as during cell separation. Only such cell preparations that exhibit a purity of at least 98% were selected.

Separation of CCR7$^+$ $T_{EM}$ cells

Either 2 or 28 days after anti-CD3/CD28 stimulation, cultured $T_{EM}$ cells were stained with mouse anti-human mAbs against CCR7. CCR7$^+$ $T_{EM}$ cells were then isolated using a FACSVantage SE with CellQuest Pro software.

Cell culture

Isolated $T_{EM}$ and $T_{CM}$ cells were transferred into 24- or 48-well plates (TPP and Corning Costar) and cultured in X-VIVO 20 (Cambrex) supplemented with 10% human AB serum (Sigma-Aldrich), 100 U/ml recombinant human IL-2 (Promocell), 50 ng/ml Zienam (MSD), 50 ng/ml Erythromycin (Abbott), 50 ng/ml vancomycin (Abbott), and 2.5 μg/ml amphoterin B (Invitrogen). The medium was changed every 2 days.

Proliferation assay

For thymidine incorporation assay, cells were activated by incubation on petri dishes coated with 1 μg/ml anti-CD3 (clone OKT3; American Type Culture Collection) and 1 μg/ml anti-CD28 (clone 9.3; DFKZ-Heidelberg) mAbs for 18 h. Afterward, triplicates of the cells were transferred into 96-well plates and cultured for 17 h as described above. The medium was supplemented with 1 μCi/well $[^{3}H]$thymidine. Thymidine incorporation by proliferating cells was measured using a 1205 liquid scintillation counter (PerkinElmer).

Results

Isolation and characterization of $T_{EM}$ and $T_{CM}$ cell subsets

Gated CD8$^+$ and CD4$^+$ memory T cells from the peripheral blood of healthy donors were analyzed for CCR7 and CD62L expression to define CCR7$^+$CD62L$^-$ $T_{EM}$ and CCR7$^+$CD62L$^+$ $T_{CM}$ cell subsets. Proportions of $T_{CM}$ and $T_{EM}$ cell subsets from peripheral blood of healthy donors were separated by FACS. One representative dot plot of 14 experiments is shown (A). D. Proportions of $T_{CM}$ (■), $T_{EM}$ (□), and other memory subsets (CD62L$^+$CCR7$^-$, or CD62L$^-$/CCR7$^-$; □ among CD8$^+$ or CD4$^+$ memory T cell populations from PBMCs ex vivo displayed as means and SD of 14 healthy donors.

Intracellular staining

Intracellular staining with mouse anti-human mAbs against Ki-67, perforin or IFN-γ were performed after permeabilization and fixation of sorted cells with a Cytofix/Cytoperm Kit (BD Biosciences) according to the manufacturer’s protocol. Isotype controls were performed for all applied intracellular stainings as suggested by the mAb suppliers.

FACS

Sorted $T_{EM}$ and $T_{CM}$ cells ($5 \times 10^5$) were first treated with human Ig (Endobulin) to block unspecific binding sites. Staining was performed using the above listed mouse anti-human mAbs. Cells were measured by using a FACSScan (BD Biosciences) or FACSVantage SE with CellQuest software (version 3.3 or version 4.0.2). Analysis of data was performed using FlowJo software (version 4.3; Tree Star).

CFSE staining

For CFSE staining, cells were washed in PBS (Biochrom) supplemented with 0.1% BSA (Sigma-Aldrich). Afterward, cells were incubated with 2 μM CFSE (Invitrogen) for 10 min at room temperature; then the cells were washed in X-VIVO 20 medium containing 10% AB serum and cultured as described above.
CD8+ T_EM

Stimulation:
- anti CD3/CD28

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CD4+ T_EM

Stimulation:
- anti CD3/CD28

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C

CD4+78+, day 4

CD4+78+; day 4

CD62L

CCR7+ cells [%]

D

day 4

CD4+78+, day 4

CD4+78+; day 4

CD62L

CCR7+ cells [%]

