

# CD4<sup>+</sup> T Cells: Guardians of the Phagosome

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## SUMMARY

CD4<sup>+</sup> T cells are key cells of the adaptive immune system that use T cell antigen receptors to recognize peptides that are generated in endosomes or phagosomes and displayed on the host cell surface bound to major histocompatibility complex molecules. These T cells participate in immune responses that protect hosts from microbes such as *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Leishmania major*, and *Salmonella enterica*, which have evolved to live in the phagosomes of macrophages and dendritic cells. Here, we review studies indicating that CD4<sup>+</sup> T cells control phagosomal infections asymptotically in most individuals by secreting cytokines that activate the microbicidal activities of infected phagocytes but in a way that inhibits the pathogen but does not eliminate it. Indeed, we make the case that localized, controlled, persistent infection is necessary to maintain large numbers of CD4<sup>+</sup> effector T cells in a state of activation needed to eradicate systemic and more pathogenic forms of the infection. Finally, we posit that current vaccines for phagosomal infections fail because they do not produce this “periodic reminder” form of CD4<sup>+</sup> T cell-mediated immune control.

## INTRODUCTION

The number of microbes that can cause disease in mammals is extremely large (1). Different pathogens infect and reside in different niches in the body and have various mechanisms of pathogenesis. Consequently, immune functions that are protective against one pathogen may be completely ineffective against another. To deal with this challenge, the vertebrate adaptive immune system has evolved three types of lymphocytes that survey different types of pathogen-derived antigens: B cells, which use antibodies to recognize extracellular macromolecules; CD8<sup>+</sup> T cells, which use T cell receptors (TCRs) to recognize short pep-

tides from cytosolic proteins; and CD4<sup>+</sup> T cells, which use TCRs to recognize peptides from extracellular proteins that are taken up into endosomes or phagosomes. This review focuses on CD4<sup>+</sup> T cells and their important role in protecting the host from pathogens that persistently infect the phagosomes of phagocytes.

## ANTIGEN RECOGNITION BY LYMPHOCYTES

The adaptive immune system depends on lymphocytes, which exist in three main classes: B cells, CD8<sup>+</sup> T cells, and CD4<sup>+</sup> T cells (2). Each lymphocyte produces an antigen receptor from a composite gene assembled by somatic recombination of many possible gene segments on only one chromosome (3). This process, which occurs only in developing lymphocytes, ensures that millions of different antigen receptors can be produced but that each newly formed lymphocyte expresses many copies of a single antigen receptor. Each antigen receptor has a unique binding pocket that accommodates an antigen with a defined shape (4, 5). Since the gene segment shuffling mechanism that produces antigen receptors is relatively random (6), the antigen receptor binding pockets have random shapes. Thus, the set of lymphocytes in an individual is likely to contain only a few cells that by chance express antigen receptors with shapes that are highly complementary to the molecular shapes of antigens produced by a given microbe. The adaptive immune response to that microbe therefore depends on the expansion and differentiation of these few lymphocytes (7).

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## T Cell Antigen Receptor Recognition of Peptide-MHC Ligands

Immune protection from intracellular pathogens is the job of T cells (2). To understand how T cells do this job, it is important to understand the specificity of their TCRs. TCRs are composed of either alpha and beta or gamma and delta chain heterodimers that form antibody Fab-like structures on the surface of T cells (4, 8). This review focuses on alpha-beta TCRs, because the CD4<sup>+</sup> T cells that control phagosomal infections for the most part express these receptors (9).

The ligands for alpha-beta TCRs are usually short peptides bound to host cell surface proteins called major histocompatibility complex (MHC) molecules (4). MHC molecules come in two forms: MHC class I (MHCI) molecules, which are expressed on most cells of the host and present peptides that are recognized by TCRs on CD8<sup>+</sup> T cells, and MHCII molecules, which are constitutively expressed on B cells, dendritic cells (DCs), and macrophages and present peptides that are recognized by TCRs on CD4<sup>+</sup> T cells (4). MHC molecules alert the host to an infection by displaying peptides from intracellular microbes on the surfaces of host cells.

