Dissection and manipulation of antigen-specific T cell responses

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Chapter 1

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Review

Dissection of cytotoxic and helper T cell responses

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Abstract. Cytotoxic ($CD8^+$) and helper ($CD4^+$) T cells play a crucial role in resolving infections by intracellular pathogens. The development of technologies to visualize antigen-specific T cell responses in mice and men over the past decade has allowed a dissection of the formation of adaptive T cell immunity. This review gives a brief overview of the currently used detection techniques and possible future additions. Furthermore, we discuss our current understanding of the formation of antigen-specific T cell responses, with particular attention to the similarities and differences in $CD4^+$ and $CD8^+$ T cell responses, the functional heterogeneity within responder T cell pools and the regulation of $CD8^+$ T cell responses by dendritic cells and $CD4^+$ helper T cells.

Key words. $CD4^+$ T lymphocytes; $CD8^+$ T lymphocytes; T cell memory; antigen presenting cells; dendritic cells; infection; T cell differentiation; T cell migration.

Introduction

T cells are crucial for fighting pathogens such as viruses and bacteria. Cytotoxic ($CD8^+$) T cells recognize peptides bound to the major histocompatibility complex (MHC) class I molecules that are present on virtually all nucleated cells. $CD8^+$ T cells are specialized in tracking down infected cells with the aim to destroy these cells, including the residing pathogens. The main mechanism used by $CD8^+$ T cells to attack infected cells is the induction of apoptosis, either through ligand-induced death-receptor triggering, or via the release of perforins and granzymes. In addition, $CD8^+$ T cells secrete cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF) that can enhance antigen (Ag) presentation and mediate anti-pathogenic effects, for instance by interfering with viral/bacterial replication. Helper ($CD4^+$) T cells recognize peptides complexed with MHC class II molecules that are only expressed on so-called professional Ag presenting cells (APCs), such as dendritic cells (DCs), macrophages and B cells. $CD4^+$ T cells are specialized in providing supporting signals to other cell types within the immune system such as macrophages, B cells and $CD8^+$ T cells, in the form of cytokines and through direct interactions.

During T cell development in the thymus each thymocyte creates an essentially unique T cell receptor (TCR) by rearrangement of functional TCR-α and -β genes from pools of discontinuous TCR gene segments. This process of TCR gene recombination leads to the formation of a pool of T cells expressing a broad repertoire of TCRs that collectively can recognize millions of different peptide-MHC (pMHC) complexes. The process of recombination not only results in TCRs that have the potential to recognize a foreign peptide bound to MHC, but also in TCRs that fail to recognize MHC altogether, or that can recognize self-peptides bound to MHC. To mould

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the repertoire of T cells towards a pool of cells that can potentially recognize foreign pMHC complexes and that is not self-reactive, T cells undergo an intrathymic selection process. Thymocytes that express TCRs with high affinity for self-pMHC undergo apoptosis, thymocytes with no affinity for endogenous pMHC complexes die of neglect, and consequently only thymocytes that express TCR with low affinity for self-pMHC differentiate into $CD4^+$ and $CD8^+$ T cells and are exported to the periphery. In the periphery, T cells continuously migrate through lymphoid organs, where they scan the pool of peptides presented by APCs.

Techniques to visualize Ag-specific T cell responses

Traditional techniques to monitor Ag-specific T cell responses measured the capacity of pools of T cells to react to Ag, either in the form of Ag-induced proliferation, cytotoxicity or cytokine secretion. In the past decade these techniques have in large part been replaced by technologies that allow the visualization of Ag-specific T cells at the single cell level. Of these techniques, in particular the flow cytometry-based techniques, which also allow further characterization and/or isolation of Ag-specific T cells, have become valuable and are the main focus of this review.

MHC multimers

The analysis of Ag-specific T cell responses using pMHC multimers is based on the very convincing argument that if the binding of T cells to a given pMHC complex defines their Ag specificity, a recombinant version of this pMHC complex should be an ideal tool to detect such cells. Following the first description by Altman et al. some 10 years ago, pMHC-based reagents have become an invaluable tool for the analysis of Ag-specific T cell responses [1]. For the generation of pMHC class I complexes, both the MHC class I heavy chain and light chain (β2-microglobulin) are generally produced in *Escherichia coli* cells and folded in vitro in the presence of a specific peptide. For pMHC class II complexes, the complex of the MHC chains with Ag peptide is generally produced in eukaryotic expression systems, but variations on these themes are possible [2]. As the interaction between a TCR and its cognate pMHC complex is characterized by a fast off-rate [3], pMHC complexes need to be multimerized for efficient detection of Ag-specific T cells. Multimerization is most often achieved using the biotin-streptavidin interaction, resulting in the formation of tetrameric pMHC complexes [1], but other ways of multimerization are possible [2]. Coupling of a fluorochrome to the multimeric pMHC complex is used to allow the visualization of Ag-specific T cells most commonly by flow cytometry but also in situ [4] or on pMHC microarrays [5, 6].

Although MHC class II multimers can be generated by in vitro refolding of at least some MHC class II alleles, the yield of this procedure is low. The group of Kappler and Marrack provided an alternative to this strategy by covalently linking the peptide of interest to the β-chain of MHC class II, thereby ensuring binding of the peptide into the peptide-binding groove during production in eukaryotic cells [7, 8]. This approach has successfully been used to generate both human and mouse MHC class II multimers [9, 10], and modifications of this technique have now also been used to generate MHC molecules that can be complexed with a peptide of choice in vitro [11, 12].

A major advantage of MHC-multimer technology is that it allows detection of Ag-specific T cells directly ex vivo, without requiring any in vitro restimulation [1]. This can for instance be used to correlate the phenotype of Ag-specific T cells with certain disease states. Although the low frequency of Ag-specific $CD4^+$ T cells often complicates the ex vivo phenotypic characterization of these cells, it was recently shown that isolation of infrequent MHC multimer-positive $CD4^+$ T cells with magnetic beads allows their phenotypic characterization in at least some settings [11, 13]. It should be noted though that binding of MHC class II multimers to $CD4^+$ T cells is generally achieved by incubation of the cells with these reagents at 37°C for several hours and one should take into account the possibility that TCR triggering induced by MHC multimer binding may result in up- or downregulation of phenotypic markers such as early activation markers. In addition, it is noted that MHC multimers can in some cases fail to detect a fraction of Ag-specific T cells (reviewed in [2]), such as highly activated T cells that have a reduced TCR expression and T cells with a lower avidity for the relevant pMHC complex. Again, this problem appears to be more common for MHC class II multimers than for MHC class I multimers.

Functional assays

Whereas MHC multimers detect Ag-specific T cells on the basis of the pMHC-TCR interaction, functional assays monitor the effects of this interaction on T cells. At present, three techniques are available for the detection of Ag-induced cytokine production at the single cell level. The shared characteristic of these techniques is that they aim to ensure that cytokines that are produced by the cell upon antigen encounter do not dissipate into the medium upon secretion. The most frequently used functional test to detect Ag-specific T cells on a per cell basis is the intracellular cytokine staining (ICS) assay. In this assay, T cells are incubated for several hours in the presence of Ag plus an intracellular protein transport inhibitor (e. g.

brefeldin A) to ensure accumulation of the Ag-induced cytokine inside the cells. Subsequent permeabilization and staining for the cytokine of interest then allows the detection of functional Ag-specific T cells at the single cell level by conventional flow cytometry. While the ICS assay is generally used to monitor cytokine secretion following ex vivo Ag encounter, a recent study demonstrates that administration of BrefoldinA to mice also allows the measurement of in vivo cytokine production [14]. Although combined cytokine and MHC multimer stainings have been performed, the stimulation with Ag plus incubation with brefeldin A that is needed to allow cytokine detection leads to down-regulation of the TCR, often to such an extent that it hampers T cell detection by MHC multimer staining. For unknown reasons, this phenomenon is more pronounced for mouse T cells than for human T cells.