E

CD8+ T_EM

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CD8+ T_EM

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CCR7+ CD62L of CCR7+ T_EM

FIGURE 2. CCR7 and CD62L expression profile of stimulated CD8+ and CD4+ T_EM cells. Anti-CD3/CD28 stimulated T_EM cells were gated on CD8+ (A) and CD4+ (B) populations and analyzed for CCR7 expression. Cumulative data of CCR7 expression of CD8+ CCR7+ T_EM cells at various time points after stimulation. CCR7 expression of unstimulated populations is depicted as control (■). Proportions of CCR7+ T_EM cells are shown as means and SD of healthy donors (n = 3). C and D. CD62L expression by stimulated T_EM 4 days after stimulation. C. One representative dot plot; D. Cumulative data of three donors. E. CCR7+ T_EM cells were isolated by FACs 2 days after anti-CD3/CD28 stimulation (blue-outlined square; cells referred to as d2-T_EMCCR7+). Cumulative data of CCR7+ T_EM cells acquire a stable CCR7 expression profile after activation

Fractions of T_EM cells acquire a stable CCR7 expression profile after activation

It has been shown that activated T_EM cells acquire CCR7 expression (11) or exhibit a transient expression of this chemokine receptor (12). However, there are as yet no data available concerning the precise kinetics and maintenance of CCR7 expression of separated CD8+ T_EM cells of healthy individuals after stimulation. Therefore, we tested the CCR7 expression profile of purified T_EM cell populations during a course of 28 days after a single bead-mediated anti-CD3/CD28 stimulation (Fig. 2, A and B). The proportion of CCR7 expressing T_EM cells continuously increased, peaked at day 3, and declined to ~5% at day 7 after activation. CCR7 up-regulation was induced by TCR stimulation, because nonstimulated T_EM subsets showed only little CCR7 induction after 1–2 days (Fig. 2, A and B) or 4–7 days of culture (data not shown). Similar to an up-regulation of CCR7, a concomitant induction of CD62L was detected in 70–80% of CCR7+ T_EM cells until day 4 after stimulation (Fig. 2, C and D). The decline of CCR7 expression was due to CCR7 down-regulation rather than to loss of CCR7+ cells, because CCR7+ T_EM cells sorted 2 days after activation adjusted the proportion of CCR7+ cells until day 7 to levels similar to those detected in the peripheral blood without major loss of cell numbers (Fig. 2E). A fraction of ~6% CCR7+ cells within the T_EM population was detectable up to 28 days after stimulation (mean, 5.8 ± 0.6%). Their proportion was similar to that of CCR7+ cells obtained 28 days after activation of purified T_CM (mean, 16.4 ± 7.6%; data not shown). These results suggest that fractions of T_EM cells acquire a stable CCR7 expression profile after activation.

CCR7+ T_EM cells acquire a T_CM-like proliferative capacity

It was shown that T_CM cells exhibit a higher proliferative capacity than T_EM cells (1, 10, 11). We examined this observation with respect to our experimental setting. After polyclonal stimulation of sorted T_CM and T_EM cells, we similarly detected a higher proliferative potential of T_CM cells. However, T_EM cells also showed a marked and prolonged expansion compared with unstimulated populations (Fig. 3) when tested 2 days (Fig. 3A) and 7 days (Fig. 3B) after stimulation. Thus, T_EM posses a certain expansive potential. We now compared the proliferative response of CCR7+ T_EM cells to anti-CD3/CD28 stimulation with that of CCR7+ T_CM and T_EM cells using the proliferation marker Ki-67 (Fig. 3, C and D) and analysis of CFSE dilution (Fig. 3E). Because activated T_CM CD8+, CCR7+, and CCR7– d2-T_EMCCR7– cells 7 days after stimulation are shown. Proportions of CCR7+ and CCR7– d2-T_EMCCR7– cells are shown as means and SD of healthy donors (n = 2). To check the purity of isolated memory T cell subsets, CD8+ T_EM cells were analyzed for CCR7 expression immediately after cell separation (day 0). SSC, Side scatter.
CD8<sup>+</sup> TEM cells lose CCR7 expression and differentiate into T<sub>EM</sub> cells (1), gated CCR7<sup>+</sup> T<sub>CM</sub> cells were used for comparison. As shown in Fig. 3, C and D, direct ex vivo analysis revealed that T<sub>EM</sub> and T<sub>CM</sub> cells from peripheral blood do not proliferate. After stimulation, the three subsets of isolated memory T cells became positive for Ki-67 on days 2 and 3. The proliferative capacity of CCR7<sup>+</sup> T<sub>EM</sub> cells was lower than that of the two CCR7<sup>+</sup> memory subsets. At day 5 after stimulation, Ki-67 expression within all three subsets dropped to similar levels marking the end of the proliferation phase. Similarly, CFSE dilution was found predominantly in CCR7<sup>+</sup> T<sub>CM</sub> and T<sub>EM</sub> subsets starting at day 2–3 after stimulation and decreased until day 7 (Fig. 3E). These results indicate that CCR7 expressing T<sub>EM</sub> cells acquire a T<sub>CM</sub>-like proliferative capacity.

