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MECHANISMS OF MEMORY T CELL ACTIVATION AND EFFECTIVE IMMUNITY

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Abstract

Effective immunization induces the development of populations of robust effector lymphocytes specific for the immunizing antigens. Amongst them are cytotoxic/CD8⁺ T lymphocytes, which few will further differentiate into long-lived memory cells persisting in the host and exhibiting improved functional characteristics. The current model is that such memory cells can confer rapid host protection upon cognate antigen-mediated activation and direct killing of infected cells. In this chapter, we discuss work from our group and others that highlight the contribution of inflammatory cytokines to memory CD8⁺ T cell activation and of cytolysis-independent mechanisms of host protection.

Synonyms

Memory T lymphocytes; Monocytes; Inflammation; Vaccination; Protective immunity

Introduction

Formation of long-lasting antigen-specific memory CD8⁺ T cells involves complex sets of events that comprise initial activation (priming), proliferation (expansion) and massive apoptosis (contraction), after which the cells that survive give rise to long-lived memory cells (Cui and Kaech, 2010; Harty and Badovinac, 2008; McKinstry et al., 2010). Through this process, memory cells acquire multiple unique functional features which make them able not only to respond to, but also to actively provide different signals ultimately culminating in host protection. While the current view is that cognate antigen is the only essential trigger to reactivate the memory T cells, several evidence in the literature, pointed to an important role of cytokines and chemokines in these processes. Thus, in this chapter, we most specifically present results that our group and others have generated over the past years and that support such evidence.

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Non-Cognate Differentiation of Memory CD8⁺ T Cells Into Robust Effector Cells

Cytokines and Pathways

Initial hints came from early work by the Sprent group showing that CD8⁺ T cells exhibiting features of memory cells (CD44^{hi}) could proliferate in response to the cytokine IL-15 *in vitro* and *in vivo*, and independently of T cell receptor (TCR) triggering (Zhang et al., 1998). A further report from the Forman lab using mice immunized with the intracellular bacterium *Listeria monocytogenes* (*Lm*) established that in response to the cytokines IL-12 and IL-18 only, *Lm*-specific memory but not naive CD8⁺ T cells were able to differentiate into IFN- γ -secreting effector cells, which could confer some protection to immunized hosts (Berg et al., 2003). Also using *Lm* as a model, we reported that protection of vaccinated host mice during recall infection was occurring within 6–8 hours post challenge infection (Narni-Mancinelli et al., 2011). Interestingly, control of bacterial growth was correlated with the rapid clustering and activation (IFN- γ ⁺) of memory CD8⁺ T cells to the red pulp/marginal zone area of the spleen of challenged mice (Bajenoff et al., 2010), within bacteria-containing area where macrophages actively filter the blood (Aoshi et al., 2008). These ‘effector-clusters’ were exclusively formed by memory but not naive T cells, and included rapidly recruited blood-derived innate immune cells such as Ly6C⁺ ‘inflammatory’ monocytes, the most abundant subset of blood-derived monocytes (Auffray et al., 2009), and neutrophils. Quite unexpectedly, we found that the memory CD8⁺ T cells underwent comparable activation (as measured by expression of IFN- γ , Granzyme B, CD25, NKG2D etc) whether cognate antigen was present or not (Soudja et al., 2012). Investigating further the underlying mechanisms, we could show that the cytokines IL-18 and IL-15 were major drivers of early memory T cell effector differentiation respectively into IFN- γ -secreting and cytolytic (GrB⁺) cells. This involved the triggering of the inflammasome (IL-18) and the IRF3/type I IFN (IL-15) pathways. While such rapid memory T cell-differentiation did not require cognate antigen recognition by the memory cells, cellular proliferation and robust secondary expansion did not occur without T cell receptor (TCR) triggering. Similar findings were also reported by other groups (Chu et al., 2013; Kupz et al., 2012).

Innate Immune Cells

An initial study from the Lefrancois group suggested the unexpected importance of CD11c^{hi} dendritic cells (DCs) for optimal activation and expansion of memory CD8⁺ T cells in mice immunized with *Lm* and two distinct viruses, the vesicular stomatitis and the influenza viruses (Zammit et al., 2005). Our study and that from the Bedoui group investigating the mechanisms of antigen-independent activation of memory CD8⁺ T cells reported an essential role for different subsets of innate myeloid cells in providing key activating cytokines to the memory cells (Kupz et al., 2012; Soudja et al., 2012). While the latter study showed the implication of the NLR4 inflammasome inside CD11c⁺ DCs, leading to IL-18 production, we reported that Ly6C⁺ monocytes could produce both IL-18 and IL-15. Of note, Ly6C⁺ monocytes appeared as exclusive providers of IL-15 very early on -by 6–8 hours post infection-, yet other cell types such as macrophages or DCs could compensate as early as 24 hours after the challenge infection. Though substantial differences could account

