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'Stem-like' precursors are the fount to sustain persistent CD8$^+$ T cell responses

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Virus-specific CD8$^+$ T cells that differentiate in the context of resolved versus persisting infections exhibit divergent phenotypic and functional characteristics, which suggests that their differentiation trajectories are governed by distinct cellular dynamics, developmental pathways and molecular mechanisms. For acute infection, it is long known that antigen-specific T cell populations contain terminally differentiated effector T cells, known as short-lived effector T cells, and proliferation-competent and differentiation-competent memory precursor T cells. More recently, it was identified that a similar functional segregation occurs in chronic infections. A failure to generate proliferation-competent precursor cells in chronic infections and tumors results in the collapse of the T cell response. Thus, these precursor cells are major therapeutic and prophylactic targets of immune interventions. These observations suggest substantial commonality between T cell responses in acute and chronic infections but there are also critical differences. We are therefore reviewing the common features and peculiarities of precursor cells in acute infections, different types of persistent infection and cancer.

The mammalian immune system rapidly clears most infections and forms functional memory T (T_{mem}) cells, which protect the host upon reencountering the same or a cross-reactive pathogen. Nonetheless, several viruses bypass this elimination and establish either a latent chronic infection, characterized by intermittent virus reactivation, or a chronic infection, in which virus particles are continuously produced. We commonly refer to the latter as 'active chronic infections' (Box 1). Latent infections are typically caused by herpesviruses such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), or herpes simplex virus (HSV) and active chronic infections by the hepatitis B virus (HBV) and hepatitis C virus (HCV) or human immunodeficiency virus (HIV) in humans and certain lymphocytic choriomeningitis virus (LCMV) strains in mice. In active chronic infections, T cells often show the phenomenon of T cell exhaustion, referring to T cells with diminished effector functions compared to effector and memory T cells, which develop in resolved infections as reviewed elsewhere[1–7]. By contrast, pathogen-specific T cell populations in latent infections remain functional and can, for instance in CMV infections, considerably grow in size—a phenomenon known as memory inflation[8].

Irrespective of these differences, there are core principles that universally apply to T cell responses across acute and chronic infections. These involve the effective recruitment, massive expansion of pathogen-specific naive or previously formed T_{mem} cells, and the formation of short-lived, terminally differentiated effector T (T_{eff}) cells. T_{eff} cells massively decline in numbers following the acute infection phase. For acute and latent infection, it is well established that this decline coincides with the appearance a subpopulation known as memory precursor T (T_{mp}) cells (see Box 1 for nomenclature details), which retain proliferative potential and progressively develop into T_{mem} cells that then can give rise to T_{eff} cells, either by antigen reexposure following a secondary infection or following a reactivation of a latent infection[9,10]. T_{mem} cells are often further divided into: (1) central memory T (T_{cm}) cells that bear strong proliferative capacity, (2) effector memory T (T_{em}) cells, which exhibit limited survival but more immediate effector functions, and (3) stem-cell memory T (T_{scm}) cells—a population related to T_{cm} with superior re-expansion capacity (Box 1). In addition, there are tissue-confined T cells known as tissue-resident memory T (T_{rm}) cells.

Reports about a limited survival and re-expansion potential of exhausted T cell populations spurred the assumption that proliferation-competent T cells with memory potential are not formed in active chronic infections. In contrast, we know now that they also contain a small subset of proliferation-competent precursors of exhausted T (T_{pex}) cells (Box 1) that express the transcription factor T cell factor 1 (TCF-1) encoded by *Tcf7*, which are preferentially found in secondary lymphoid organs and in the blood of mice[11–16]. These precursors are essential for the overall maintenance of the terminally differentiated (exhausted) T cell population (T_{ex} cells; Box 1). Thus, the branching of activated and expanding T cells into terminally differentiated cells and into cells with proliferative potential is a universal core feature of the CD8$^+$ T cell response in acute and chronic infections and we know that these cells are formed in acute and in chronic infection irrespective of antigen persistence or clearance (Figs. 1a–c and 2) and in the context of tumors. Given the importance of these proliferation-competent precursors (T_{pex} and T_{mp} cells), we summarize the current knowledge about particularities and molecular mechanisms that apply to these precursors in acute, latent and chronic infection and cancer.

The dynamics of proliferation-competent precursors in different infections

Even though proliferation-competent T cells are an integral part of all T cell responses, their development is typically masked by the much larger terminally differentiated T_{eff} cell population. For instance, our linear illustration of T cell response kinetics in acute

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Box 1 | Glossary

Given the parallel existence of different nomenclatures for subsets of specific phenotypes of $CD8^+$ T cells, we clarify below the terms used throughout the paper:

T cell exhaustion
State of T cell differentiation, often emerging during continuing antigen exposure in chronic infection and cancer, goes typically along with reduced effector function and expression of inhibitory receptors

Also known as T cell dysfunction and chronic T cell phenotype

Polyfunctional T cells
T cells that coexpress effector molecules and cytokines such as IFNγ and TNF, antigen-expanded cells typically formed in acutely resolved infections

Also known as acute T cell phenotype

T_{ex} cells
Antigen-expanded TCF-1^- cells that express markers of T cell exhaustion

T_{pex} cells
Antigen-expanded TCF-1^+ cells that express markers of T cell exhaustion

Also known as stem-like progenitors, progenitors, memory-like T cells, proliferation-competent progenitor cells and proliferation-competent precursor cells

T_{mx} cells
Cells that coexpress features of T_{mem} cells such as CD127 expression along with markers of T cell exhaustion; these cells may stem from resolved infection or from acute infection in which T cells are very strongly stimulated

T_{eff} cells
Antigen-expanded TCF-1^- cells that arise during an acute infection and do not (or only temporally) express markers of T cell exhaustion

Also known as short-lived effector T cells

T_{mp} cells

Antigen-expanded TCF-1^+ subsets that emerge during an acute infection, do not express markers of T cell exhaustion, and preferentially give rise to memory cells

This cell subset is often referred to as memory precursor effector cells (MPECs); however, we refrain from calling them effector cells, as they diverge from a terminally differentiated effector fate

T_{mem} cells
Antigen-expanded T cells that are found in resolved (or latent) infection after the T cell contraction phase.

Subsets include:

- T_{cm} – memory population that is found in secondary lymphoid organs.
- T_{em} – memory population that is found outside of secondary lymphoid organs, bears immediate effector function and limited secondary expansion capacity.
- T_{em}-like – memory population that is found within and outside secondary lymphoid organs in the context of reactivating persistent infections, which bears immediate effector function and limited secondary expansion capacity but compared to T_{eff} cells is much more long-lived depending on IL-15 signaling.
- T_{scm} – a subset of $CD45RA^+CD45RO^-CCR7^+CD95^+$ T cells that is typically described in humans and which is distinct from T_{cm} and T_{em} cells, displaying enhanced self-renewal and multipotency.

Active chronic infection
Infection that persists with continuous virus production and virus release

Latent viral infections
Infections with mostly members of the herpesvirus family that initially cause lytic infection, followed by viral latency with limited viral gene expression; reactivation and production of virions can occur in latently infected cells

infections, which includes expansion, contraction, T_{mem} cell formation and maintenance phase (Fig. 1a), does not reflect the dynamics nor do any of the three main models discuss how T_{mp} cells are formed. These models include: (1) a bifurcation model claiming that effector and memory T cell trajectories segregate at a certain time point following an asymmetric cell division (Fig. 2a); (2) a progressive differentiation model demonstrating that more extensively dividing cells become effector and less-stimulated cells become memory precursor cells (T_{mp}; Fig. 2a); and (3) a third and not mutually exclusive model showing that some T cells transition from an effector stage into T_{mem} cells (Fig. 2a). Experimental evidence exists that supports each of these models[17–22], leaving the origin of T_{mem} cells unresolved. What is undisputed by all models is the existence of a trajectory, which connects the different types of proliferation-competent naive and memory T cells or, for instance, primary T_{mem} cells with secondary T_{mem} cells. Along this trajectory, cells transition from a resting state through a phase of rapid proliferation, followed by a partially activated T_{mp} cell state, before the cells return to quiescent T_{mem} cells, which only occasionally homeostatically proliferate. Upon reencountering antigen, T_{mem} cells again move through these differentiation phases. Because of this repeating nature, the dynamics of proliferation-competent cells can be viewed as a circular process (Fig. 2b).