**CCR7<sup>+</sup> T<sub>EM</sub> cells exhibit a weak effector capacity characteristic for T<sub>EM</sub> cells**

A characteristic attribute of CD8<sup>+</sup> T<sub>EM</sub> cells is their ability to express perforin. In contrast, CD8<sup>+</sup> T<sub>CM</sub> cells show marginal perforin expression levels (1, 11). To characterize the effector capacity of CCR7<sup>+</sup> T<sub>EM</sub> cells, their perforin expression after anti-CD3/CD28 stimulation was compared with that of CCR7<sup>+</sup> T<sub>EM</sub>- and CCR7<sup>+</sup> T<sub>CM</sub> cells (Fig. 4, A and B). The ex vivo analysis of T<sub>EM</sub> and T<sub>CM</sub> cells from peripheral blood indicated that T<sub>EM</sub> cells, in contrast to T<sub>CM</sub> cells, express prestored perforin granules. The perforin expression of CCR7<sup>+</sup> T<sub>EM</sub> cells showed a strong increase at days 1 and 2 and then slightly decreased to 60% at day 7 after activation. In contrast, the perforin expression of CCR7<sup>+</sup> T<sub>EM</sub> cells did not rise after stimulation but dropped to 15% at day 7 (Fig. 4A). As shown by the dot blot of Fig. 4B, the majority of perforin-expressing T<sub>EM</sub> cells were CCR7<sup>+</sup> at day 1 after stimulation. Interestingly, CCR7 up-regulation was detected in both perforin<sup>-</sup> T<sub>EM</sub> and perforin<sup>+</sup> T<sub>EM</sub> cells (Fig. 4B). This suggests that CCR7 expression is regulated independently from the presence or absence of prestored perforin granules. Similar to CCR7<sup>+</sup> T<sub>EM</sub> cells, CCR7<sup>+</sup> T<sub>CM</sub> cells showed a marginal perforin expression with a maximum proportion of 11% at day 7 after stimulation.

We also tested the capacity of T<sub>EM</sub> cells to secrete IFN-γ with respect to CCR7 expression. Interestingly, early cytokine secretion was restricted to low proportions of CCR7<sup>+</sup> cells in both populations (Fig. 4C) and exerted by CD8<sup>+</sup> as well as CD8<sup>+</sup> T cells (Fig. 4D). The results indicate that CCR7-expressing T<sub>EM</sub> cells exhibit a weak effector capacity, which is characteristic for T<sub>CM</sub> cells.