An alternative technique for the visualization of Ag-specific T cells based on cytokine secretion is the ELISPOT assay. In this assay, T cells are stimulated with peptide for a brief period, while being trapped in an agar gel. Subsequently, Ag-induced secretion of cytokines is detected by antibody staining. The main advantage of this technique is that it is extremely sensitive, enabling the detection of cells that are present at very low frequencies. However, because ELISPOT does not allow the further characterization and isolation of the Ag-specific T cells, its value is mostly restricted to the counting of Ag-specific T cells.

A third approach for the detection of cytokine-secreting T cells at the single cell level makes use of a cell surface affinity matrix consisting of a bispecific antibody recognizing the cytokine of interest and the CD45 molecule that is widely expressed on lymphocytes [15]. The advantage of this approach is that the cytokine-secreting cells maintain their viability during the assay, allowing T cell isolation (using flow cytometric cell sorting or magnetic bead isolation) and further characterization.

Apart from these three assays that detect T cell function by monitoring cytokine production, Ag-specific $CD8^+$ T cells may also be detected at the single cell level based on Ag-induced degranulation. In this assay, cells are stained with an antibody specific for CD107 (LAMP-1), a molecule that is present in the membrane of cytotoxic granules and is exposed on the cell surface upon Ag-induced degranulation [16]. Although cell surface exposure of CD107 is a marker for degranulation, CD107-positive T cells are not necessarily cytotoxic, as cytotoxicity depends on the protein content of the cytotoxic granules [17]. As is the case for the cytokine capture assay, viable Ag-specific T cells may be isolated by monitoring Ag-induced degranulation.

It is important to note that the several functional assays and MHC multimer staining should be considered complementary rather than substitute technologies. Specifically, the total number of Ag-specific T cells may be substantially greater than the number of Ag-specific T cells capable of performing a given effector function. For instance, T cells that have only partially differentiated upon Ag encounter may not be able to perform specific effector functions, and even when fully differentiated, the capacity of $CD4^+$ T cells to produce specific cytokines is dependent on the differentiation pathway that was followed (further discussed below). Finally, during chronic infections, the capacity to produce specific cytokines may be lost. Specifically, the parallel use of MHC multimer staining and intracellular cytokine staining has revealed that in HIV-infected individuals HIV-specific $CD8^+$ T cells do not disappear, but rather lack the potential to produce IFN-γ [18]. Thus, before setting out to measure Ag-specific T cell frequencies, one should consider whether an analysis of Ag-specific T cell numbers or of Ag-specific T cell activity is most relevant for a particular study, and in most cases the parallel use of MHC multimer staining and functional assays is likely to be most valuable.

Counting Ag-specific T cells

The first important observation made through the use of MHC multimer and intracellular IFN-γ staining was that the frequencies of Ag-specific $CD8^+$ T cells present during an acute viral infection are much higher than previously thought [19–23]. Earlier studies had established that the fraction of T cells that went through cell division during an acute infection was high. However, it had long been assumed that the majority of these cells were so-called bystander T cells that did not recognize epitopes associated with the ongoing infection. MHC multimer analysis of $CD8^+$ T cell responses during acute infections disproved this hypothesis by demonstrating that the vast majority of proliferating cells that are found during an acute infection are specific for the infectious agent [19, 23]. At the peak of the Ag-specific $CD8^+$ T cell response, up to 70% of $CD8^+$ T cells were found to be Ag-specific in mice infected with lymphocytic choriomeningitis virus (LCMV) or influenza virus.

Although studies using MHC class I multimers showed that the frequencies of Ag-specific $CD8^+$ T cells had greatly been underestimated, studies using MHC class II multimers suggest that this may not be the case for $CD4^+$ T cell responses. Only in a few instances can Ag-specific $CD4^+$ T cells be readily detected directly ex vivo, for example in synovial fluid of patients with rheumatoid arthritis or lyme disease [24, 25] or in draining lymph nodes of vaccinated mice [26, 27]. In murine viral infection models such as Moloney murine sarcoma/leukaemia virus (MoMSV) and LCMV infection, reasonably high frequencies of $CD4^+$ T cells specific for the immunodominant epitope (approximately 1% and 10% respectively)

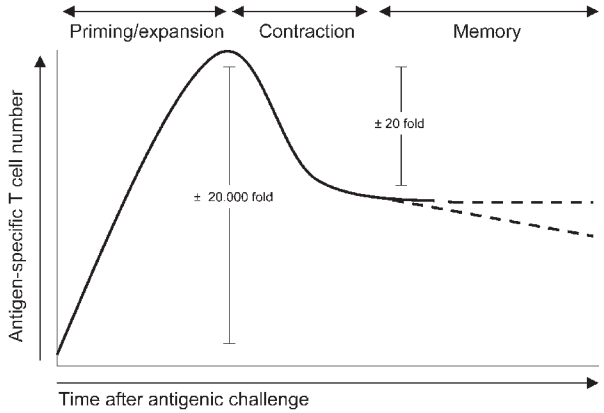

Figure 1. Ag-specific T cell responses are characterized by three phases. In the priming/expansion phase, T cells proliferate and differentiate into effector cells that have cytotoxic capacity and express high levels of IFN-γ. In the following contraction phase, Ag-specific T cell numbers decline to 5–10% of the numbers observed at the peak of the response. The remaining population forms the memory T cell pool that is maintained a long time and mediates long-term protection. The numbers indicate the fold increase/decrease in Ag-specific T cell numbers during the priming/expansion and contraction phase, respectively.

have been detected [10, 28]. However, in other cases in vitro restimulation of blood cells is often needed to allow for the detection of MHC class II multimer-positive cells [12, 25, 29, 30].

Furthermore, even in cases where $CD4^+$ T cell responses can be detected directly ex vivo, the size of these responses is substantially smaller than that of the $CD8^+$ T cell responses that occur in parallel. Specifically, quantification of $CD4^+$ and $CD8^+$ T cell responses by MHC multimer staining in mice undergoing MoMSV or LCMV infection showed that at the peak of the T cell response about 20-35 times more Ag-specific $CD8^+$ T cells are found than Ag-specific $CD4^+$ T cells [10, 28]. Similar results were obtained when LCMV-specific $CD4^+$ and $CD8^+$ T cell responses were analyzed by intracellular IFN-γ staining [31]. Intracellular IFN-γ staining has likewise been used to demonstrate that Ag-specific $CD4^+$ T cell responses are much less prominent than Ag-specific $CD8^+$ T cell responses during infection with the intracellular bacterium *Listeria monocytogenes* (LM) in mice and Epstein Barr virus (EBV) and cytomegalovirus (CMV) infections in humans [32–34]. This difference in the magnitude of Ag-specific $CD4^+$ and $CD8^+$ T cell responses could in theory be due to the fact that MHC class I complexes have a broader expression pattern than MHC class II complexes, which are primarily expressed by APC. More likely, in vitro experiments by Foulds et al. demonstrated that the smaller size of Ag-specific $CD4^+$ T cell responses may be explained by a difference in the size of the proliferative burst that $CD4^+$ and $CD8^+$ T cells undergo upon Ag encounter [35]. This difference is likely to be at least in part cell-intrinsic as it can be documented in simplified in vitro culture systems. However, it remains possible that the capacity of $CD4^+$ and $CD8^+$ T cells to respond to environmental signals such as cytokines and/or co-stimulatory molecules plays an additional role in vivo.