for this discrepancy, including the experimental systems that were used, we favor the idea that both antigen-presenting cell (APC) types are indeed contributing to reactivating the memory cells. As initially proposed by Busch and colleagues (Neuenhahn et al., 2006), and further established by the Murphy lab (Edelson et al., 2011), amongst CD11c⁺ DCs, the CD8α⁺ DCs are required to carry live blood bacteria to spleens, thus it is certainly possible that DCs act as initiators while Ly6C⁺ monocytes, which undergo massive mobilization from the bone-marrow (Serbina and Pamer, 2006), quickly amplify and sustain the cytokinetic signals.

In addition to providing activating cytokines and along the lines of the early work from Lefrancois and colleagues (Zammit et al., 2005), we also found that Ly6C⁺ monocytes contribute to antigen-dependent expansion of the memory CD8⁺ T cells (Soudja et al., 2012). Selective depletion of Ly6C⁺ monocytes in immunized mice, substantially decreased memory CD8⁺ T cell expansion during the challenge infection, as also shown following CD11c⁺ DC elimination. Both of these observations may indeed be accounted for by indirect 'inflammatory' effects, rather than by direct presentation of cognate antigen to the memory cells, as also proposed by Badovinac and Harty (Wirth et al., 2011). The contribution of antigen-presentation by DC and/or Ly6C⁺ monocytes to memory T cell activation and expansion still remains to be defined.

In summary, all these reports supported the notion that APCs which act as early sentinel cells of the immune system, in particular DCs and Ly6C⁺ monocytes, play an essential role for optimal cytokine- as well as cognate antigen-mediated activation of memory CD8⁺ T cells *in vivo*. This mechanism also contributes to host mechanisms of innate immune protection.

Effective Memory T Cell-Mediated Immunity

Ly6C⁺ monocytes and CD11c⁺ DCs are required to promote optimal memory T cell reactivation during a recall infection by acute intracellular pathogens such as *Lm* or viruses like the lymphochorionmeningitis virus (LCMV Armstrong) or the Vaccinia virus. However, how each of these signals translates into effective protection of the hosts is still incompletely understood. The prominent view proposes that reactivated memory CD8⁺ T cells rapidly express cytolytic effector functions (such as perforin, Granzyme and Fas) that allow for direct killing of pathogen-infected cells, representing the major mechanism of host protection (Harty et al., 2000). Reactivated memory T cells are also shown to secrete important amounts of proinflammatory cytokines and chemokines such as IFNγ, CCL3, CCL4 and CCL5, which promote immune cell activation and recruitment (Dorner et al., 2002; Sallusto et al., 2000). Ultimately, effective protection of vaccinated hosts likely involves both direct and indirect mechanisms orchestrated by memory T cells, yet we and others have sought to most accurately define the relative contribution of the possible different mechanisms. Of note, which effector mechanisms will be most important will depend on each infection as previously reviewed elsewhere (Harty et al., 2000).

Numerous studies, in particular from the Harty lab, have used both mice lacking essential effector molecules (such as IFN-γ, TNF-α, Perforin, CD95/Fas), as well as adoptive transfer

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experiments of memory CD8⁺ T cells purified from mice immunized with *Lm* as model (Badovinac and Harty, 2000; Harty and Bevan, 1995; White et al., 2000a; White et al., 2000b). Wild-type or knockout immunized mice were challenged and their ability to clear the infection compared. Likewise, memory CD8⁺ T cells from knockout or WT immunized mice were purified and transferred to naïve recipient mice that were subsequently challenged to assess the contribution of distinct effector mechanisms to host protection. Several major conclusions could be drawn from these studies, specifically that (i) not one but multiple mechanisms accounted for host protection, (ii) all immunized knockout mice listed above turned out to be protected against recall infection and (iii) a mechanism that required TNF- α yet was independent of memory T cell-cytolytic activity and IFN- γ was implicated (White et al., 2000a). Further investigations using potent TNF- α neutralizing reagents *in vivo*, by several groups including ours, established the importance of TNF- α for the protection of immunized hosts during the recall infection (Narni-Mancinelli et al., 2007; Neighbors et al., 2001). While discrepant with the initial studies using mice lacking TNF- α or its receptor TNFRI (p55) (White et al., 2000a), it did underline that knockout mice can develop compensatory mechanisms that substantially differ from that of WT mice. Alternative explanation, though not exclusive, may be that adoptively transferred memory T cells conferred protection to recipient mice through substantially distinct mechanisms than those of vaccinated mice undergoing the challenge infection.