**CCR7<sup>+</sup> T<sub>EM</sub> and T<sub>CM</sub> acquire functional capacity characteristic for T<sub>EM</sub> cells on loss of CCR7 expression**

Consistent with the model of progressive differentiation, fractions of activated T<sub>CM</sub> cells lose CCR7 expression after proliferation and differentiate into T<sub>EM</sub> cells (1, 8). Similarly, when we analyzed the perforin expression of CCR7<sup>+</sup> or CCR7<sup>−</sup> T<sub>CM</sub> cells 7 days after anti-CD3/CD28 stimulation, cells that had lost CCR7 exhibited a significantly higher perforin expression than CCR7<sup>+</sup> T<sub>CM</sub> cells.
cells (Fig. 5A). These data indicate that TCM cells losing their CCR7 expression acquire effector capacity. As already shown, CCR7+ TCM cells exhibit a weak effector capacity, which is characteristic for TCM cells. CCR7 expression of TCM cells peaks at day 3, and most of these cells lose their chemokine receptor expression after proliferation. To analyze whether CCR7− TEM cells losing their CCR7 expression acquire effector capacity, CCR7− expressing TEM cells were separated 2 days after anti-CD3/CD28 stimulation (cells referred to as d2-TEMCCR7−). Seven days after activation, we measured the perforin expression of CCR7− or CCR7+ d2-TEMCCR7− cells (Fig. 5B). Similar to TCM cells, CCR7− d2-TEMCCR7− derived cells showed significantly higher perforin expression than CCR7+ d2-TEMCCR7− derived cells. This suggests that CCR7− TEM cells losing their chemokine receptor expression acquire effector capacity.

**A**

![Comparison of perforin expression in stimulated CD8+ T EM and T CM cell subsets.](image)

**B**

![Histograms show perforin expression of CD8⁺CCR7⁺ T EM, CCR7− T EM, and T CM cells at various time points after activation. Dashed lines represent isotype controls. Values are given as percent.](image)

**C**

![Anti-CD3/CD28-stimulated T EM and T CM cells were analyzed for CCR7 and perforin expression. Dot plot showing the proportion of IFN-γ-expressing gated CD8⁺ (light gray bars) and CD8⁺ (dark gray bars) memory T cell subsets 1 day after polyclonal stimulation. Labeled histograms shown in A correspond to the quadrants marked with asterisks in B. *, CCR7⁺ T EM; **, CCR7− T EM.](image)

**D**

![Cumulative data of four experiments showing the proportion of IFN-γ-expressing gated CD4⁺ and CD8⁺ memory T cell subsets 1 day after polyclonal stimulation. Labeled histograms shown in A correspond to the quadrants marked with asterisks in B.](image)

**T EM cells with stable CCR7 expression conserve a functional capacity characteristic for TCM cells**

T EM cells that show stable CCR7 expression >28 days after activation were further tested for their capacity to express perforin after renewed activation. For this, CCR7⁺ T EM cells were isolated 28 days after anti-CD3/CD28 stimulation (cells referred to as d28-T EMCCR7⁺) and compared with ex vivo isolated memory subsets. Because fractions of activated TCM cells lose CCR7 expression after proliferation and differentiate into TEM cells (1), the perforin expression profile of d28-T EMCCR7⁺, CCR7⁺ T EM and CCR7⁺ T CM as well as of CCR7− T EM cells was analyzed 7 days after secondary (in case of d28-T EMCCR7⁺ cells) or primary anti-CD3/CD28 stimulation, respectively (Fig. 6A). Isolated, d28-T EMCCR7⁺ cells showed no changes in their perforin expression profile after reactivation (compare perforin expression of d28-T EMCCR7⁺ and CCR7⁺ T EM cells). The additional comparison with CCR7⁺ T CM and CCR7− T EM cells indicated that T EM cells with stable CCR7 expression exhibit a perforin expression profile characteristic for TCM cells. We also tested the proliferative capacity of d28-T EMCCR7⁺ cells (Fig. 6B). For this purpose, the cells were labeled with CFSE and analyzed for loss of CFSE due to proliferation, 7 days after activation. d28-T EMCCR7− cells maintained a strong proliferative potential. In contrast, sorted and activated d28-T EMCCR7− cells showed no proliferative potential (Fig. 6B). Taken together, the results demonstrate that T EM cells with long term stable CCR7 expression maintain TCM-like functional capacity.
Discussion

Because different memory T cell subsets were originally defined in human T cells based on their CCR7 expression profile (1), the lineage relationship of populations referred to as TCM and TEM cells is under discussion. One model proposes a linear differentiation process from TCM to TEM (8), but there are studies indicating a reverse phenotypic differentiation of these cells (11, 12). Also, investigations performed within a mouse model suggested a differentiation from TEM to TCM cells (13). The isolation of memory T cell subsets in the latter studies, however, differed from the original definition (1) based on the expression of the cell adhesion molecule CD62L. It is still unclear whether memory T cell subsets defined through either CCR7 or CD62L represent identical populations.