The smaller size of immunodominant Ag-specific $CD4^+$ T cell responses may in part be compensated by a greater diversity of the Ags that are recognized within a pathogen-specific $CD4^+$ T cell response. $CD8^+$ T cells that recognize different epitopes presented on the same APC appear to compete with each other in at least some mouse models [36–38]. In contrast, one study demonstrated that $CD4^+$ T cells that recognize distinct Ags presented by the same APC can help each other [39]. As a consequence of intraclonal competition, $CD8^+$ T cell responses may generally be restricted to a restricted set of epitopes, whereas a pathogen-specific $CD4^+$ T cell response could conceivably recognize a broader repertoire of epitopes, each of which would only constitute a small part of the response.

Whereas the use of MHC class I multimer and IFN-γ staining has demonstrated that dividing/effector $CD8^+$ T cells that are present during an ongoing immune response are Ag-specific rather than bystander cells, it is still unclear whether the same holds true for Ag-specific $CD4^+$ T cell responses. Specifically, $CD4^+$ T cells that recognize the immunodominant epitope of the MoMSV virus only accounted for about 1% of the $CD4^+$ T cells that were present in MoMSV-induced lesions [10]. If T cells that recognize the immunodominant epitope constitute a substantial part of the total pathogen-specific $CD4^+$ immune response, as has been described for $CD8^+$ T cell responses (but see note on interclonal help above), a significant percentage of the lesion-infiltrating $CD4^+$ T cells are likely not to be Ag-specific. Interestingly, during LCMV infection at least 80% of the $CD4^+$ T cells that divide in the spleen have been shown to be specific for two immunodominant MHC class II restricted epitopes of LCMV [28]. At first glance, these data appear in contradiction with the analysis of the MoMSV-specific $CD4^+$ T cell response. Conceivably, the involvement of bystander $CD4^+$ T cells might be small during LCMV infection and large during MoMSV infection. Alternatively, a large fraction of $CD4^+$ T cells that are attracted to an effector site might not be pathogen-specific, whereas the majority of dividing $CD4^+$ T cells present in lymphoid organs during an infection are specific for this pathogen.

Three separate phases during Ag-specific T cell responses

Upon activation, Ag-specific T cells rapidly expand and differentiate into effector cells that express high levels of effector cytokines and in case of $CD8^+$ T cells also

have cytotoxic capacity (fig. 1) [40–44]. Following this activation/expansion phase, a contraction phase ensues, in which Ag-specific T cell numbers decline to 5–10% of the number observed at the peak of the response (reviewed in [45]). The remaining population forms the memory T cell pool that stays present for longer periods of time (memory phase), and can mediate long-term protection (reviewed in [46]). These three phases of clonal expansion, contraction and memory formation are found in response to many different types of acute infection and for different Ags within the same pathogen, indicating a common pathway for memory T cell formation.

Activation/expansion phase

Immature APC require two ingredients to become capable of stimulating a robust T cell response (fig. 2A). First, at the site where pathogens invade the body, APCs, primarily DCs, can internalize pathogen-infected dying cells and in some cases also free pathogen, providing a source of Ag that can subsequently be presented in the context of MHC molecules [47]. Second, APCs possess a set of receptors that recognize molecules or molecular patterns that are associated with infection or cell death. A first class of these receptors is formed by the pattern-recognition receptors, such as the Toll-like receptors [47–49] that recognize pathogen-associated molecular patterns (PAMPs). In addition, APCs appear to express receptors that recognize endogenous indicators of 'danger' (such as uric acid) that are released by dying or infected cells [50, 51]. Triggering of these receptors leads to activation of APCs [47–51] and induces the release of inflammatory mediators such as IFN-α/β, TNF and IL-1 by tissue (-resident) cells, leading to further activation of DCs [48]. Upon activation, APCs gain an increased capacity to migrate to lymphoid organs, allowing them to subsequently activate naïve T cells [47].

Activation of naïve T cells upon APC encounter not only depends on the number of pMHC complexes that are present on the APC and the affinity of the TCR for the pMHC complex (pMHC-TCR interaction, 'signal 1'), but also on signals provided by the APC in the form of costimulation ('signal 2') and cytokines ('signal 3') (fig. 2B). Costimulation can be defined as triggering of receptors on T cells by costimulatory ligands expressed by activated APCs that leads to enhanced TCR-driven expansion. The best-defined costimulatory molecules belong either to the immunoglobulin-like CD28 family or to the TNF receptor family. The costimulatory molecules and ligands of these families are differently expressed throughout an infection. For instance, whereas some receptors are expressed on naïve T cells (such as CD27 and CD28), other costimulatory molecules are only expressed on the cell surface at different time points

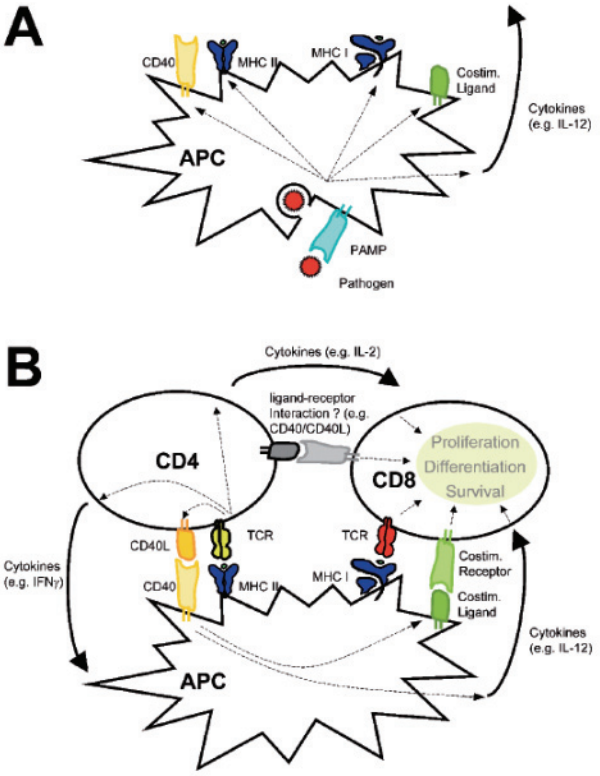

Figure 2. Activation of Ag-specific $CD8^+$ T cells by APC. (A) Ag uptake by APCs. Upon pathogen encounter, APCs internalize pathogen-infected dying cells/free pathogen, providing a source of Ag that can subsequently be presented in the context of MHC molecules. In addition, APCs express receptors that recognize indicators of 'danger' (such as pattern recognition receptors), the triggering of which leads to APC activation. (B) APC-T cell interaction. Depending on the amount and type of pathogen, APCs require help from $CD4^+$ T cells to activate $CD8^+$ T cells. This help by $CD4^+$ T cells is provided via signals, such as CD40L and IFN-γ, that stimulate APCs to increase the expression of costimulatory ligands and the secretion of stimulatory cytokines (such as IL-12). In addition, $CD4^+$ T cells may directly activate $CD8^+$ T cells by membrane-bound ligands and/or cytokines.

after T cell activation (e. g. OX40, 4-1BB) [52]. The consequences of costimulation range from enhanced survival and increased proliferation to differentiation of Ag-specific T cells and can vary between different costimulatory molecules. For instance, engagement of CD28 enhances IL-2 production [53], whereas signaling through inducible costimulator (ICOS) leads to increased production of IL-10 [54]. In the absence of costimulation, Ag-loaded APCs are generally non-stimulatory and may even tolerize responding T cells [55].