In active chronic infections, the continuation of antigen exposure interrupts this circle and counteracts the return of proliferation-competent T_{pex} cell to the quiescent T_{mem} cell state. Instead, T_{pex} cells continuously proliferate to reproduce themselves and to generate T_{ex} cells (Fig. 2c). Of note, bromodeoxyuridine (BrdU) incorporation studies revealed that proliferation occurs at a much slower pace than in the acute infection phase. This slower pace likely accounts for the contraction phase that is detectable even in active chronic infection after a certain time and despite abundant antigen being present[23–25] (Fig. 1c). Another possible reason for this slowdown of proliferation is a reduced level of activation, owing to negative regulation by co-inhibitory receptors[26].

In contrast, latent infections show a mixed phenotype that combines features of resolved and active chronic infections. Here, the intervals and the magnitude of pathogen reactivation determine the response pattern. With more frequent reactivation of the proliferation-competent precursors, a larger population of T cells with a T_{em} cell phenotype is induced[27–29] in a process known as memory inflation (Fig. 1b). Of note, the proliferation-competent cells established in CMV infections do not exhibit signs of exhaustion in healthy individuals. Instead, they resemble T_{cm}-like cells established in resolved infection[8]. This observation indicates that the extent of (recurrent) antigen exposure is a critical determinant in

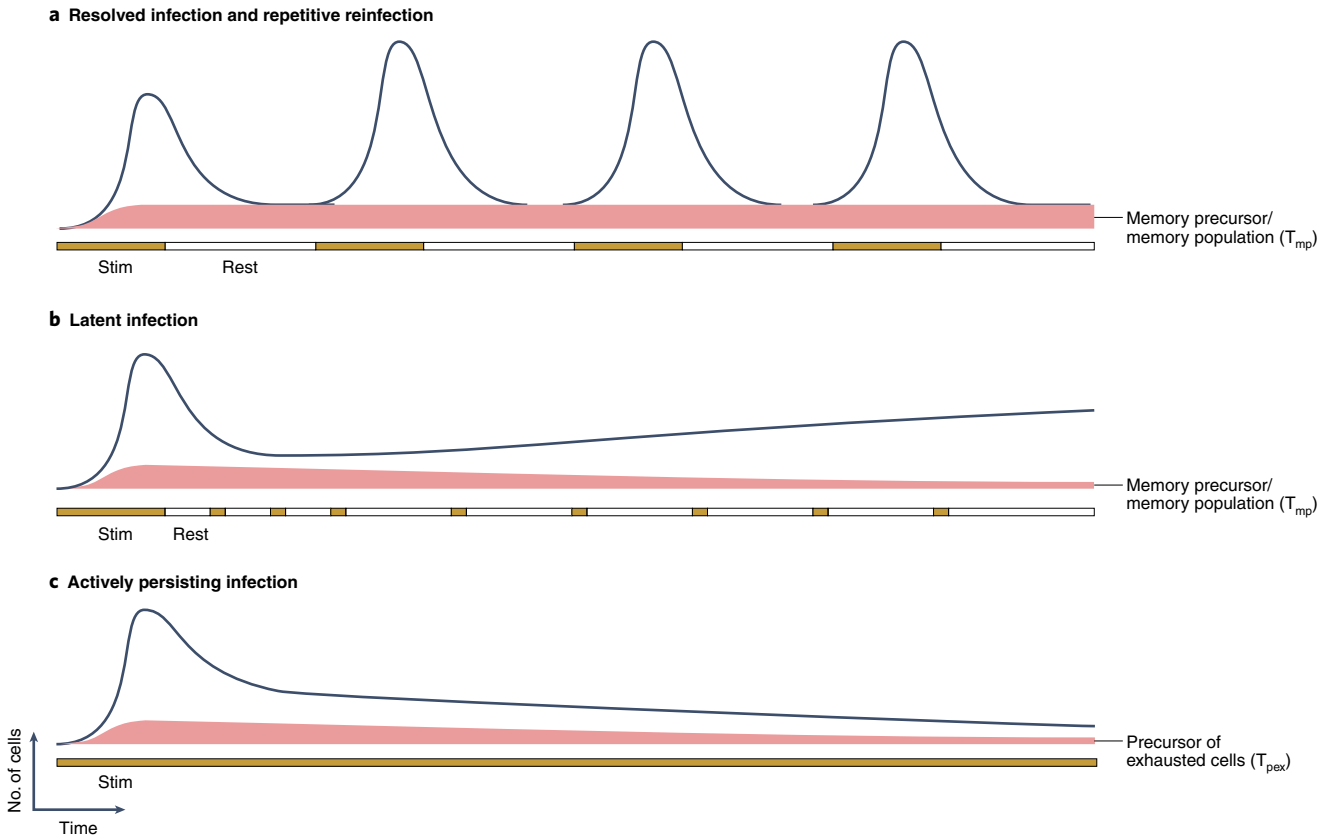


Fig. 1 | Illustration of the dynamics of antigen-specific T cell populations following reoccurring infections versus latent or active chronic infections. **a**–**c**, White area under the curve indicates the total magnitude of the antigen-specific T cell response, while the red area under the curve indicates the fraction of proliferation-competent precursor cells. Yellow intervals illustrate the frequency of reactivation. The black line in **b** increases over time to indicate the rise in terminally differentiated effector cells during inflating T cell responses.

shaping the phenotypic and functional profile of T cells in persisting infections[25].

Specific features of T_{pex} cells and their descendants

The identification of T_{pex} cells resolved the long-standing question of how short-lived, terminally differentiated T_{ex} cells are maintained long term and how T_{ex} cells could be reinvigorated via immune checkpoint blockade (ICB). In murine chronic LCMV infection, T_{pex} cells express TCF-1, CXCR5, Ly108, CD73, ID3 and low levels of CD127 and CD62L, while they lack expression of TIM-3 (refs. [13,14,30]). TCF-1 is essential for the formation and function of this precursor population. Interestingly, its absence results in a normal primary T cell expansion (Fig. 2c), but T cells steadily decline over time[14]. This response resembles the pattern seen with TCF-1-deficient T cells in acute LCMV infection, where effector but not functional T_{mp} cells are formed without TCF-1 (refs. [31,32]). Recently, T_{pex} cells were further divided into TCF-1$^+$Ly108$^+$CD69$^+$ triple-positive T_{pex}^1 cells, which then transition into further differentiated TCF-1$^+$Ly108$^+$CD69$^-$ double-positive T_{pex}^2 cells. Likewise, T_{pex}^2-derived terminally differentiated cells can be divided into CD69$^-$Ly108$^-$ (T_{ex}^{Int}) cells that generate the most terminally differentiated CD69$^+$Ly108$^-$ T_{ex} cell population[33] (Fig. 2a). Similarly, exhausted cell populations were also divided into TCF-1$^+$Ly108$^+$ precursors, Ly108$^-$CX3CR1$^-$ double-negative terminal cells, and into CD4-help-dependent CX3CR1$^+$ cells[34,35], or into TCF-1$^+$CD101$^-$TIM3$^-$ precursors, CD101$^-$TIM3$^+$CX3CR1$^+$ transitional cells and CD101$^+$TIM3$^+$ double-positive terminal cells[36]. Despite the use of different markers, all studies depict similar differentiation concepts for exhausted T cell populations.