In almost all cells of the body, the peptides that bind to MHCI molecules are derived exclusively from cytosolic proteins (10). Newly translated proteins are constantly being clipped by proteasomes in the cytosols of all host cells. Some of the resulting peptides are pumped by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum, where 8- to 9-amino-acid peptides with certain key anchor residues bind to newly synthesized MHCI molecules (11). The resulting peptide:MHCI (p:MHCI) complexes are transported to the cell surface. Certain dendritic cells can shuttle internalized extracellular proteins from endosomes to the cytosol, where proteasomes produce peptides that enter the endoplasmic reticulum through TAP and are loaded onto MHCI molecules (12). This exceptional capacity is known as cross-presentation.

The p:MHCII ligands for TCRs on CD4<sup>+</sup> T cells are formed from extracellular proteins that are taken up by cells that express MHCII molecules (10). Dendritic cells and macrophages take up extracellular proteins into endosomes by macropinocytosis or particles by phagocytosis. B cells are very inefficient at this process and can efficiently internalize only proteins that bind to their surface antibody. Endosomes or phagosomes containing internalized proteins then fuse with vesicles containing proteases such as cathepsins, which cleave the internalized proteins into peptides. These vesicles also contain newly synthesized MHCII molecules that bind ~9-amino-acid peptides that have certain key anchor residues. The p:MHCII complexes are then trafficked to the cell surface.

MHC molecules are loaded with peptides from host proteins in uninfected individuals (13). These host p:MHC complexes play a critical role in T cell development in the thymus (14). Developing T cells rearrange *Tcrα-V*, *Tcrα-J*, *Tcrβ-V*, *Tcrβ-D*, and *Tcrβ-J* segments such that each cell expresses a unique alpha-beta TCR (15). Unlike antibodies, TCR V domains have an inherent germ line-encoded affinity for MHC molecules, which causes strong signaling and clonal deletion of many developing T cells in the thymus (16). The only cells that are not deleted are those that produce TCRs with weakened binding to self-p:MHC molecules. Weak TCR binding to self-p:MHC molecules is needed for survival and

lineage commitment: thymic T cells with TCRs with low affinity for self-p:MHCI molecules become CD8<sup>+</sup> T cells, whereas cells with TCRs with low affinity for self-p:MHCII molecules become CD4<sup>+</sup> T cells (14). The result of this process is a diverse set of T cells, all with TCRs with weak affinity for self-p:MHC molecules, a few of which are likely to have high affinity for a host MHC molecule when complexed with a given foreign peptide.

The utility of the CD8<sup>+</sup> T cell-MHCI system becomes apparent when considering intracellular infections such as those caused by viruses. Viral proteins are processed in the cytosol, and viral p:MHCI complexes are displayed on the cell surface of any infected cell, marking it for recognition and killing by CD8<sup>+</sup> T cells. There is almost nowhere in the body for viruses to hide, since most cells of the body express MHCI molecules (17, 18).

Not all intracellular microbes, however, infect the cytosol; some infect the phagosomes of phagocytes, for example, *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, and *Salmonella* species (19). These microbes are not well controlled by CD8<sup>+</sup> T cells (9, 20, 21), probably because these microbes are not abundant in the cytosols of infected cells and therefore do not lead to efficient production of microbial p:MHCI complexes. Proteins from these microbes, however, are processed in the phagosome, loaded onto MHCII molecules, and shuttled to the cell surface, marking the infected cells for recognition by CD4<sup>+</sup> T cells (22–26). The importance of this fundamental aspect of antigen presentation is evidenced by that fact that CD4<sup>+</sup> T cell-deficient individuals have a preferential susceptibility to phagosomal infections (27, 28).

## CD4<sup>+</sup> T CELL RESPONSE

### General Aspects of the CD4<sup>+</sup> T Cell Response

We first review some general information about how CD4<sup>+</sup> T cells respond to p:MHCII ligands before delving into the mechanisms used by these cells to control phagosomal infections. After leaving the thymus, a newly minted CD4<sup>+</sup> T cell, now called a naive T cell, enters a secondary lymphoid organ (lymph nodes, spleen, and mucosal lymphoid organs) from the blood and percolates through a meshwork of MHCII-expressing dendritic cells (29). This search process optimizes the likelihood that a naive T cell will encounter the p:MHCII ligand that its TCR has a high affinity for no matter where in the body that ligand happens to be produced. The recirculation of naive T cells is facilitated by the expression of CD62L and CC chemokine receptor 7 (CCR7), which bind to ligands expressed exclusively on endothelial cells in secondary lymphoid organs (29). If a naive T cell does not encounter its high-affinity p:MHCII ligand, it leaves that secondary lymphoid organ and migrates to a different one to continue the search (30). The cell remains in the G<sub>0</sub> phase of the cell cycle and expresses small amounts of CD44 and large amounts of CD45RA during the search process, which goes on for 2 to 3 months in mice before the cell dies (31).