Studies analyzing the maintenance of CCR7 expression after activation of separated TEM cells as well as the functions of CCR7+ TEM cells are important for the characterization of the differentiation potential of TEM cells. On the basis of the expression of CCR7 and CD62L, we isolated TEM cells of high purity and analyzed their phenotype and function during a period of up to 4 wk after polyclonal stimulation in vitro.

Our results show that on activation of TEM cells, fractions of at least 6% acquire stable CCR7 expression for at least 4 wk (Fig. 2). This change to stable CCR7 expression is unlikely to be an in vitro artifact, because the proportions of peripheral blood TCM cells in vivo are also not much higher (Figs. 1, B and C, and 2, A and B), and proportions of CCR7+ TEM cells 28 days after activation are similar in cultures of sorted TCM and TEM cells (our unpublished observations). Additionally, the functional properties of d28-TEMCCR7+ are very similar to those of freshly isolated TCM cells (Fig. 6). It should therefore be taken into consideration that studies on human TCM cells can deal with mixed populations of both primary TCM cells and secondary TEM-like cells derived from activated TEM cells.

A comparison of CCR7+ TEM and TCM cells revealed that both subsets possess a high proliferative capacity after stimulation, which declines at day 5, marking the end of the proliferation phase. In contrast, TEM cells that remain CCR7+ proliferate only marginally (Fig. 3). These data indicate that CCR7+ TEM cells acquire a proliferative capacity that is characteristic for TCM cells, most likely representing cells at intermediate stages of differentiation.

Almost 30% of C8+ TEM cells, tested directly ex vivo, expressed perforin. On activation, TEM cells showed diverse differentiation processes. Fractions of perforin+CD8+ TEM cells became perforin+. These cells remained CCR7+. In contrast, in stimulated TEM cells that acquired the expression of CCR7, perforin expression dropped to a TCM-like level 7 days after activation (Fig. 4A). These data indicate that fractions of stimulated TEM cells acquire a functional capacity that is characteristic for TCM cells.

When TEM cells were activated, during the first days CCR7+ cells increased expression of perforin or IFN-γ, thus indicating...
immediate effector capacity, whereas a fraction of cells first be-
came CCR7+ (peak at day 3), then expressed Ki-67, and acquired
proliferative activity as seen by CFSE dilution. Until day 7, many
of the CCR7+ TEM cells reverted to a CCR7− phenotype with
expression of TCM properties. Thereby, activation of TEM cells provides a sequence of two
effector phases: an immediate phase provided by a limited number
of nonproliferating TEM cells; and a second phase provided by a
high number of clonally expanded TEM cells. The acquisition of
Teff-like phenotypic, proliferative, and functional characteristics
of fractions of activated TEM cells indicates that not only TCM cells
as proposed (8) but also TEM cells contain populations at interme-
diate stages of differentiation. The acquisition of a stable CCR7
expression profile and a TCM-like functional capacity of fractions
of activated TEM cells could assure a self-renewal potential and
long term maintenance of TEM cells.

Our results demonstrate that upon activation CD8+ TEM cells
exhibit a dynamic differentiation. Fractions of TEM cells acquire
phenotypic and functional characteristics of TCM cells. After pro-
liferation, most of these cells lose CCR7 expression and acquire
effector capacity. In contrast, a proportion of at least 6% main-
tained TCM properties, suggesting that fractions of TEM cells retain
a flexible imprinting of genes characteristic of cells at intermediate
stages of differentiation possibly associated with a self-renewal
potential of these cells.

Acknowledgments
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Disclosures
The authors have no financial conflict of interest.

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