A key feature of T_{pex} cells is that they are stably committed to exhaustion and they transmit an exhausted phenotype to their progeny. Thus, even when T_{pex} cells from a chronic infection are experimentally transferred into new hosts experiencing an acute infection, their progeny retains an exhausted phenotype[37]. Similar observations were made following ICB. While the treatment boosts the overall T cell response and their effector capacity in chronic infection and tumors, the progeny of the reactivated T_{pex} cells still shows core features of exhausted T cells[38]. We know now that this stability is enforced by epigenetic imprints that, once established, cannot be overcome by reactivation of the T_{pex} cell or by checkpoint inhibition[37–40]. Several observations have indicated that a full commitment to T cell exhaustion requires time, such that an early transfer of virus-specific CD8$^+$ T cells exposed to a chronic infection into a new setting of an acute infection prevents the acquisition of an exhausted phenotype[41]. Similar observations were made in the context of tumor-reactive T cells in mice[40]. Nonetheless, transcriptional and certain epigenetic differences between T_{pex} and T_{mp} cells can be detected as early as 5 days after infection[42]. Thus, the commitment toward T cell exhaustion begins early but requires time to stabilize.

This commitment to the generation of T cell exhaustion involves distinct metabolic states[43,44] and epigenetic mechanisms, including the activity of methyltransferases such as Dnmt3a[39], and also require continued high-level antigen exposure and strong T cell antigen receptor (TCR) stimulation that lead to NFAT-induced transcriptional networks. The transcription factors such as NR4A1, NR4A2 and TOX are essential for inducing or enforcing T cell exhaustion[45–51]. In addition, interleukin-2 receptor (IL-2R) signaling was shown to promote T_{ex} cell differentiation at the expense of Tpex cell

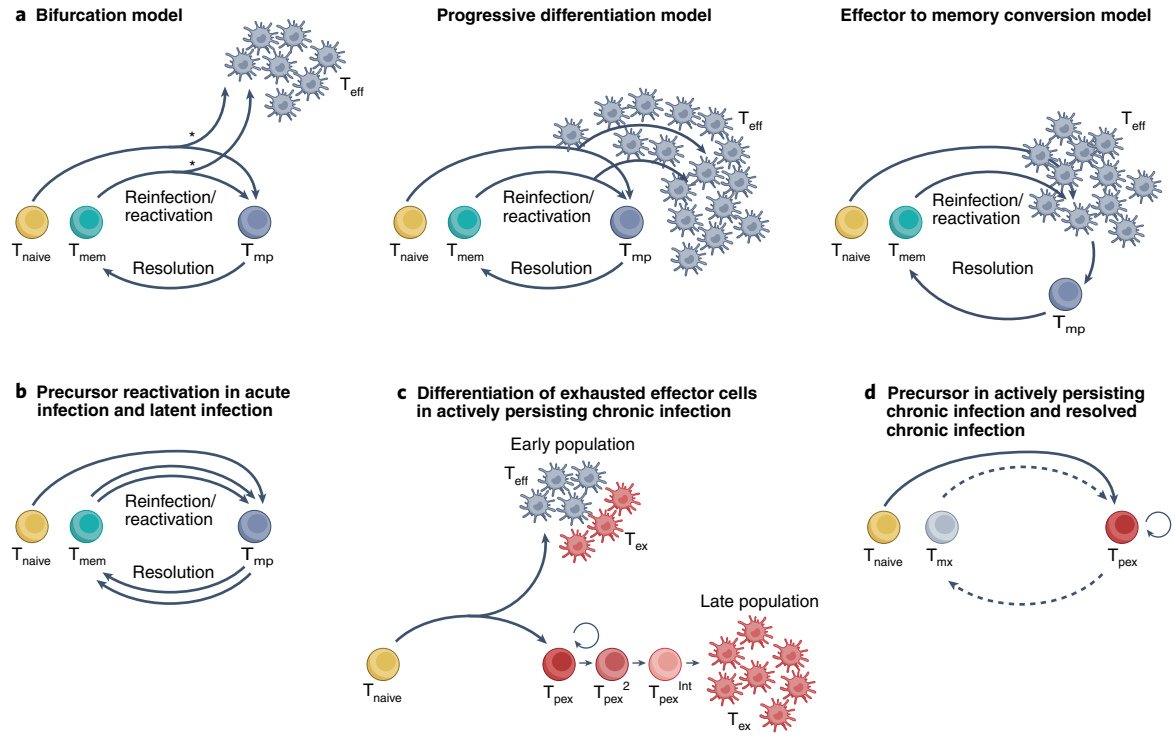


Fig. 2 | The life cycle of precursor T cells in acute and chronic infection. In both acute and chronic infections, naive T cells differentiate during the T cell expansion phase into short-lived effector cells and proliferation-competent precursor cells. We refer to these latter cells in acute infections as T_{mp} cells and in active chronic infection as T_{pex} cells. **a**, Three possible scenarios for the generation of effector and memory T cells are: the bifurcation model according to which effector and memory T cells are formed independently of each other (left), the progressive differentiation model proposing that cells with fewer rounds of division form T_{mp} cells, while more excessively proliferating cells generate primarily T_{eff} cells (middle), and the progression of memory T cells through an effector stage (right). **b**, Upon resolution of the acute infection, T_{mp} cells transition into T_{mem} cells, which themselves upon reinfection can differentiate again into T_{mp} cells and subsequently into secondary T_{mem} cells. **c**, The initial mixed short-lived T_{eff} (blue) and T_{ex} (red) cell population is generated independently of TCF-1-expressing T_{pex} cells, but this initial population becomes replaced at later time points by T_{ex} cells derived from a self-maintaining T_{pex} population. T_{pex} cells are permanently exposed to antigen (albeit being considerably shielded against activation by the expression of co-inhibitory receptors), leading to their frequent (or permanent) activation, proliferation and differentiation into Tex cells. The latter follows a trajectory including T_{pex}^{2} and T_{ex}^{Int} subpopulations. **d**, In case of an eventually occurring resolution of a chronic infection, or due to a loss of a particular epitope due to virus escape, T_{pex} cells likely transition into a resting population, which combines features of memory and of exhausted cells. These T_{mem} cells with signs of T cell exhaustion (T_{mx}) appear to have the capacity to regenerate T_{eff} cells with an exhausted phenotype upon secondary stimulation (gray dashed arrows).

formation[52], while IL-21R signaling at late time points prompted T_{pex} cell formation[53]. The transcription factor TOX is particularly interesting as its absence results in a T cell population with higher functional capacity and markedly reduced signs of exhaustion, including lower PD-1 expression. While the mechanisms by which TOX exerts this function remain unclear, it appears to be involved in establishing the exhausted phenotype in T_{pex} cells (discussed below)[46]. Nonetheless, it needs to be underlined that we are still far from fully understanding the molecular mechanisms that induce T cell exhaustion.

The functional similarities between T_{pex} and T_{mp} cells in chronic and acute infections, respectively, suggest that they are related populations. Although this notion holds true for certain aspects such as their ability to regenerate themselves and to generate more differentiated cells (stem-cell-like properties), there are also notable differences. Unlike T_{mem} cells, T_{pex} cells show markers and features of T_{ex} cells including elevated levels of programmed death protein 1 (PD-1) and compromised tumor necrosis factor (TNF) and interferon-γ (IFNγ) secretion. T_{pex} cells also differ from T_{mem} cells in forming and surviving independently of $CD4^+$ T cell help[34,35], which is required to form or maintain T_{mem} cells in resolved infections[10]. By contrast, T_{ex} cell differentiation and, in particular, the CX3CR1 subset strongly depends on CD4 help and the provision of interleukin (IL)-21 (refs. [34,35]), while T_{eff} cells in acute LCMV infections can be formed independently of CD4 help[54]. Thus, T_{pex} and T_{ex} cells show divergent CD4 help requirements compared to T_{mem} and T_{eff} cells in resolved infections.