The naive T cell undergoes a dramatic transformation if it encounters a dendritic cell displaying the relevant high-affinity p:MHCII ligand. This occurs during infection, as dendritic cells at the infection site take up microbial proteins and migrate to the draining lymph nodes, and free microbial proteins are carried by lymph or blood to secondary lymphoid organs for uptake by resident dendritic cells (32). In either case, dendritic cells in secondary lymphoid organs produce and display microbial p:MHCII complexes. On average, about 1 naive CD4<sup>+</sup> T cell in a million, about 50 cells in a mouse, expresses a TCR capable of strong bind-

ing to any given microbial p:MHCII complex (33). During the relevant infection, these 50 cells interact with dendritic cells displaying the relevant microbial p:MHCII complex, receive TCR signals, produce growth factors, divide many times, and produce several hundred thousand progeny in 1 week (34, 35). These progeny are termed effector cells, which lose the naive phenotype by increasing the expression of levels CD44 and CD45RO and reducing the expression level of CD45RA (36).

Early *in vitro* studies showed that effector T cells differentiate into specific subsets during this period of rapid division. The best-understood effector cell subsets are known as T helper 1 (Th1), T helper 2 (Th2), T helper 17 (Th17), and T follicular helper (Tfh) cells (37). These effector cell subsets form in response to infection-specific signals from the innate immune system and perform specific functions. For example, worm infections cause epithelial cells to produce interleukin-33 (IL-33) and cause group 2 innate lymphoid cells to produce IL-4 (38). Effector T cells that are exposed to these cytokines express the GATA3 transcription factor, which enforces Th2 cell differentiation (37). When stimulated by the relevant p:MHCII ligand, Th2 cells produce IL-4, IL-5, and IL-13, which activate eosinophils and goblet cells to expel worms (39). Extracellular bacterial infections cause dendritic cells to produce IL-6 and transforming growth factor  $\beta$ , which cause effector cells to induce the retinoic acid-related orphan receptor  $\gamma$ t transcription factor, which promotes Th17 cell differentiation (40). Th17 cells produce IL-17, which stimulates epithelial cells to produce chemokines that recruit neutrophils to engulf and kill bacteria. Extracellular bacterial infections also cause the production of IL-6 and IL-21, which induce the expression of the Bcl6 transcription factor, which drives Tfh differentiation in combination with inducible T cell costimulator (ICOS) signals from ICOS ligand-expressing B cells (41). Tfh cells localize to B cell-rich follicles and germinal centers and help B cells undergo antibody affinity maturation and isotype switching. Antibodies then facilitate killing of extracellular microbes via complement activation, antibody-dependent cellular cytotoxicity, or opsonization (42). Certain intracellular infections cause macrophages and dendritic cells to produce the cytokine IL-12 or type 1 interferon (IFN), which causes effector cells to express the T-bet transcription factor, which promotes Th1 cell differentiation (43). Th1 cells produce gamma interferon (IFN- $\gamma$ ) and tumor necrosis factor (TNF), which activate phagocytes to kill intracellular microbes (44).

### CD4<sup>+</sup> T Cell Response to Acute Infection

**Generation of effector cells.** Although much of the evidence for CD4<sup>+</sup> effector T cell differentiation comes from *in vitro* culture systems, recent studies of acute infections by attenuated *Listeria monocytogenes* bacteria or lymphocytic choriomeningitis virus (LCMV) have shed light on this process as it occurs for p:MHCII-specific T cells *in vivo*. Naive CD4<sup>+</sup> T cells with TCRs specific for MHCII-bound peptides from either infection proliferate to produce effector cell populations consisting of about equal numbers of Th1 and Tfh cells in the secondary lymphoid organs by 1 week after infection (35, 45, 46). IL-12 likely drives the differentiation of Th1 cells during infection with attenuated *Listeria monocytogenes* bacteria (47), while type 1 IFN serves this function during LCMV infection (48). In contrast, Tfh cell formation during LCMV infection and probably *Listeria monocytogenes* infection depends on IL-6 and IL-21 (49).