Building on these observations, we further explored the mechanisms of TNF- α -dependent protective immunity. In agreement with prior work (Cook et al., 1999), memory CD8⁺ T cells could secrete the proinflammatory cytokine CCL3, yet very rapidly following challenge infection, which promoted the differentiation of blood-derived phagocytes -both Ly6C⁺ monocytes and neutrophils- into TNF- α and reactive oxygen species (ROS) producing cells (Narni-Mancinelli et al., 2007). This oxidative burst enhanced antimicrobial autophagy which was correlated with intracellular pathogen killing (Narni-Mancinelli et al., 2011). Collectively, these results provided solid proof of concept supporting the importance of innate immune effector cells for rapid memory CD8⁺ T cell-mediated protection in vaccinated hosts. CCL3, however, was released by the memory cells only upon cognate antigen triggering, suggesting that other, possibly earlier, signals were contributing to innate immune cell mobilization.

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From prior work by us and others (Berg et al., 2003; Kupz et al., 2012; Soudja et al., 2012), IFN- γ secreted by memory T cells in response to IL-18, IL-12 and IL-15, appeared as a strong candidate. Production of IFN- γ by pathogen-specific memory T cells indeed starts already by 4 hours post recall infection and can occur in a cognate-antigen independent manner. Through series of advanced genetic depletion and bone-marrow chimera experiments, results from our lab provided compelling evidence that IFN- γ from memory T cells, but not other lymphocytes such as NK or NK T cells, was most essential in instructing innate myeloid and lymphoid cell activation and differentiation into robust microbicidal effector cells. IFN- γ signaling to myeloid cells such as Ly6C⁺ monocytes, neutrophils, macrophages and DCs was required for them to secrete proinflammatory cytokines (TNF- α) and chemokines (CXCL9, CXCL10, CCL2, CXCL1), express the inducible nitric oxid synthase (iNOS), and upregulate costimulatory (CD80, CD86, CD40) and antigen-presenting molecules. While the importance of IFN- γ as a key modulator of immune

responses has long been investigated and documented in humans and mice models (Hu and Ivashkiv, 2009), such links to the mechanisms of memory T cell-mediated protection in a relevant *in vivo* model of vaccination/infection had not been previously established. Analysis of Ly6C⁺ monocytes genetic expression program in vaccinated mice undergoing challenge infection revealed an overall 'IFN- γ -skewed' program, notably with genes encoding for the guanylate binding protein (gbp) GTPases involved in microbial killing (Kim et al., 2011; Yamamoto et al., 2012). Importantly, both Ly6C⁺ monocytes, CD11c⁺ DCs and subsets of tissue macrophages contributed to effective protection of vaccinated hosts during the recall infection, in an IFN- γ -dependent manner. Another key aspect to outline that directly results from IFN- γ modulation is the secreted levels of chemokines such as CXCL9, CXCL10 and others, which are essential for immune cell recruitment to infected foci. Elegant studies from the Germain and the von Andrian labs have illustrated the importance of such mechanism orchestrated by memory CD8⁺ T cells for rapid pathogen containment near the peripheral entry portal of lymph-borne bacterial and viral pathogens (Kastenmuller et al., 2013; Sung et al., 2012).

Interestingly too, we could extend some of our findings to a relevant mucosal model of infection, using mice vaccinated intravaginally by attenuated TK⁻ herpes simplex virus 2 (HSV-2). We found that Ly6C⁺ monocytes and neutrophils underwent better recruitment and activation during recall infection with WT virulent HSV-2 than that of non-vaccinated counterparts, a finding that is particularly relevant in the context of prior and recent literature on the importance of mucosal resident memory T (T_{RM}) cell-derived IFN- γ for protective immunity against HSV-2 (Ariotti et al., 2014; Gebhardt et al., 2009; Iijima and Iwasaki, 2014; Schenkel et al., 2014; Schenkel et al., 2013). In all of these studies, T_{RM} were reported to carry the very initial immune 'alarming' functions, using IFN- γ as the most essential lymphokine.

In summary, results from several studies largely conducted in our lab support the notion that indirect mechanisms of protection, distinct from the 'usual' cytolytic effector mechanisms expressed by CD8⁺ T cells, are significant contributors to the protection of vaccinated hosts during virulent pathogen infection. This mechanism implicates the coordinated orchestration of innate immune cell activation and differentiation by the memory T cells. Altogether, this body of work reveals the importance for such indirect mechanisms of protection, and further emphasizes their need to be accounted for when evaluating vaccine efficacy.