While we clearly understand now that T_{pex} cells feed into the pool of $CD8^+$ T_{ex} cells in an antigen-dependent manner, it is less frequently considered that T_{pex} cells can survive following antigen withdrawal[37,55]. In fact, we consider that T_{pex} cells transition in the absence of antigen into cells with self-renewal capacity similar to that of conventional memory cells. In case of antigen reencounter, they then mount a recall response and produce terminally differentiated progeny (Fig. 2d)[14,37]. Related to this, it was recently identified that healthy humans harbor antigen-specific T cells that combine features of T_{mem} cells and exhaustion. These features include the expression of PD-1 and TIGIT inhibitory receptors and enhanced chromatin accessibility at exhaustion-related genomic sites, for instance, in the *TOX*, *PDCD1* and *TIGIT* genes. These exhaustion traits are stable following activation and effector differentiation in vitro[56] and are associated with enhanced response to immunosuppressive transforming growth factor-β (TGFβ) and reduced anti-leukemic activity when these cells are used as hosts for chimeric antigen receptor (CAR) expression in a humanized model of leukemia[56]. Intriguingly, the TCR clonal repertoire of these memory cells was largely non-overlapping with that of $PD\text{-}1^-TIGIT^-$ T_{mem} cells. This finding implies that a specific set of antigens and/or stimulation

conditions generates this specific cell population. Presently, we lack a name for these cells and we propose to refer to them as descendants of T_{pex} or T_{mx} cells (for T_{mem} cells with exhausted features). While more research is needed to understand their biology, we consider that these cells might originate from resolved active chronic infection (Fig. 2d), possibly from latently persisting pathogens, or from an acute infection with very strong T cell stimulation that induces T cell phenotypes seen otherwise in active chronic infection. In fact, different reports indicate that severe acute respiratory syndrome coronavirus 2 infection induces signs of T cell exhaustion[57]. This transition of T_{pex} cells into T_{mx} cells might at first glance appear as a rare scenario but these cells are readily found in humans. Of note, similar features can be seen in antigen-escape situations or pharmacologically resolved active human chronic infections, both of which support the formation of CD127-expressing cells featuring signs of T cell memory and exhaustion, simultaneously[55].

Molecular determinants of precursor T cells. A large number of phenotypic markers, distinct transcriptional networks and specific epigenetic and metabolic profiles impact the differentiation of exhausted T cell populations[1,3,7,58]. Most of these features were established irrespective of the subsets of exhausted T cells. It is therefore important to identify how these mechanisms operate in T_{pex} cells. Here, we will mainly focus on a selection of molecules that were shown to affect the establishment or maintenance of the TCF-1$^+$ T_{pex} cells.

PD-1, encoded by *Pdcd1*, was the first co-inhibitory receptor found to be constitutively expressed on exhausted T cells[59]. Its expression is regulated by the transcription factors NFAT and T-bet following T cell activation[60,61]. *Pdcd1* deletion can result in lethal CD8$^+$ T cell-mediated immunopathology[59,62]. PD-1 acts directly on T_{pex} cells, as adoptive transfer of LCMV-specific PD-1-deficient CD8$^+$ T cells revealed compromised long-term maintenance combined with enhanced differentiation into T_{ex} cells, suggesting that PD-1 is at least partly preserving T_{pex} cells[63]. This conclusion was confirmed by the demonstration that PD-1 stabilized the T_{pex} cell population[64].

TCF-1 is a hallmark transcription factor for the formation and/or maintenance of T_{pex} cells[2]. It playshas a central role in the early bifurcation of terminal effector and T_{pex} cells by repressing the former and stabilizing the latter. This T_{pex}-stabilizing function relies on promoting EOMES and c-MYB expression, which antagonize terminal differentiation and potentially promote survival by supporting BCL-2 expression[64]. In addition, TCF-1 induces BCL-6 expression in virus-specific CD8$^+$ T cells and this counteracts terminal differentiation by antagonizing type 1 interferon signaling[16], while absence of TCF-1 impacts T cell maintenance without affecting the expression of exhaustion-related genes. Of note, it is important to mention that TCF-1 also plays a key role in other cells that bear strong proliferative potential such as naive, T_{mem} and T_{mp} cells.

ID3, a transcriptional regulator, is expressed in T_{pex} cells and sustains their survival by decreasing susceptibility to Fas–Fas ligand-mediated cell death[65]. Within T_{pex} cells, regulatory regions of the *Id3* gene were more accessible than in T_{ex} cells, whereas *Id2* regulatory regions were more accessible in T_{ex} cells[42,66].

The transcription factors IRF4 and BATF are highly expressed in T_{ex} cells in response to sustained TCR stimulation in a largely NFAT-dependent manner, promoting a transcriptional and metabolic profile of terminal exhaustion. This includes PD-1 expression, reduced glycolysis and oxidative phosphorylation and repression of TCF-1. Deletion of one *Irf4* allele promoted memory-like T cell development in chronic LCMV infection[67]. Overexpression of BATF in murine CAR T cells early after activation results in preferential cooperation with IRF4 to promote effector cell differentiation at the expense of entering the exhaustion lineage[68]. Similar data were obtained by overexpressing the AP-1 family member c-Jun, a transcriptional partner of BATF and IRF4, in human CAR T cells[69].

The transcription factor TOX (thymocyte selection-associated high-mobility group box protein) is induced by strong TCR stimulation in conjunction with calcineurin and NFATC2 signaling[45–47]. Multiple studies identified TOX as key factor for the establishment and maintenance of exhausted T cells in chronic viral infection or cancer, but not for the formation of T_{eff} cells and T_{mem} cells in acute infection[46,47,49]. Mechanistically, TOX supports imprinting of an exhausted phenotype in T_{pex} cells, which is then passed on to their progeny. Absence of TOX results in the retention of an acute phenotype in T_{pex} cells, in a failure to maintain the T_{pex} cell population, and over time in a loss of the T_{ex} cell population in chronic infection and in tumors[46,48,70]. This loss may involve direct regulation of TCF-1 (ref. [70]), but there is no tight dependence as TCF-1 is expressed normally in TOX-deficient cells in acute infections[46]. Alternatively, reduced inhibitory receptor levels, such as PD-1, in the absence of TOX may lead to an overstimulation and terminal differentiation of T_{pex} into T_{ex} cells. TOX expression seems to be particularly important for the instruction or epigenetic fixation of an 'exhaustion program', as delayed TOX inactivation (20 days after infection) did not show phenotypical or functional consequences[46].

EGR2 (early growth response 2) is an anergy-associated transcription factor selectively expressed in CD8$^+$ T_{pex} cells in chronic viral infections and tumors. Akin to TOX, EGR2 stabilizes the exhausted transcriptional state on the transcriptional and epigenetic levels[71]. In addition, EGR2 maintains an exhausted signature in the TCF-1$^-$ descendants of TCF-1$^+$ precursors via epigenetic repression of AP-1 family transcription factors[71].

EOMES and T-bet are key transcription factors involved in effector, memory and exhausted CD8$^+$ T cell differentiation[11,72,73]. In T_{ex} cells, an increased ratio of nuclear EOMES to T-bet is found compared to T_{mem} cells in mice and humans[74,75], leading to weaker repression of *Pdcd1* as opposed to memory cells with a higher ratio of T-bet to EOMES. Enforced nuclear localization of T-bet in T_{ex} cells leads to effector-like differentiation, contrasting with the high EOMES/T-bet ratios that are indicative of terminally exhausted CD8$^+$ T cells and which are responsible for shielding memory-like CD8$^+$ T cells from differentiating into terminal T_{eff} cells[74].

BACH2 and BATF are transcription factors involved in opposing functions in the generation of early T_{pex} cells. BACH2 was shown to be upregulated in T_{pex} cells[42,70]. BACH2-deficient CD8$^+$ T cells were impaired in the generation of early T_{pex} cells, whereas overexpression of BACH2 had the opposite effect[70]. Mechanistically, overexpression of BACH2 inhibited the function of IRF4 and AP-1 family members (such as BATF) that support proliferation and effector cell differentiation[42] and instilled the differentiation of memory-like CD8$^+$ T cells by repressing the expression of *Prmd1*, which encodes the transcription factor BLIMP-1 (ref. [70]). During later phases of chronic LCMV infection, BATF and to some extent T-bet activity induced the differentiation of T_{pex} cells toward a CX3CR1$^+$ effector-like population[76], a transitory state that eventually feeds into T_{ex} cells[36].