These studies provide the important insight that two different

polarized Th cell subsets, Th1 cells and Tfh cells, can differentiate simultaneously in the same infection. It is possible that individual naive T cells become activated in different niches in secondary lymphoid organs that contain either IL-12 or IL-6 and IL-21. Alternatively, some effector cells may receive ICOS signals from B cells and become Tfh cells, while others do not (45, 50). Another possibility relates to the strength of the signal that a CD4<sup>+</sup> T cell receives through its TCR. *In vitro* stimulation with very low or very high doses of peptide favors the generation of IL-4-producing T cells, while moderate doses of peptide favor IFN- $\gamma$  production (51, 52). *In vivo* studies showed that cell-mediated immunity (associated with Th1 cells) is favored at low antigen doses (53), while humoral immunity (associated with Tfh cells) is favored at high antigen doses (54). Naive T cells that experience moderate TCR stimulation during attenuated *L. monocytogenes* infection or after peptide injection have a tendency to become Th1 cells, while strong or prolonged TCR signaling favors Tfh cell generation (55–58). Consistent with this finding, immune control of *Leishmania major* infection, which depends on Th1 immunity, is induced more efficiently by immunization with a low dose of *Leishmania* protein than with a high dose (59). Strong TCR signaling inhibits the expression of the IL-12 receptor (60) and IL-2 receptor signaling (61), both of which are needed for maximal Th1 cell differentiation (35, 45, 62, 63). Thus, it is possible that strong TCR signaling suppresses the Th1 cell fate, thereby promoting the Tfh cell fate.

Studies on attenuated *L. monocytogenes* infection also revealed that some Th1 effector cells leave secondary lymphoid organs and migrate to nonlymphoid tissues such as the liver, while Tfh cells are confined to the secondary lymphoid organs (55). Although not examined in this case, in other systems, Th1 cells express the active form of P-selectin glycoprotein 1, which binds to CD62E and CD62P, and CXC chemokine receptor 3 (CXCR3), which binds to CXC chemokine ligand 9 (CXCL9) and CXCL10 (64). CD62E, CD62P, CXCL9, and CXCL10 are displayed on endothelial cells in inflamed nonlymphoid tissues, thereby facilitating the extravasation of Th1 cells (65).

**Formation of memory cells.** Some of the effector cells survive acute infection to become memory cells. The number of *L. monocytogenes* or LCMV p:MHCII-specific effector cells in the body declines by about 90% in secondary lymphoid organs via apoptosis over a 2-week period after the peak (34, 35, 46, 66). The loss of effector cells coincides with clearance of the infection, and it was recently found that deprivation from p:MHCI molecules may cause some CD8<sup>+</sup> T cells to die during the contraction period (67). Thus, it is possible that the 10% of effector cells that survive to become memory cells are those that happen to interact with a cell displaying the relevant p:MHC molecules during a critical period of the contraction phase. Effector cell survival into the memory phase is associated with expression of the IL-7 receptor (68) or the TNF receptor superfamily member CD27 (34), which enhances expression of prosurvival members of the Bcl-2 family.

The CD4<sup>+</sup> memory T cells that survive the contraction period after *L. monocytogenes* or LCMV infection retain the CD44<sup>high</sup> phenotype of effector cells and consist of Th1 and Tfh-like subsets (35, 46, 69–71). It is likely that Th1 memory cells derive from Th1 effector cells (72) and that Tfh memory cells derive from Tfh effector cells (35, 71). Th1 memory cells lack expression of CCR7 (35) and thus resemble so-called effector memory cells (73), while Tfh-like memory cells express CCR7 (35) and resemble central



memory cells (73). Most of the memory cells in either subset are not cycling (34) but are maintained for prolonged periods in the G<sub>0</sub> or G<sub>1</sub> phase of the cell cycle by the cytokine IL-7 (74). However, some of the memory cells in the population divide infrequently in response to the cytokine IL-15 (34, 75). In contrast, if the relevant infection occurs a second time, memory cells receive TCR signals and rapidly enter the cell cycle, with Th1 memory cells producing Th1 effector cells and Tfh memory cells producing Th1 and Tfh effector cells (35, 69, 70).