Cytokines produced by APCs can also affect both the size and the quality of the ensuing T cell response. For example, recognition of virus and bacteria-derived PAMPs leads APCs to produce IL-12 that drives the generation of so-called type 1 T helper cells (Th1 cells), which express IFN-γ and promote cell-mediated immunity [56, 57]. In contrast, recognition of parasites stimulates APCs to pro-

duce IL-4, IL-10 and TGF-β. These cytokines drive the generation of so-called type 2 T helper cells (Th2 cells), which express IL-4, IL-5 and/or IL-13 [56, 57], and that stimulate antibody-mediated immunity and host defense against parasitic infections.

Visualization of T cell priming using T cells from TCR transgenic mice and two-photon microscopy of intact lymph nodes has revealed that the contacts between Ag-expressing DC and Ag-specific T cells can be divided in three phases [58, 59]. During the first phase (from ~6–12 h after immunization) DC-T cell interactions are dynamic, ranging from 2 to 10 min, and T cells start to express early activation markers such as CD44 and CD69 [58, 59]. In the absence of Ag, the motility of the T cells and DC is extensive, enabling an individual DC to interact with an estimated 500-5000 T cells per hour [60, 61]. The contacts in the second phase (from ~12 to 20 h after immunization) are long-lasting (>1 h) and T cells reach maximum expression levels of activation markers and begin to produce effector cytokines such as IL-2 and IFN-γ [58, 59]. Stable interactions are not observed in the absence of Ag [58]. In the third phase (1–2 days after immunization) T cell-APC interactions are dynamic again, which coincides with the onset of proliferation [58, 59].

Based on an estimate of the frequency of Ag-specific T cells before Ag encounter and the number of Ag-specific T cells present at the peak of an anti-LCMV specific $CD8^+$ T cell response, the peak rate of division was calculated to be 6-8 h [19, 23]. More recent experiments using 5- and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE), a fluorescent dye that binds to free amines of the cell and is equally divided amongst daughter cells, show that activated $CD8^+$ T cells have a division rate of 4–6 h [62, 63], whereas activated $CD4^+$ T cells have an estimated division rate of 6–8 h [64]. This indicates that under optimal conditions a single T cell can give rise to an offspring of ~1,000 T cells in a 2-day period.

During proliferation, activated T cells progressively gain effector functions, and at the end of the expansion phase T cells express high levels of cytokines and in case of $CD8^+$ T cells have acquired cytotoxic capacities [40–44]. Interestingly, it has been shown that a brief period of Ag stimulation (8–48 h) can be sufficient to commit $CD8^+$ and $CD4^+$ T cells to proliferate and differentiate independent of further antigenic stimulation [65–70]. This indicates that at least some of the properties of the progeny of a T cell are programmed before a T cell undergoes its first round of division. This autonomous program of differentiation may serve to allow APC to activate a higher number of Ag-specific T cells as compared with a situation in which T cells and their progeny would require prolonged APC contact.

In studies that simultaneously quantified Ag-specific $CD4^+$ and $CD8^+$ T cell responses in a single host, it was found that the kinetics of the Ag-specific $CD4^+$ and $CD8^+$ T cell responses do not necessarily coincide. Whereas in the LCMV- and LM infection models Ag-specific $CD4^+$ and $CD8^+$ T cells were found to peak at the same time point [28, 31, 34], in other infections, such as MoMSV in mice and CMV and EBV in humans, the Ag-specific $CD4^+$ T cell response was found to peak earlier [10, 32, 33]. These data suggest that depending on the pathogen, the kinetics of Ag-specific $CD4^+$ and $CD8^+$ T cell response may differ. It is currently unclear which properties of the pathogen determine the kinetics of Ag-specific $CD4^+$ and $CD8^+$ T cell responses. It seems quite possible that differences in the kinetics of MHC class I and II-restricted Ag presentation, depending for instance on viral tropism or the presence of free versus cell-associated pathogen, may be a causal factor, but any direct evidence for this is lacking.

Contraction

During the contraction phase, the number of Ag-specific T cells declines by massive apoptosis to 5-10% of those at the peak of the response. The contraction phase may serve two goals. First, it may limit immunopathology either due to inadvertent cross-reactivity of the activated T cells with endogenous pMHC complexes, or in cases of high Ag load. Second, and perhaps more important, contraction of T cell responses may be required to preserve the ability to respond to other pathogens. In many experimental systems, the onset of $CD8^+$ T cell contraction correlates with pathogen clearance, suggesting that Ag-specific $CD8^+$ T cells 'sense' that the pathogen has been cleared. However, recent experiments using attenuated strains of LM or administration of antibiotics during LM infection show that the contraction kinetics are independent of the duration of infection, indicating that the decay in Ag-specific $CD8^+$ T cell numbers is programmed before Ag load reduces [71]. Further experiments indicated that cell death during the contraction phase is controlled by inflammation and IFN-γ levels early after infection [72], which may be due to a direct effect of IFN-γ on $CD8^+$ T cells [73]. Although signals delivered early after infection may in part program the kinetics of T cell contraction in the following days, other studies have demonstrated that contraction of T cell responses is not entirely autonomous and extrinsic factors such as cytokines do contribute. For instance, the administration of IL-2 during the contraction phase has been shown to sustain the number of effector T cells [74]. In addition, costimulatory signals such as signals derived from CTLA-4, PD-1 and BTLA may further modulate the contraction of Ag-specific T cell responses [75–77]. In line with this idea, we recently showed that signals provided by CD70 may also influence the kinetics of contraction as in CD70Tg mice, in which the CD27 coreceptor is continuously triggered, contraction of the Ag-specific

T cell pool is somewhat delayed [78]. Although it is unclear by which mechanisms cytokines and costimulatory molecules influence the contraction of Ag-specific T cell responses, alterations in the expression of the usual suspects of pro- and anti-apoptotic molecules seem a safe bet. Evidence for a role of pro-apoptotic molecules has been provided by the finding that the contraction phase of Ag-specific $CD8^+$ T cells is prolonged in mice lacking the Bcl-2 family member Bim [79].