BLIMP-1 is a zinc-finger-containing transcriptional repressor whose role in promoting terminal differentiation was initially described for B cells. In the chronic LCMV infection, Blimp-1 promoted the terminal differentiation of virus-specific CD8$^+$ T cells and positively regulated expression of many co-inhibitory receptors. Conditional deletion of *Prdm1* in CD8$^+$ T cells promoted the formation of T_{pex} cells[77].

Nuclear receptor subfamily 4 group A (NR4A) factors also have roles in CD8$^+$ T cell fate decisions. In the setting of cancer-reactive CD8$^+$ T cells, NR4A-deficient tumor-infiltrating CD8$^+$ T cells exhibited lower TIM-3 expression as compared to their wild-type counterparts and showed increased functionality with respect to TNF and IFNγ expression after restimulation. NR4A-deficient tumor-infiltrating CD8$^+$ T cells exhibited a gene expression profile characteristic of T_{eff} cells, evidenced by low TCF-1 expression, suggesting

that NR4A transcription factors serve to inhibit terminal differentiation and hence to maintain the T_{pex} cell population[78].

Taken together, a number of regulatory pathways can promote or impair the generation and maintenance of the T_{pex} cell population, including cell surface receptors (PD-1) and transcription factors or regulators (TOX, TCF-1, ID3, EGR2, BACH2, BATF, IRF4, BATF, BLIMP-1, NR4As and ratios of EOMES to T-bet). Given the importance of T_{pex} cells to sustain CD8$^+$ T cell responses in chronic infection and cancer, the discovery of additional regulatory pathways is warranted.

Precursors in exhausted human T cells. Chronic HCV infection in humans differs in many aspects from chronic LCMV infection in mice, including that HCV infections are organ specific rather than systemic. Nevertheless, both infections display key similarities that have, in a bidirectional fashion, nurtured our mechanistic understanding of T cell exhaustion[14,37,46,55,79,80]. For example, T cell responses in HCV also branch into TCF-1$^+$CD127$^+$Bcl-2$^+$ antigen-specific CD8$^+$ T cells alongside EOMES^{hi}CD127$^-$CD38$^+$ terminally differentiated cells[14,55]. As in chronic LCMV infection, the TCF-1$^+$CD127$^+$ population expands more than TCF-1$^-$CD127$^-$ cells upon antigen stimulation, suggesting a similar potential of TCF-1$^+$ cells to maintain HCV-specific T cell populations. Comprehensive transcriptome analysis indicates that the TCF-1$^-$CD127$^-$ subset closely resembles LCMV-specific T_{ex} cells, with conventional memory signatures being absent. Vice versa, TCF-1$^+$CD127$^+$ cells express several markers typically detected in T_{pex} cells in chronic LCMV infection[37]. At the same time, the expression of exhaustion-specific markers can be detected in TCF-1$^+$CD127$^+$ and TCF-1$^-$CD127$^-$cells as seen among LCMV-specific T_{pex} cells and T_{ex} cells[13,42,46,79–82]. In addition, virus-specific CD8$^+$ T cells with a phenotype similar to T_{pex} cells have also been identified in human chronic HBV[83] and HBV/HDV infection[84,85], underscoring similar precursor-mediated T cell maintenance mechanisms across different chronic human viral infections. Detailed unbiased single-cell RNA-based profiling even revealed a CD127^{int} cell cluster that is situated between T_{pex} and T_{ex} cells[82], which further resembles the abovementioned diversity of exhausted T cells in chronically infected mice[33]. These developmental relationships are also underscored by TCR clones shared between these subclusters[82]. We therefore recommend to refer to HCV-specific and HBV-specific T cells in chronic infections that express hallmark features of T cell exhaustion also as T_{pex} cells and T_{ex} cells, respectively.

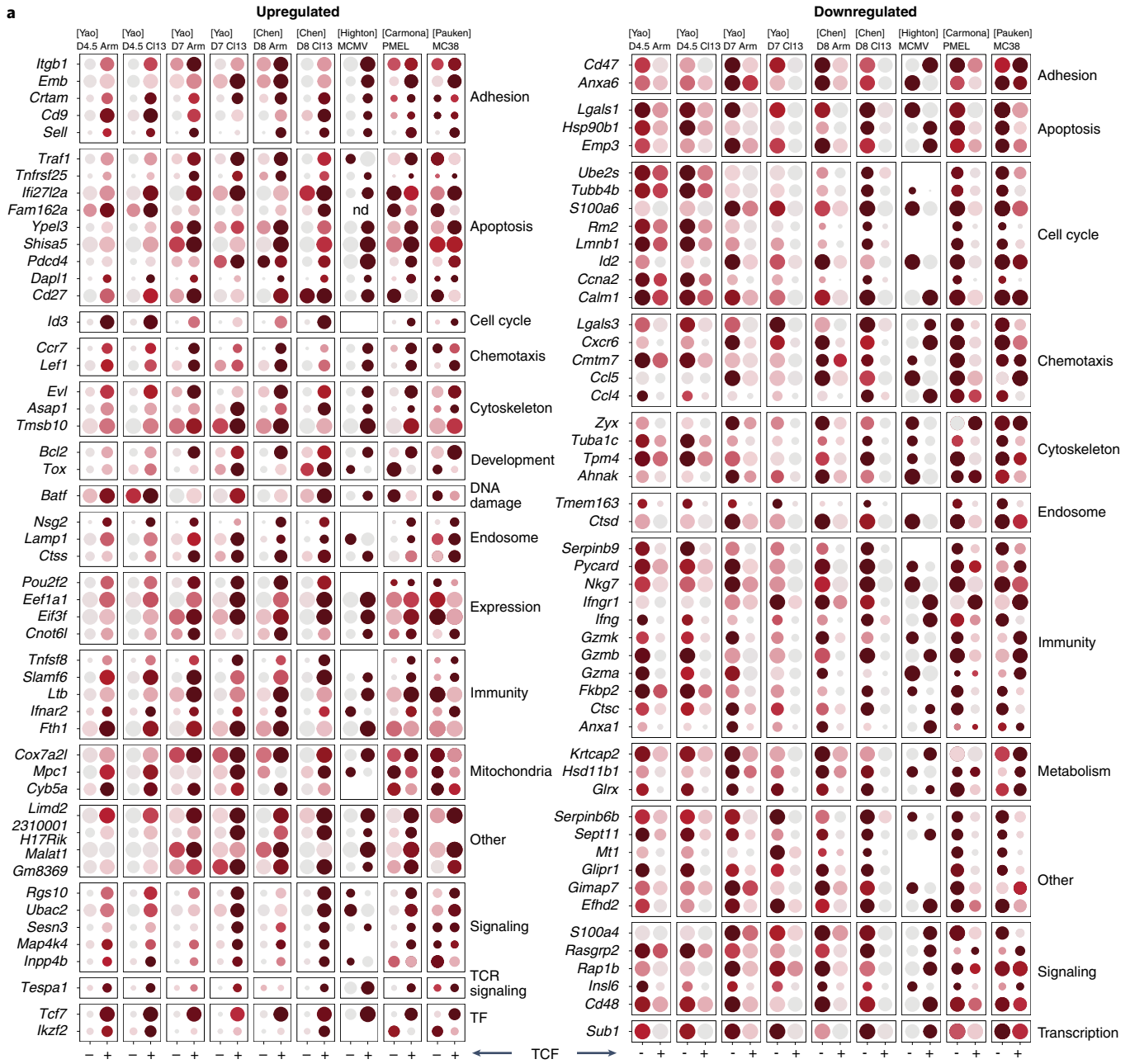
HCV infection provided also unique insights regarding what happens with exhausted T cell populations upon infection resolution via direct-acting antivirals (DAAs). This treatment results in a loss of the TCF-1$^-$CD127$^-$PD-1$^+$ terminally differentiated T_{ex} cell population, as it was seen in mice following transfer of virus-specific CD8$^+$ T cells from chronic infection into antigen-free hosts[23,37]. Nonetheless, the selective maintenance of quiescent cells with T_{mem} cell features such as CD127 and TCF-1 expression, secondary expansion capacity but also signs of T cell exhaustion is a typical outcome of DAA-resolved HCV infection[55,79–82]. As mentioned above, we suggest using the term T_{mx} for these T_{pex}-derived T_{mem} cells.