Surprisingly, the combination of IL-7-dependent survival and IL-15-dependent proliferation is not sufficient to maintain CD4<sup>+</sup> memory T cells induced by acute infections. Levels of CD4<sup>+</sup> memory T cells induced by attenuated *L. monocytogenes* or LCMV infection slowly decline, with a half-life of about 60 days in mice (34, 35, 46, 66). This decline is in contrast to the seemingly perfect numerical stability of CD8<sup>+</sup> memory T cells and plasma B cells (76, 77). Thus, at the population level, murine CD4<sup>+</sup> memory T cells induced by acute infections are remarkably “forgetful” compared to other memory cells. This could be due to the fact that CD4<sup>+</sup> T cells undergo less IL-15-driven homeostatic proliferation than CD8<sup>+</sup> T cells due to a lower expression level of the IL-15 receptor (34, 75, 78). A second, non-mutually exclusive possibility is that weak TCR signals from self-p:MHCII ligands are important for the survival of CD4<sup>+</sup> memory T cells, and competition for these ligands reduces survival when CD4<sup>+</sup> T cell populations specific for the same ligand are large (79, 80). In any case, murine CD4<sup>+</sup> T cells do not conform to the textbook notion that a single transient exposure to antigen is sufficient to induce lifelong immune memory. Although human CD4<sup>+</sup> T memory cells appear to be long-lived without the eliciting antigen (81), it is difficult to rule out the involvement of periodic TCR stimulation with environmental peptides that cross-react with the eliciting peptide (82).

### CD4<sup>+</sup> T Cell Response to Phagosomal Infection

**Generation of effector cells.** Although work on attenuated *L. monocytogenes* and LCMV infections elucidated many interesting aspects of the *in vivo* CD4<sup>+</sup> T cell response, it did not reveal much about the role that these cells play in protective immunity because CD8<sup>+</sup> T cells are mainly responsible for protecting the host from these microbes (83, 84). The primary role of CD8<sup>+</sup> T cells in the control of these cytosolic infections is likely related to the efficient production of microbial p:MHCI complexes by infected cells. In contrast, CD4<sup>+</sup> T cells are probably the key controllers of primary infections by microbes such as *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Leishmania major*, and *Salmonella enterica* (27), because these organisms replicate in the phagosomes of macrophages and dendritic cells, where p:MHCII complexes are formed (85–88). Not only are CD4<sup>+</sup> T cells the main controllers of these infections (9, 21, 89–91), they also appear to do so with little help from other cells of the adaptive immune system. For example, despite becoming activated (92), gamma-delta T cells appear to play no positive role in controlling phagosomal infections (9, 21), and CD8<sup>+</sup> T cells play only a small or no role (9, 20, 21, 93). Mice lacking CD1 control *M. tuberculosis* infection normally, demonstrating that natural killer T (NKT) cells are not required (94). In addition, a role for B cells or antibodies has not been clearly established for the control of phagosomal infections. Several groups reported that mice lacking B cells had a defect in the control of primary *M. tuberculosis* infection (95, 96), whereas another group reported no defect (97). Several other studies found

that mice lacking B cells had mild deficits in controlling phagosomal infections, but these defects were attributed to poor priming of T cells rather than antibody production (98–100). In summary, although CD8<sup>+</sup> T cells and B cells may play minor roles in the control of phagosomal infections, their importance in this regard pales in comparison to that of CD4<sup>+</sup> T cells.

We now review research on the CD4<sup>+</sup> T cell response to the phagosomal pathogens *M. tuberculosis*, *L. major*, and *S. enterica*. These organisms are the focus of this review because the large body of research on each one is conducive to identifying common elements of the CD4<sup>+</sup> T cell response to phagosomal pathogens. In the case of *S. enterica*, special attention is given to studies of Nramp1-sufficient hosts, which generate persistent infections, unlike Nramp1-deficient hosts, which are abnormally susceptible and die very early after infection (86). Emphasis is also placed on studies in which T cells specific for MHCII-bound peptides from phagosomal pathogens were tracked, because these studies provide insight into the T cells that specifically control these infections. Because of this focus, most of the studies reviewed here were performed in mouse model systems where relevant reagents, such as p:MHCII tetramers, are available for tracking relevant CD4<sup>+</sup> T cells (101).