Memory phase

After the contraction phase, Ag-specific T cells are maintained at levels that are greatly elevated compared with frequencies in naïve mice, and these remaining cells are termed memory T cells. In mice infected with LCMV or LM, Ag-specific $CD4^+$ memory T cell numbers were found to gradually decline, whereas Ag-specific $CD8^+$ memory T cell numbers remained stable at ~5–10% of the T cell levels found during the peak of the response [28, 34]. The maintenance of memory T cell numbers is based on the balance between the low death rate amongst memory T cells, and production of new offspring by homeostatic proliferation of memory T cells. The homeostatic proliferation of both $CD4^+$ and $CD8^+$ memory T cells has been shown to occur in the absence of MHC molecules [80–85] and after ablation of TCR expression on memory T cells [86] and is thus considered Ag independent. Homeostatic proliferation of memory T cells is driven by cytokines such as IL-7 and IL-15 [87–93] and the difference in memory cell maintenance between $CD4^+$ and $CD8^+$ T cells may be explained by their distinct sensitivity to IL-7 and IL-15. Specifically, whereas IL-7 and IL-15 both contribute to $CD8^+$ T cell memory maintenance, IL-7 seems to play a more dominant role in the maintenance of $CD4^+$ T cell memory [87–93].
Although $CD8^+$ memory T cell numbers generally remain stable, heterologous infections have been found to impact on the stability of $CD8^+$ T cell memory. Memory T cells that do not cross-react with Ag that are present during the heterologous infection decline in number [94]. However, cross-reactivity of memory T cells with Ag from two different (un)related pathogens is not uncommon and can lead to increased $CD8^+$ memory T cell levels upon heterologous infection [94, 95].
Formation of T cell memory serves to mold the T cell repertoire towards Ags that have been encountered previously. Compared with primary T cell responses, secondary (memory) T cell responses are more rapid [45, 96], thereby resulting in faster pathogen clearance. The more rapid build-up of an army of effector cells is in large part due to the higher precursor frequency of memory T cells, but also due to a faster response upon TCR triggering, a shorter cycling time and increased survival of dividing memory T cells compared with naïve T cells [45, 96–98]. In addition to this numerical aspect, memory T cells are more rapidly capable of performing specific effector functions, such as cytolysis and cytokine secretion, upon TCR triggering [97, 98].

Lineage relationships between naïve, effector and memory T cells

It has long been debated whether memory T cells are derived from effector T cells or from naïve T cells that have not passed through an effector cell stage. Two recent studies argue that $CD8^+$ memory T cells are the progeny of effector T cells, but the debate may not be settled. In these studies, it was found that at the peak of the primary $CD8^+$ T cell response, two subsets of cells can be identified that express different levels of the IL-7 receptor α-chain (CD127) [99, 100]. Of these cells, only the pool of T cells that express high levels of CD127 can give rise to long-lived memory cells [99, 100], and the majority of these $CD127^+$ T cells were shown to have effector T cell properties, such as the capacity to kill and produce IFN-γ [99]. These results argue in favor of a linear differentiation model in which naïve $CD8^+$ T cells differentiate into memory T cells by passing through an effector stage. However, it remains possible that $CD8^+$ memory T cells develop from the minority of $CD127^+$ cells that do not have effector T cell properties. Perhaps more important, these studies do not establish whether the capacity of a T cell to enter the memory T cell pool is instilled during early Ag encounter (e. g. by the quality of the APC), or is controlled at a later phase.
One study has provided evidence that expression of the CD8αα homodimer on effector T cells may also be used as a marker for $CD8^+$ memory T cell precursors [101]. However, it is presently unclear how CD8αα expression is connected to T cell survival (as is the case for the memory precursor marker CD127), and this will require further study. A study by the group of R. Seder, in which $CD4^+$ T cells were separated based on the capacity to produce IFN-γ, indicated that IFN-γ-producing $CD4^+$ T cells do not proliferate upon adoptive transfer, whereas IFN-γ negative cells do [102]. At face value, these data argue that $CD4^+$ memory T cells are not derived from $CD4^+$ effector T cells. It is, however, unclear to what extent the in vitro peptide stimulation and method to isolate the IFN-γ-producing cells might have influenced the destiny of the cells.

Role of CD4 help in $CD8^+$ T cell responses

Depending on the nature of the antigenic challenge, APC also require signals from $CD4^+$ T cells to 'help' primary

$CD8^+$ T cell responses. In settings in which the Ag is introduced in the absence of inflammatory mediators (e. g. soluble proteins and Ag-loaded cells), APCs are generally unable to efficiently prime naïve $CD8^+$ T cells without the help of $CD4^+$ T cells. In these cases CD40 ligation on APCs has been shown to bypass this requirement for $CD4^+$ T cell help, which led to the concept that CD40L-expressing $CD4^+$ T cells license CD40-expressing APCs (by upregulating costimulatory molecules and cytokines) to stimulate $CD8^+$ T cells. In line with this concept, it has been demonstrated that $CD4^+$ and $CD8^+$ T cells need to recognize Ag on the same APCs [103, 104].

Experiments with live pathogens, such as LM or LCMV, showed that primary $CD8^+$ T cell responses do not necessarily depend on $CD4^+$ T cells [105–108]. This is likely to be due to the ligation of Toll-like receptors on APCs, thereby bypassing the requirement for $CD4^+$ T cell help. Because T cell responses against certain other pathogens are $CD4^+$ T cell dependent [109, 110], one may assume that these pathogens are intrinsically less potent in inducing inflammatory signals. Furthermore, while in one study in which a high dose of LM was used, $CD4^+$ T cell help was found to be dispensable [107, 108], in a second study that used a lower dose of LM, the presence of $CD4^+$ T cell help was essential [111]. These data suggest that the requirement for $CD4^+$ T cell help may not only depend on the type of pathogen but may also be more profound at lower pathogen doses.

Although there is now solid evidence that $CD4^+$ T cells can provide help to $CD8^+$ T cells via APC licensing, this does not necessarily invalidate the traditional model in which $CD4^+$ T cells directly 'help' $CD8^+$ T cells. Specifically, it has been suggested that $CD4^+$ T cells provide help to $CD8^+$ T cells via secretion of IL-2. This model is largely based on the observations that IL-2 is a potent T cell growth factor in vitro and that activated $CD4^+$ T cells produce copious amounts of IL-2. However, it remains to be elucidated whether $CD4^+$ T cell-derived IL-2 enhances $CD8^+$ T cell responses in vivo. Help of $CD4^+$ T cells to $CD8^+$ T cells may conceivably also occur by direct cell-cell contact. For example, it has been reported that ligation of CD40 expressed on $CD8^+$ T cells by CD40L expressed on $CD4^+$ T cells can boost $CD8^+$ T cell responses, although this is not observed in all model systems [104, 112, 113].

In both non-inflammatory and inflammatory conditions, memory $CD8^+$ T cell responses were found to be dependent on the presence of $CD4^+$ T helper cells during the primary response. Whether these $CD4^+$ T cells are required to instill the capacity for secondary expansion in $CD8^+$ T cells during the priming/expansion phase is not fully resolved. Work from four independent groups demonstrated that when $CD4^+$ T cells were depleted during priming, memory $CD8^+$ T cells displayed an impaired secondary expansion, suggesting that the helper T cells somehow program $CD8^+$ T cells during acute infection, and that subsequent availability of help has no influence [104, 105, 107, 108]. However, a more recent study from the Bevan group indicates that $CD4^+$ T cells are not required during but rather after the priming phase for the maintenance and optimal function of memory $CD8^+$ T cells [114]. The discrepancy between these reports necessitates further investigation and might be explained by differences between the experimental setup. Notably, the latter study analyzes the role of $CD4^+$ T cell help using TCR transgenic $CD8^+$ T cells rather than endogenous $CD8^+$ T cells, and there are indications that under certain conditions the use of TCR transgenic cells may not be optimal (see further).