Conclusion

This book chapter summarizes recent developments from our groups and others investigating mechanisms of *in vivo* memory T cell-activation and effective host protection that are taking place in vaccinated hosts during an acute challenge infection (See also summary Figure). Current evidence reveal the complexity of these processes and suggest the implication of multiple steps of innate and adaptive cell cross-talks that ultimately lead to microbial pathogen containment and killing, and host protection. Cognate memory T cell antigens, cytokines and chemokines, and the differentiation of robust microbicidal innate and adaptive effector cells are all contributing to an optimal and effective immune response. Further

studies, in particular using relevant models of mucosal immunizations, will be necessary to fully dissect and understand these mechanisms, and harness them for potential therapies.

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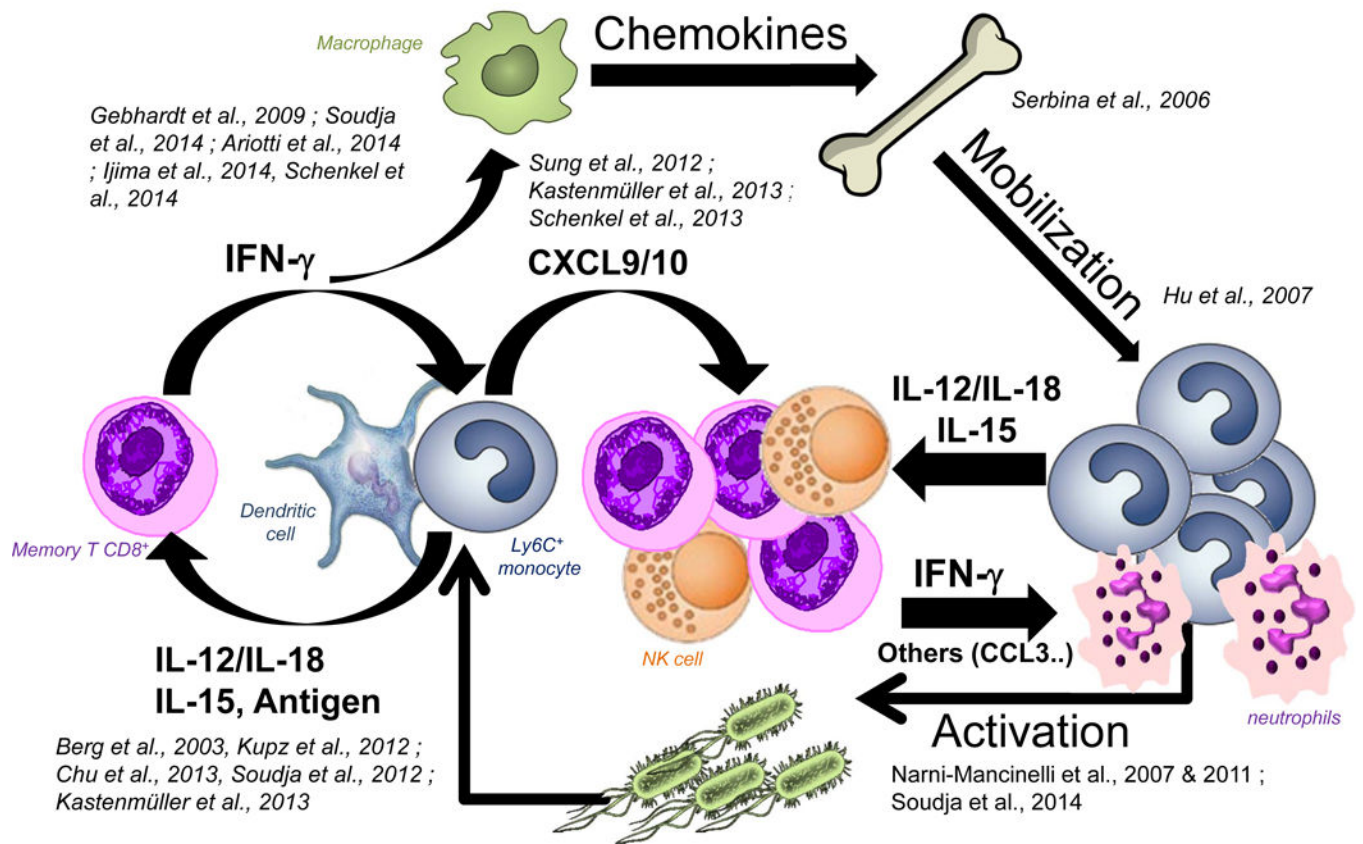


Figure.
Crosstalk Between Memory T cells and Innate Immune Cells Occurring During Recall Infection of Vaccinated Hosts