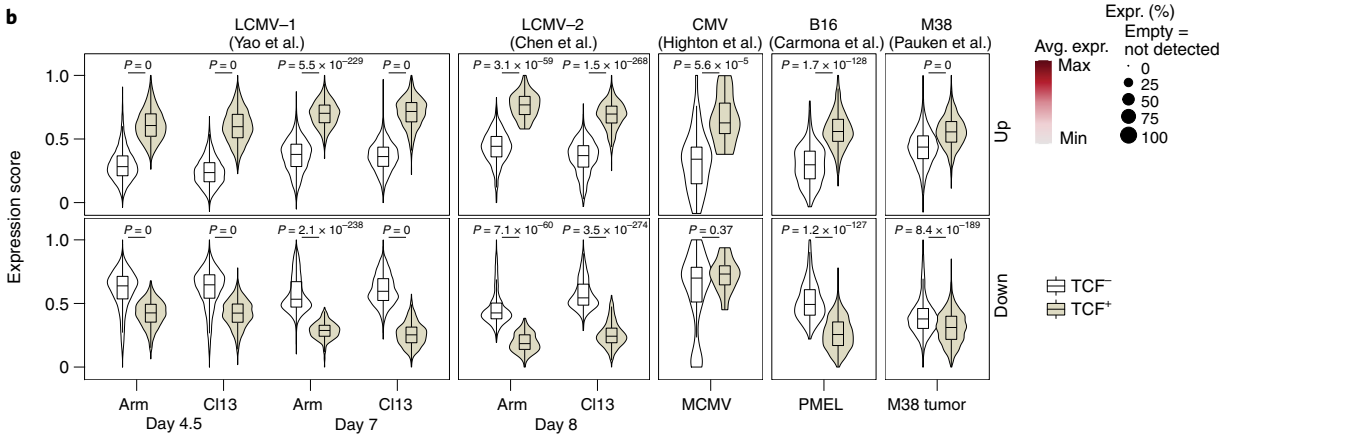
The retention of an exhausted signature among TCF-1$^+$ cells in DAA-treated individuals mirrors the phenotypic stability and epigenetic enforcement of exhausted T cells seen in other instances[2,37–40,79–82,86,87], including initial observations in mice[37] and among tumor-specific T cells[38,40,86]. A series of recent publications refers to this epigenetic commitment in T_{pex} cells as the acquisition of molecular 'scars'[79–82]. Interestingly, this 'scaring' or commitment is not observed among bona fide HCV-specific memory CD8$^+$ T cells formed in HCV infections that were spontaneously and rapidly resolved by the immune system[46], but it is evident among TCF-1$^+$ cells specific for epitopes that were lost due to antigen escape. Noteworthy, viral escape occurs rather early during the first 6 months of HCV infection[88], indicating that the epigenetic imprinting takes place in this time period. Of note, while variant-specific T cells still acquire features of T cell exhaustion, their molecular commitment is less prominent than that of T_{pex} cells specific for conserved epitopes[79,80,82]. This observation implies a progressive acquisition of features of T cell exhaustion as opposed to the relatively rapid fixation of T cell exhaustion in LCMV infection, whereby the systemic nature of the LCMV infection may be a factor speeding up this commitment. However, in line with recent reports of early onset of the commitment toward exhaustion in T_{pex} cells, it could also mean that the early infection period, during which very high antigen levels are present, has the highest potential to initiate the acquisition of a strong exhausted phenotype[42,46]. Of note, a related study of antiretroviral treatment in HIV infection suggests some differences, that is, while the treatment went along with increases in the TCF-1-expressing population, there was a prominent loss of PD-1 expression[89]. This finding implies that the induction of exhaustion and its epigenetic fixation depends on factors that are yet to be identified and not only on prolongated antigen exposure. Here, it needs to be considered that antiretroviral treatment was applied to the specific HIV study cohort >2 years after having an uncontrolled HIV infection. However, as this fixation occurs also in tumors[38,40,86], it remains one of the key challenges in the field of immunology—how the underlying processes and mechanisms can be overcome.

Precursors in latent reactivating viral infections. The majority of the world population is infected with CMV[90,91]. It differs from other chronic infection as it perseveres mainly in form of vial latency with very limited expression of viral gene products during latency (reviewed in refs. [92–94]). However, sporadic reactivation from latency can reinitiate lytic replication, which is in most cases well controlled by established potent CMV-specific immunity.

Human CMV (HCMV) and murine CMV (MCMV) infections trigger two divergent types of CD8$^+$ T cell responses that differ in their kinetics, phenotypical composition and maintenance requirements. While CD8$^+$ T cells specific for some CMV antigens follow the 'classical' expansion, contraction and establishment of stable memory kinetics, CD8$^+$ T cells specific for certain CMV-derived epitopes follow an atypical kinetics with continued expansion and accumulation of functional, effector-like CMV-specific CD8$^+$ T cells (termed 'memory inflation') in the circulation, secondary lymphoid organs and peripheral tissues[8,95–97]. Because of the latter, the

Fig. 3 | Gene expression signatures of TCF-1-positive, antigen-specific T cells. a, Total P14 T cells were obtained 4.5 and 7 days after infection with LCMV Armstrong or clone 13 by Yao et al.[70] and analyzed by single-cell RNA sequencing. We reanalyzed these data and after clustering we identified the cluster expressing TCF-1 (*Tcf7*). We compared expression levels of genes in this cluster to the expression levels of the same genes in all other clusters. Depicted are the top genes that were either significantly upregulated or downregulated across both time points and the LCMV Armstrong versus clone-13 infection. We then compared the expression of these genes to the expression of the same genes in TCF-1$^+$ and TCF-1$^-$ clusters in a similar LCMV dataset published by Chen et al.[64], a dataset based on CMV-specific cells published by Highton et al.[150], and two datasets of tumor-specific T cells published by Carmona et al.[151] and Pauken et al.[152]. Shown are scaled expression levels (color intensity) and the percentage of expressing cells (circle size) for the top 50 upregulated (left) and downregulated (right) genes. Genes are grouped according to common biological functions. **b**, Gene signatures from **a** were tested for their ability to separate TCF-1$^+$ and TCF-1$^-$ T cells in datasets derived from the publications indicated above. TF, transcription factor.