Heterogeneity in effector T cell populations

Ag-specific T cells are heterogeneous with respect to many different properties, such as the effector functions they can perform and the migration pattern that they display. The Th1/Th2 paradigm is one of the best examples of functional heterogeneity within the $CD4^+$ T cell pool. Th1 and Th2 T cell populations arise from the same uncommitted naïve $CD4^+$ T cell population. Recent evidence indicates that the nature of the pathogen encountered by an APC determines which Notch ligand is expressed by that APC, and that these Notch ligands are the initial factors that instruct Th1 and Th2 differentiation [115, 116]. Th2-promoting stimuli induce expression of the Notch ligand Jagged on APCs that triggers the Notch receptor on helper T cells and induces IL-4 transcription and expression of the transcription factor GATA-3 [115]. In contrast, the Notch ligand Delta (via a pathway that probably involves T-bet) provides a signal for helper T cells to develop into Th1 cells producing high amounts of IFN-γ [115, 116]. The fate of T helper cells that have started to produce either IFN-γ or IL-4 is then reinforced through a positive feedback loop. Specifically, triggering of the IFN-γ receptor induces the expression of the transcription factor T-bet that further drives Th1 differentiation by increasing the accessibility and transcription of the *Ifng* locus. Vice versa, triggering of the IL-4 receptor induces the expression of the transcription factor GATA-3, which further drives Th2 differentiation by increasing the accessibility and transcription of the *Il4* locus. In addition, T-bet and GATA-3 are believed to induce the binding of repressive factors to the opposing locus (reviewed in [117, 118]). Collectively, these studies are providing a detailed picture of how the initial 'sensing' of the pathogen by APCs determines the activity of $CD4^+$ T cells. It seems likely that similar mechanisms control aspects such as the homing potential of helper and cytotoxic T cells (see below), but there the details are still sketchy. Furthermore, in addition to the polarizing role of APCs,

other cell types of the innate immune system, such as NK cells, are also likely to influence the functional characteristics of T helper cell responses by producing polarizing cytokines [119].

Heterogeneity in homing potential of effector T cells

The presence of pathogen at a peripheral site should not only yield an army of pathogen-specific T cells, but should preferably also provide cues to guide these cells to the site of pathogen invasion. A general mechanism that promotes accumulation of activated T cells at inflamed sites is formed by the inflammation-induced expression of extravasation-promoting signals on endothelial cells, such as chemokines and ligands for adhesion molecules. Whereas adhesion molecules initiate the first contact between T cells and the endothelium, the subsequent binding of T cells to chemokines is believed to initiate a process that allows the actual extravasation of T cells [120]. The molecules involved in this migration step can be distinct for different tissue sites. For instance, the α4β7 integrin and chemokine receptor CCR9 guide lymphocytes to the lamina propria of the small intestine and the mucosal epithelium [121–123], whereas E-selectin ligands (E-lig) and P-selectin ligands (P-lig) guide T cells to inflamed skin (fig. 3) [124, 125].

But how is the expression of these chemokine receptors and adhesion molecules on T cells regulated? Initial reports showed that the route of Ag entry determines which tissue-specific homing molecules are expressed by T cells [126, 127]. Subsequent studies demonstrated that T cells that were activated in vitro by DC from gut-draining lymph node (DLN) or skin DLN express chemokine receptors/adhesion molecules associated with gut and skin homing, respectively, and preferentially migrate to the corresponding effector site [128–130]. The notion that the Ag-presenting DC rather than the DLN in which Ag encounter takes place is responsible for the programming of homing potential receives further support from a recent study by Calzascia et al. [131]. This study demonstrates that T cells activated within the same DLN, but by DCs that drain from two different peripheral sites obtain distinct chemokine receptor/integrin expression profiles that correlate with the site of Ag entry [131]. In line with these findings, Iwata et al. recently reported that retinoic acid, a factor that is uniquely produced by intestinal DC, imprints gut-tropism into T cells (fig. 3) [132].

If programming plays an important role in determining the in vivo migration of Ag-specific T cells, one would predict that T cells found at different effector sites would be the progeny of distinct naïve T cells. What is the evidence for this? The group of Pamer showed that the TCR Vβ repertoire of H2-K^b-restricted LM-specific T cells

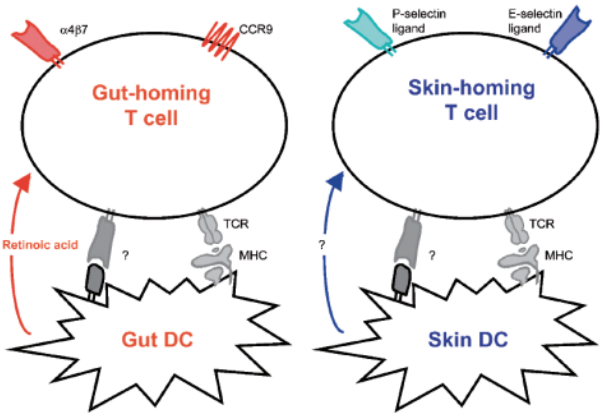

Figure 3. DC-induced acquisition of gut/skin-homing molecules during T cell activation. Retinoic acid produced by gut DCs induces expression of gut-homing molecules CCR9 and α4β7 integrin on T cells. Skin DCs, via unknown mechanisms, mediate upregulation of skin-homing molecules E- and P-selectin ligands on T cells.

in the spleen and intestinal mucosa of mice are distinct [133]. This observation is consistent with the possibility that DCs involved in primary T cell activation may program these T cells and their progeny to accumulate at either site. However, the evidence is obviously indirect. Furthermore, two other recent studies observed no significant differences in the TCR Vβ repertoire of influenza virus- and LCMV-specific CD8$^+$ T cells that were isolated from different lymphoid organs and non-lymphoid organs (liver, lung and peritoneum) [134, 135], suggesting that individual T cells can generate progeny that migrate to different sites (but see note below). The observation that T cells that appear to be the progeny of the same cell can migrate to different sites may be due to the fact that in contrast to gut tropism, liver, lung and peritoneum tropism may not be imprinted in T cells. Alternatively, recent in vitro evidence suggests that the migration properties of T cells can also be reprogrammed upon incubation with DCs derived from DLN of the opposing tissue [128, 130].

Finally, it should be noted that it is far from clear whether TCR-β chain sequencing can reliably tell whether effector T cells present at distinct sites are derived from the same naïve T cell. Thymocytes undergo a substantial proliferative burst following TCR-β chain recombination, and of the few Ag-specific T cells within the naïve T cell repertoire of a single mouse, a substantial number will have the same β chain. Thus, a similarity in TCR-β chain sequences of different T cell populations may simply be a reflection of the preponderance of that TCR-β chain in the naïve Ag-specific T cell repertoire and better techniques of tracing kinship in T cell populations will be required to elucidate these types of issues.