All three of the prototypical phagosomal infections have similar features. In each case, the microbes enter and replicate in macrophages and dendritic cells at the initial site of infection and then spread to associated secondary lymphoid organs and eventually to blood, probably within phagocytes (9, 86, 102, 103). The number of microbes in all body sites increases steadily from a small number to a peak about 3 weeks after infection and then declines to a lower set point that is maintained for the life of the host. CD4<sup>+</sup> T cells have no effect on the growth of the microbes for the first 2 to 3 weeks after infection but are then critical for reducing the number of microbes to the set point (9, 21, 104). Phagosomal pathogens often persist during the period of CD4<sup>+</sup> T cell-mediated control in organoids called granulomas, which form at the initial site of infection and consist of tight clusters of phagocytes interspersed with epithelial cells and surrounded by T cells (85).

Phagosomal infections induce CD4<sup>+</sup> T cell responses that get off to a slow start compared to the responses induced by acute bacterial and viral infections. In each case, macrophages and dendritic cells at the initial site of infection—lung for *M. tuberculosis*, skin for *L. major*, and Peyer’s patches for *S. enterica*—take up microbes into phagosomes (103, 105–107). The infected macrophages and dendritic cells then migrate to the draining lymph nodes (105, 108–110), where activation of CD4<sup>+</sup> T cells with specific TCRs is first detected 1 to 2 weeks after infection (111–114) (Fig. 1). The relevant p:MHCII-specific CD4<sup>+</sup> T cells eventually produce a large number of effector cells that peak in the secondary lymphoid organs after 2 to 3 weeks, when the infection peaks. Some of the effector T cells that are generated in the secondary lymphoid organs migrate to nonlymphoid organs, including the original sites of infection (111, 114, 115) (Fig. 1). In the case of *M. tuberculosis*, granulomas eventually form in the lungs (116), and the T cells are contained within them (117). Granuloma-like structures also eventually form in the skin sites of *L. major* infection (118, 119) and the livers of *S. enterica*-infected mice (120).

Several factors may account for the slow tempo of the CD4<sup>+</sup> T cell responses to phagosomal infections. First, in all three cases, it is likely that only a small number of microbes make it to the secondary lymphoid organs early after infection. The initial inocu-

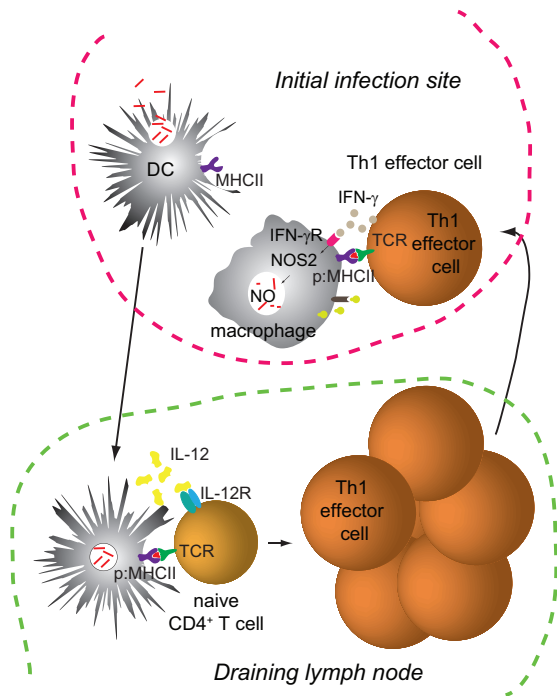


FIG 1 Early events after a phagosomal infection. The schematic illustrates events occurring at the initial site of infection and a draining lymph node about 1 to 2 weeks after infection.