a
Upregulated
Downregulated
[Yao] D4.5 Arm
[Yao] D4.5 Cl13
[Yao] D7 Arm
[Yao] D7 Cl13
[Chen] D8 Arm
[Chen] D8 Cl13
[Highton] MCMV
[Carmona] PMEL
[Pauken] MC38
Itgb1
Emb
Crtam
Cd9
Sell
Adhesion
Traf1
Tnfrsf25
Ifi27l2a
Fam162a
Ypel3
Shisa5
Pdcd4
Dapl1
Cd27
nd
Apoptosis
Id3
Cell cycle
Ccr7
Lef1
Chemotaxis
Evl
Asap1
Tmsb10
Cytoskeleton
Bcl2
Tox
Development
Batf
DNA damage
Nsg2
Lamp1
Ctss
Endosome
Pou2f2
Eef1a1
Eif3f
Cnot6l
Expression
Tnfsf8
Slamf6
Ltb
Ifnar2
Fth1
Immunity
Cox7a2l
Mpc1
Cyb5a
Mitochondria
Limd2
2310001 H17Rik
Malat1
Gm8369
Other
Rgs10
Ubac2
Sesn3
Map4k4
Inpp4b
Signaling
Tespa1
TCR signaling
Tcf7
Ikzf2
TF
Cd47
Anxa6
Adhesion
Lgals1
Hsp90b1
Emp3
Apoptosis
Ube2s
Tubb4b
S100a6
Rrm2
Lmnb1
Id2
Ccna2
Calm1
Cell cycle
Lgals3
Cxcr6
Cmtm7
Ccl5
Ccl4
Chemotaxis
Zyx
Tuba1c
Tpm4
Ahnak
Cytoskeleton
Tmem163
Ctsd
Endosome
Serpinb9
Pycard
Nkg7
Ifngr1
Ifng
Gzmk
Gzmb
Gzma
Fkbp2
Ctsc
Anxa1
Immunity
Krtcap2
Hsd11b1
Glrx
Metabolism
Serpinb6b
Sept11
Mt1
Glipr1
Gimap7
Efhd2
Other
S100a4
Rasgrp2
Rap1b
Insl6
Cd48
Signaling
Sub1
Transcription
TCF


b
LCMV–1 (Yao et al.)
LCMV–2 (Chen et al.)
CMV (Highton et al.)
B16 (Carmona et al.)
M38 (Pauken et al.)
Expression score
Up
Down
Avg. expr.
Max
Min
Expr. (%)
Empty = not detected
0
25
50
75
100
TCF⁻
TCF⁺
Arm
Cl13
Day 4.5
Day 7
Day 8
MCMV
PMEL
M38 tumor

CMV-specific CD8+ T cell population is dominated by inflationary CD8+ T cells during latency, which exhibit an effector memory-like phenotype (T_{em}-like CD62L−CD127−KLRG1+CD27lo). Inflationary CD8+ T cells exert potent effector functions and have, from a bioengineering standpoint, gained considerable interest for T cell-based vaccines against viral infections and tumors[98–105]. The inflationary CD8+ T cell response depends on sporadic reactivation events, on antigen processing via the constitutive proteasome[106,107] and on antigen presentation by latently infected non-hematopoietic cells[108,109].

The large population of inflationary MCMV-specific CD8+ T cells is maintained at a remarkably stable level for the lifetime of the infected mouse. This pertains not only to the numbers of inflationary CD8+ T cells, but also to their overall antigen avidity[110], at least over an observation period of 5 months. Considerably longer exposure periods tend to go along with decreased avidity[29]. A dynamic process is responsible for the apparent stable maintenance of the inflationary CD8+ T cell response, as the half-life of terminally differentiated inflationary T cells is 6–8 weeks in the circulation and 10–12 weeks in the lung[111,112], implying that at least every 3 months 50% of the inflationary pool becomes replenished[113]. This replenishment depends on a small, lymph node (LN)-residing subset of the inflationary cells that exhibits a T_{cm} cell phenotype (CD62L+IL-7Rα+ and high proliferative potential)[108,114]. It is also conceivable that CD62L−CX3CR1int cells contribute to the process of inflation[115,116]. Recently, the T_{cm} subpopulation of inflationary CD8+ T cells was shown to express TCF-1 (ref. [9]) and depletion of TCF-1+ cells during established infection severely curtailed the pool of inflationary cells[9]. Moreover, there is large clonal overlap between inflationary TCF-1+ and TCF-1− cells of the same specificity, with highly abundant TCF-1+ clones also being highly abundant within the effector-like population[9]. Both document a critical role of TCF-1+ precursors for feeding the more differentiated T_{em}-like pool in inflationary, MCMV-targeting T cell responses. Whether in humans a proportion of HCMV-specific inflationary CD8+ T cells expresses TCF-1 remains to be formally shown[117]. A comparative analysis of the TCR repertoire in humans revealed only a partial overlap of HCMV-specific CD8+ T_{cm} (IL-7Rα+) and T_{em} (IL-7Rα−) cells. However, as comparative clonal analyses in humans and in particular in tissues such as the spleen and lymph nodes (LNs) are difficult to perform, it is conceivable that under-sampling might explain this partial clonal overlap[117]. Nonetheless, HCMV-specific CD8+ T cells isolated from LNs have a higher proliferative capacity compared to cells isolated from the blood[118], suggesting that also in humans secondary lymphoid organs host HCMV-specific memory cells with superior proliferation potential.

Role of tumor-specific precursor T cells

The identification of T_{pex} cells and their superior response to blocking the interaction of PD-1 and its ligand, PD-L1, in murine chronic infections had obvious implications for cancer treatment, and prompted a search for similar cells in human cancer. Initially, a hierarchy of differentiation among PD-1+CD8+ T cells in non-small cell lung cancer (NSCLC) was identified, where the CXCR5+TCF-1hiTIM-3− subpopulation was more functional and gave rise to differentiated CXCR5−TCF-1loTIM-3+ T_{ex} cells after ex vivo TCR stimulation[119]. Subsequent studies demonstrated that antigen-specific TCF-1hi T_{pex} cells are superior to TCF-1lo T_{ex} cells in mediating regression of tumors upon PD-1 blockade in mice[120,121]. This difference appears to depend on genes typically expressed by murine T_{mem} cells or human T_{scm} cells[122–125].

Nonetheless, it is still debated which T cell subset is preferentially targeted by ICB in humans. Increased baseline abundance of TCF-1+ CD8+ T cells in melanoma lesions predicted response to ICB targeting PD-1, CTLA-4 or both[126]. Accordingly, intratumoral CD8+ T_{pex} cells, but not terminally differentiated T_{ex} cells, were recently shown, along with peripheral T cells, to be the major source of effectors infiltrating NSCLC after anti-PD-1 and chemotherapy[127]. In other studies, however, pretreatment abundance of intratumoral T cells expressing high levels of the T_{ex} markers PD-1, TIM-3 or CXCL13 predicted long-term response to ICB in NSCLC[128–130]. These contrasting results could be explained by the different approaches used to define T cell subsets. Alternatively, the abundance of T_{ex} cells, making up the majority of tumor-specific cells[131–135], could simply reflect an ongoing immune response that relies on precursors originating from other sites (for more details, see ref. [136]). Initial evidence challenging the concept that anti-PD-1 immunotherapy mainly targets tumor-resident T cells came from murine models, where regression of MC38 colon carcinomas was abrogated by blockade of sphingosine 1-phosphate receptor 1 (S1P1)-mediated T cell egress from secondary lymphoid tissues by the FTY720 inhibitor[137]. The 'immunological' response to anti-PD-1 ICB, that is, the appearance of Ki-67+ proliferating CD8+ T cells, is visible in the circulation of patients with melanoma as soon as 7 days following administration[138]. These proliferating cells are also clonally related to tumor-infiltrating CD8+ T cells[139], possibly suggesting recruitment from secondary lymphoid organs. This hypothesis has been corroborated by recent data showing that T cell clones found in basal and squamous cell carcinoma lesions following anti-PD-1 ICB were, for the vast majority, not present before treatment, thus indicating clonal replacement[140]. These results were, in part, confirmed also in NSCLC[127]. In mice, tumor-draining LNs are a preferential reservoir and site of T_{pex} cell stimulation[141,142]. Moreover, T_{pex} cells are preferentially present in LNs compared to lung tumors or the adjacent peritumoral space[56]. Accordingly, administration of ICB via intratumoral, intradermal or intrapleural injection causing antibody accumulation in tumor-draining LNs, expanded the pool of intratumoral T_{pex} cells, identified in this case by *Slamf6* expression[143], and was associated with enhanced tumor regression compared to routes (that is, intravenous or intraperitoneal) resulting in systemic distribution in preclinical tumor models[143,144].