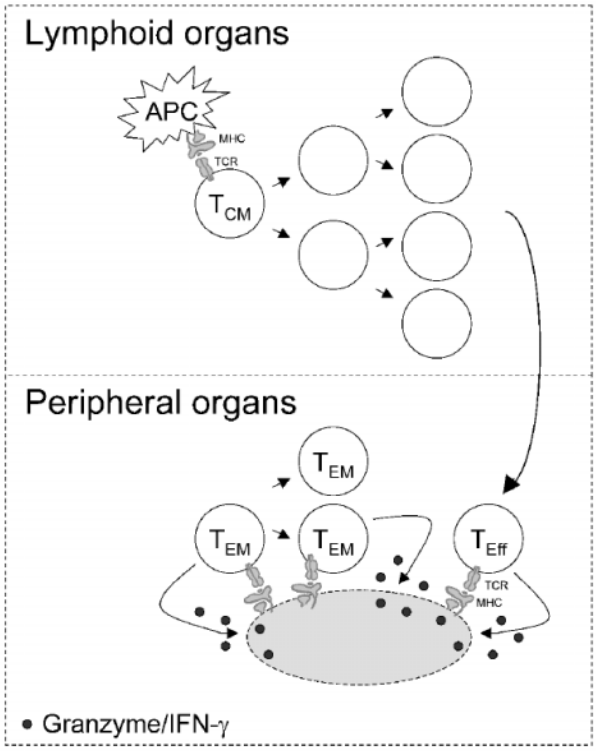

Figure 4. A model for immune-protection by central and effector memory T cells. Upon re-encounter of a pathogen, memory T cells in the periphery (effector memory T cells, T_{EM}) may function as a first line of defense by performing immediate effector functions upon Ag encounter. Should they become overwhelmed by pathogen, backup is provided by memory T cells in lymphoid tissues (central memory T cells, T_{CM}), which expand upon Ag encounter and differentiate into effector T cells.

Heterogeneity in effector function/homing potential of memory T cells

Based on the analysis of $CD4^+$ memory T cells isolated from human blood, Sallusto et al. proposed a concept in which two types of memory T cells, central and effector memory T cells, would co-exist. In this model (fig. 4), central memory T cells (T_{CM}) were defined as resting memory T cells that express CCR7 and CD62L (two molecules that are both involved in the homing of T cells to lymphoid organs) [136, 137], and that produce IL-2 and low levels of IFN-γ. Effector memory T cells (T_{EM}) were defined by lack of CCR7 and CD62L, residence in peripheral tissues and secretion of high levels of IFN-γ and low levels of IL-2 [138]. In this model, it was suggested that T_{EM} cells function as a first line of defense at mucosal sites, whereas T_{CM} cells are the back-up troops that have the capacity to proliferate and differentiate rapidly into effector cells upon renewed Ag encounter.

This model has a very clear appeal and since it was first proposed, many studies have analyzed the characteristics of both $CD4^+$ and $CD8^+$ 'T_{CM}' and 'T_{EM}' cells, which were distinguished based on differences in expression of the lymph node homing receptors CCR7 or CD62L [17, 139–142]. Before delving further into the concept of distinct memory T cell subsets, it is important to note that data from several studies suggest that the use of phenotypic markers, such as CCR7 and CD62L, to define T cells that have different migration capacities has some limitations. Although it was suggested that $CCR7^+$ T cells are not likely to migrate to peripheral organs, studies in mice and humans showed that a substantial fraction of tissue-infiltrating lymphocytes do express CCR7 [140, 143]. In addition, a fraction of T cells in human lymph nodes appear to be negative for CCR7 [143], which may be explained by the fact that T cells with low to non-detectable levels of CCR7 are still able to migrate to CCR7 ligands in a CCR7-dependent fashion [144]. Furthermore, the expression of both CCR7 and CD62L was found to be dynamic rather than static. Expression of both molecules is transiently upregulated upon activation, followed by a gradual decline [145, 146], and CD62L expression is reduced upon ligand encounter, due to shedding [147, 148]. Together, these data indicate that studying T_{CM} and T_{EM} subsets based on phenotypic markers does not equal the analysis of T_{CM} and T_{EM} subsets as based on location. Furthermore, because T_{CM} and T_{EM} cells have been proposed as T cell subsets with a role at distinct sites, isolation of the two subsets based on location may be more appropriate.

Going back to the data, what is the evidence for the T_{CM}/T_{EM} concept? *Leishmania major*-specific central memory $CD4^+$ T cells and ovalbumin-specific central memory $CD4^+$ T cells, defined based on CD62L expression and localization, respectively, produce high levels of IL-2 and low levels of IFN-γ, whereas their effector memory T cell counterparts express high levels of IFN-γ and low levels of IL-2 [142, 149]. In contrast, Unsoeld et al. found that LCMV-specific TCR transgenic $CD4^+$ central and effector memory T cells that were defined based on CCR7 expression show no difference in IFN-γ production [140]. These discrepancies might be due to differences between different pathogens or might depend on the experimental setup. Although for $CD4^+$ T cells the different memory T cell subsets were found to differ in their capacity to produce IL-2 and IFN-γ in at least some studies, for $CD8^+$ T cells no clear differences in IFN-γ production have been found between T_{CM} and T_{EM} cells [17, 140, 141, 150]. Only one study described that more IL-2 is produced in central as compared with effector memory $CD8^+$ T cells [141]. Data of Masopust et al. indicate that central and peripheral $CD8^+$ memory T cell subsets may differ in cytotoxic capacity. Specifically, vesicular stomatitis virus (VSV) and LM-specific $CD8^+$ memory T cells isolated from peripheral organs were found to have increased cyto-

toxic function compared with their splenic counterparts [150]. However, $CD8^+$ T_{EM} cells isolated from the lung of RSV, Sendai or influenza virus-infected mice exert no cytotoxicity directly ex vivo [151, 152]. In addition, there is controversy about the properties of memory $CD8^+$ T cell subsets in the LCMV model. A study by Wolint et al. showed that $CD62L^-$ LCMV-specific memory $CD8^+$ T cells have increased cytotoxic capacity compared with $CD62L^+$ $CD8^+$ memory T cells [17]. In contrast, two other studies found no difference in cytotoxic capacity between LCMV-specific central memory and effector memory $CD8^+$ T cells that were defined based on either CCR7 or CD62L expression [140, 141]. These discrepancies might again be due to differences in experimental setup. For instance, the latter two studies analyzed the properties of transferred TCR transgenic T cells rather than those of endogenous T cells.

One of the most important questions raised by the existence of different Ag-specific memory T cell subsets is their importance in protecting a host upon re-encounter of a pathogen. Zaph et al. found that *Leishmania major*-specific $CD62L^-$ $CD4^+$ T_{EM} cells are able to reduce pathogen load upon secondary *Leishmania major* infection within 3 weeks of infection, whereas a reduction in pathogen load by $CD62L^+$ $CD4^+$ T_{CM} cells required 6 weeks [142]. This would be consistent with the proposed concept that T_{EM} cells can function as a first line of defense and T_{CM} cells are the back-up troops. However, many other explanations are possible as well.

The data regarding the capacity of $CD8^+$ T_{EM} and T_{CM} cells to protect a host upon secondary infection look quite different. Wherry et al. showed that spleen-derived $CD62L^+$ $CD8^+$ memory T cells, but not $CD62L^-$ and lung-derived (70% CD62L low) $CD8^+$ memory T cells could protect naïve mice upon challenge with vaccinia virus, suggesting that $CD8^+$ T_{CM} cells are more important to protect mice from re-infection than T_{EM} cells [141]. Wherry et al. argued that this difference is likely due to the fact that $CD8^+$ T_{EM} cells have a decreased proliferative potential as compared to $CD8^+$ T_{CM} cells. However, this finding does not necessarily exclude a role for $CD8^+$ T_{EM} cells as a first line of defense. Depending on the pathogen load at a peripheral site early after infection, the different T cell subsets may alter the course of infection. Specifically, if large amounts of pathogen enter the host, locally residing T_{EM} cells that have a limited capacity to expand are likely to be insufficient. If, however, the local pathogen load is initially low, memory T cell populations that have the ability to rapidly kill the invading pathogen could make a lasting impression. Based on this argument it may be reasoned that most experimental systems in which the role of T_{EM} and T_{CM} cells are studied tend to underestimate the contribution of T_{EM} cells, because the amounts of pathogen that are introduced are likely to be higher than in most naturally occurring infections.

Lineage relationship between different memory T cell subsets

The idea that under certain conditions a particular memory T cell subset or combination of subsets would be best in mediating protection against a certain pathogen also raises the question how these T cell subsets develop and how they are maintained. Based on in vitro experiments a 'progressive differentiation model' was proposed, which suggests that depending on the strength and duration of the stimulus received by a naïve T cell, these cells will either fully differentiate into effector T cells that will then become T_{EM} cells, or arrest at an intermediate differentiation state and become T_{CM} cells [153]. Based on the finding that IL-7 and IL-15 induce differentiation of T_{CM} into T_{EM} in vitro [154, 155], it was suggested that a proportion of T_{CM} might subsequently differentiate into T_{EM} to replenish the effector memory pool without the requirement for Ag [153].

In contrast to the proposed differentiation of T_{CM} cells into T_{EM} cells, Wherry et al. showed that T_{CM} cells, as defined by CD62L expression, differentiate into T_{EM} in the presence of Ag, but not under homeostatic conditions [141]. $CD8^+$ $CD62L^-$ T_{EM} cells, on the other hand, were found to regain the expression of $CD62L^+$ in an Ag-independent fashion over a time period of 6–10 weeks, which made the authors conclude that naïve T cells might progressively differentiate following a linear, naïve -> effector cells -> T_{EM} cells -> T_{CM} cells, pathway [141]. However, a study by Bouneaud et al. recently showed that T_{EM} cells that have regained CD62L expression behave like T_{EM} cells rather than T_{CM} cells in that they are unable to mount a proliferative response upon a secondary challenge, indicating that acquisition of one of the characteristics of T_{CM} cells is not necessarily coupled to acquisition of other T_{CM} characteristics [139]. In addition, a recent paper by the group of Lefrancois shows that depending on the number of transferred TCR transgenic T cells, $CD8^+$ T_{EM} cells either do or do not develop into T cells with a T_{CM} phenotype [156]. Specifically, when only low numbers of TCR transgenic T cells were transferred, T_{EM} cells maintained their low CD62L phenotype, suggesting that T_{EM} cells do not differentiate into T_{CM} under physiological conditions. On a more general note, this latter study stresses that results obtained using transfer of high numbers of TCR transgenic T cells should be interpreted with caution.

A study by Klonowsky et al. that analyzed the migration patterns of $CD8^+$ memory T cells using parabiotic mice showed that $CD8^+$ memory T cells in peripheral tissue as well as in lymphoid organs originate from blood-borne $CD8^+$ memory T cells that continuously seed tissues after contraction of the immune response [157]. As the origin of the blood-borne $CD8^+$ T cells remains unclear, these data may be explained by three different models. First, memory T cells might form one large pool of T cells in

which individual cells continuously change their migration properties and localization, perhaps as a consequence of environmental signals. Second, these data might be interpreted as evidence for a blood-borne/lymphoid pool of memory T cells (for instance the memory T cell pool in the bone marrow [158]) that continuously gives rise to T cells that seed peripheral tissues. Third, the memory T cell pool might consist of several smaller pools of T cells that travel back and forth from the blood into the tissue, of which each pool has its specific tissue tropism.

Conclusions and future perspectives

The introduction of techniques to visualize Ag-specific T cells at the single cell level has dramatically improved our understanding of the regulation of Ag-specific $CD4^+$ and $CD8^+$ T cell responses during acute infections. Although $CD4^+$ and $CD8^+$ T cell responses go through the same phases of expansion, contraction and memory maintenance, Ag-specific $CD4^+$ T cell responses are generally much smaller in size than Ag-specific $CD8^+$ T cell responses. Additional differences between $CD4^+$ and $CD8^+$ T cell responses have been reported, such as differences in kinetics and memory maintenance. However, these differences are not observed in all infection models, indicating that they are unlikely to be due to intrinsic differences between $CD4^+$ and $CD8^+$ T cells, but rather depend on the type and amount of pathogen used. This is analogous to findings on the role of $CD4^+$ T cell help in primary $CD8^+$ T cell responses, where also the infectious agent, and more specifically the inflammatory milieu that the infectious agent creates, determines whether the primary $CD8^+$ T cell response depends on $CD4^+$ T cell help or not.

Clearly not only the Ag-specific interaction but also additional pathogen-derived cues are involved in shaping the ensuing T cell response. Rather than directly receiving signals via pathogens, T cells receive most of their signals via APCs in the form of Ag presented in MHC molecules, costimulatory molecules and cytokines. Interestingly, a brief interaction of a T cell with a stimulating APC not only determines the short-term action of that particular T cell, but also instructs what will happen to its progeny. This includes its capacity to proliferate and differentiate into effector cells, its ability to react to certain chemokines and possibly also its survival into the memory T cell pool. This type of fate determination may be based on mechanisms similar to those driving the development of the Th1 and Th2 subsets, where polarization was found to be dependent on a cytokine feed-back loop that promotes the differential expression of transcription factors and progressive changes in chromatin structure. For instance, the finding that precursors of $CD8^+$ memory T cells express the IL-7 receptor, which is involved in regulating homeostatic proliferation of memory T cells, suggests that triggering of this receptor might drive the differentiation of these cells. However, many of the molecular mechanisms that underlie the programming of the expansion, differentiation, migration and memory formation of T cells remain to be elucidated. The existence of different subsets within the $CD4^+$ and $CD8^+$ T cell pool raises the question how these subsets are related. Although adoptive transfer of different T cell subsets has been used to provide some answers, many questions remain open. Rather than studying the fate of a pool of (supposedly) homogenous adoptively transferred T cells, one ideally would like to track the progeny of individual T cells. For this purpose, we are developing a novel approach in which individual T cells are tagged with a genetic barcode using retroviral transduction. This tagging of individual cells with unique identifiers coupled to a microarray-based detection system may conceivably be used to further improve our understanding of the family relationships between distinct cell populations.

The insights gained on the development of Ag-specific $CD4^+$ and $CD8^+$ T cell responses upon acute infection should be valuable for the improvement of vaccination strategies. Studies from the past years have taught us how many Ag-specific T cells we have and what they look like. In future experiments, we should focus on which subsets of Ag-specific T cells we need to control a given pathogen, and how we can best generate this subset by vaccination.

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