It should be noted that the majority of the human studies mentioned so far analyzed polyclonal T cell subsets with limited information about their specificity. More recently, a comprehensive analysis of transcriptomes, TCR diversity and antitumor reactivity of several melanoma and NSCLC-specific CD8+ T cells, including those responding to neo-antigens, revealed that these cells display a phenotypic and gene expression profile distinct from bystander CD8+ T cells, in particular pertaining to expression of exhaustion-related markers and transcription factors[134,135]. Tumor-reactive clones were heterogeneous at the single-cell transcriptomic level, featuring mainly T_{ex} cells but also T_{pex} cells expressing TCF-1 and proliferating/activated cells[134]. Among tumor-reactive CD8+ T cells, expression of *IL7RA, TCF7* and *GZMK*, previously related to T_{pex} cells[56,127,145], were associated with major tumor regression in three of six patients with NSCLC treated with neoadjuvant anti-PD-1 ICB[135].

A major question in the field of cancer immunology and chronic infection is why cancer progresses and infections continue despite long-lived and potent T_{pex} cell responses being present in the body and at the site of the lesion. This paradox could be explained, at least in part, by the epigenetic stability of the exhausted T cell population[38–40,86], including T_{pex} cells[17,56]. In fact, our understanding of epigenetic mechanisms that enforce an exhausted phenotype also urge conception of new approaches to improve functionality of T cells during immunotherapies. Using drugs capable of specifically modifying the exhaustion-enforcing epigenetic landscape would be warranted, but this approach is challenged by poor specificity so far. As it is established early after activation, preventing, rather than reverting exhaustion by pharmacological intervention at a yet uncommitted stage might be a promising strategy to improve functionality of adoptively transferred TCR- or CAR-engineered T cells. In this regard, culturing T cells with antioxidants[124], glycogen synthase-3β (GSK-3β)[146], AKT[147] and MEK inhibitors[148], among

other molecules, preserved stemness and prevented terminal differentiation and expression of inhibitory receptors via downregulation of the mTOR signaling pathway and glycolytic metabolism. Similarly, overexpression of c-Jun, which is thought to compete with the formation of AP-1–IRF complexes involved in terminal differentiation and exhaustion, resulted in transcriptional rewiring in cultured CAR T cells, and enhanced stemness and functionality in xenogeneic tumor models[69]. A different strategy based on transient interruption of CAR-mediated signaling or inhibition of CAR proximal kinases during T cell expansion in vitro was also able to revert the chromatin accessibility landscape associated with exhaustion[149]. Collectively, these data support the notion that acquisition of terminal differentiation and exhaustion traits are major barriers to the successful outcome of ICB and adoptive T cell therapies.

Common signature genes of proliferation-competent cells

As outlined above, studies in LCMV infection have pioneered the identification of proliferation-competent T_{mp} cells in acute infections and T_{pex} cells in chronic infections. Subsequently, related populations were identified in different infections and tumors. While T_{mp} and T_{pex} cells differ in many critical aspects, they share the expression of a large number of genes. We extracted T_{pex} cell-defining genes from a representative single-cell sequencing dataset generated by Yao et al.[49] ('Data availability and analysis') and have identified 117 genes that are upregulated in TCF-1$^+$ compared to TCF-1$^-$ cells and 290 that are downregulated in TCF-1$^+$ compared to TCF-1$^-$ cells. We illustrate the top 50 differentially expressed genes in Fig. 3a. These include those genes encoding adhesion molecules, negative regulators of apoptosis and promoters of cell expansion and survival and cell cycle regulators. In addition, we noticed differential expression of several genes, whose function still needs to be characterized in chronic infections and tumors. In line with previous reports, the TCF-1$^+$ population shows higher expression levels of *CCR7*, while *CCL5*, *CXCR6* and *LGALS3* show higher expression levels in TCF-1$^-$ cells. Finally, TCF-1$^-$ cells show higher expression of *GZMA*, *GZMB* and *GZMK* than TCF-1$^+$ cells. We propose that many of these genes represent a core signature of TCF-1$^+$ cells across many conditions, as they were also enriched in TCF-1$^+$ cells in CMV infection and in the tumor settings (Fig. 3b). This analysis strongly underlines that a common and robust molecular wiring orchestrates the differentiation between precursors (T_{pex}) and terminally differentiated (T_{ex}) cells across very different conditions.

Conclusions, outlook and imminent questions

The central role of T_{pex} cells in sustaining antiviral and tumor-directed immunity underscores the importance of this population for immunotherapy. A better understanding of their biology is, in our opinion, a prerequisite to advance immunotherapy. Questions on how to augment the often-limited T cell response after immunotherapy are inevitably linked to the biology of the T_{pex} cell population, which we only partially understand so far. A particular challenge is to find solutions to potentiate the capacity of T_{pex} cells to give rise to T_{eff} cells without compromising long-term persistence of T_{pex} cells. Indeed, the currently used checkpoint inhibition strategies are optimized for short-term effects, while the long-term consequences for T_{pex} cells and possibly negative effects on this population were insufficiently explored so far. The expression of signature genes of T cell exhaustion alongside classical T_{mem} cell traits also bears tremendous importance for immunotherapeutic interventions. It signals that T cell exhaustion-specific mechanisms and their epigenetic fixation should already be prevented at the level of the T_{pex} cell population. Presently, we lack any effective strategies to do so without compromising the T_{pex} cell population. Because of these and other aspects mentioned in this review, we see a major need to focus T cell exhaustion-related research directly at investigating the biology of T_{pex} cells.

Data availability and analysis

Raw read counts were obtained from NCBI under the accession numbers GSE119940 (ref. [49]), GSE131535 (ref. [64]), GSE128147 (ref. [150]), GSE116390 (ref. [151]) and GSE158520 (ref. [152]). Only cells with more than 1,000 genes detected and with unique molecular identifier counts less than three standard deviations above the mean were kept for downstream analysis, with the exception of the dataset from Highton et al.[150], where cells with more than 500 genes detected were kept. Contaminating cells were filtered out based on the cluster expression of the marker genes *Cd14*, *Ly6d*, *H2-Aa*, *Cst3*, *Fcer1g*, *Fcgr3* and *Lyz2*. Raw counts were normalized for each sample separately using the R package sctransform (v0.3.2)[153] (with glmGamPoi method). Downstream analysis was performed with the R package Seurat (v4.0.1)[154]. Principal-component analysis was calculated on the top 1,000 highly variable genes; *k*-nearest neighbor graph and uniform manifold approximation and projection were computed on the first 20 principal-component analysis dimensions. Clusters with a high percentage of cells expressing *Tcf7* were labeled as the TCF-1$^+$ subpopulation. In the dataset from Highton et al.[150], cells with normalized expression higher than 4 were labeled as TCF$^+$ cells. In the case of the dataset from Yao et al.[70], anchors between replicates were identified on the top 1,000 highly variable genes and integration was performed on the first 20 dimensions. The integrated data were then used for cluster identification. For differential expression analysis instead, the data from all samples were merged and normalized using the R package sctransform (v0.3.2)[153] (with glmGamPoi method). The TCF-1$^+$ signatures were generated by contrasting the TCF-1$^+$ versus the TCF-1$^-$ subpopulation using the function FindConservedMarkers with the Wilcoxon rank-sum test for differential expression followed by Bonferroni correction for multiple testing. The R package biomaRt (v2.46.3)[155] was used to retrieve the Gene Ontology terms associated with each gene from the TCF$^+$ signatures and they were grouped by common terms.

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Together, the studies by He et al., Im et al. and Utzschneider et al. identified a subset of virus-specific CD8⁺ T cells, characterized by the expression of TCF-1 or CXCR5, that exhibits proliferative capacity and stem-cell traits such as self-renewal and the ability to differentiate into terminally differentiated cells. This population preferentially expanded after blockade of the PD-1 inhibitory pathway.

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Competing interests

D.Z. has a consulting agreement and research collaboration agreement with Pieris Pharmaceuticals related to manipulation of T_{pex} cells. E.L. receives research grants from Bristol Myers Squibb and is inventor on a patent describing methods for the generation and isolation of T_{scm} cells.

Additional information

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