lum of *M. tuberculosis* or *L. major* organisms is low in the most common models (9, 102), and the number of *S. enterica* organisms that enter the mucosal lymphoid organs is also very small (121), likely because most of the bacteria die in the stomach after ingestion. In addition, it has been reported that phagosomal pathogens inhibit antigen processing and the formation of p:MHCII complexes (122–128), although this effect not been observed in all studies (129, 130). Thus, only a few of the relevant CD4<sup>+</sup> T cells may become activated early after infection, because the number of infected phagocytes initially capable of presenting p:MHCII complexes is very small, or the infected phagocytes display very few p:MHCII complexes per cell (127). Second, *M. tuberculosis* p:MHCII-specific regulatory T cells become activated early after infection and slow the activation of effector T cells (114). The effector cells eventually take over, however, because the regulatory cells disappear by an IL-12-dependent mechanism (114).

**Memory cell survival.** Another difference between the immune responses to persistent phagosomal infections and those to acute infections relates to the numerical stability of memory cells. The number of microbe p:MHCII-specific effector cells induced by phagosomal infections drops about 10-fold after the peak, as in the case of acute infections (111, 113, 115). Unlike the case of acute infections, however, the number of specific memory cells then remains constant in all body sites for hundreds of days (111, 113, 115). This remarkable stability is associated with low-level persistence of the phagosomal pathogen, as evidenced by the decline in the number of *S. enterica* p:MHCII-specific CD4<sup>+</sup> T cells after persistent infection was cleared with antibiotics (113, 125). Thus, phagosomal pathogen p:MHCII-specific CD4<sup>+</sup> T cell populations are maintained by a nonclassical antigen-dependent mechanism. This mechanism has also been observed for polyomavirus p:MHCII-specific CD4<sup>+</sup> T cells in mice with persistent infection (131).

It is important to note that only a small number of cells in the specific CD4<sup>+</sup> T cell population proliferate in response to the relevant p:MHCII ligand in secondary lymphoid organs or granulomas at any one time during the persistent phase of infection (113, 115). This phenomenon is again likely related to the fact that only a small number of infected phagocytes are present in the body during this phase. Thus, specific CD4<sup>+</sup> memory T cells probably proliferate infrequently because they rarely encounter a phagocyte displaying the relevant p:MHCII ligand. The infrequency of this proliferation probably explains why the CD4<sup>+</sup> T cell population is maintained at a stable level rather than increasing steadily over time (113). Indeed, p:MHCII-driven proliferation must perfectly balance the death rate to keep the population at a stable level. This model also implies that CD4<sup>+</sup> T cells with TCRs specific for MHCII-bound peptides from phagosomal microbes spend long periods of time during persistent infection without encountering the relevant p:MHCII ligand. These long rest intervals could explain why these CD4<sup>+</sup> T cells do not experience the activation defects exhibited by the “exhausted” T cells (113, 115) observed in individuals with systemic chronic infection (132).

**Th1-mediated control of phagosomal infection.** The p:MHCII-specific CD4<sup>+</sup> T cell populations present during the persistent phase of *M. tuberculosis*, *S. enterica*, and *L. major* infections have the CD44<sup>high</sup> memory cell phenotype and are dominated by Th1 cells (111, 113–115). This makes sense because all three microbes are potent inducers of IL-12 by cells of the innate immune system, and IL-12 is a major driver of Th1 cell differentiation (47, 133–135). It is less clear why Tfh cells do not form or are not maintained efficiently during these infections as in the case of acute infection with attenuated *L. monocytogenes* bacteria, which is also a strong IL-12 inducer (47). The fact that results for attenuated *L. monocytogenes* were obtained by injecting a large number of bacteria directly into the blood (35) is one possibility. This regimen provides many extracellular bacteria for uptake by antigen-specific B cells in the spleen. These B cells could present p:MHCII complexes to effector CD4<sup>+</sup> T cells, steering them toward the Tfh fate, since antigen presentation by B cells favors Tfh cell development by providing continuously high levels of cognate p:MHCII (57). In contrast, very few free microbes likely enter the body after *M. tuberculosis*, *S. enterica*, or *L. major* infections, and the ones that do quickly enter a phagocyte, thereby limiting B cell access to the microbes. In this situation, very few of the effector cells would be diverted to the Tfh fate, and most would default to the Th1 fate. Poor access to antigen may also explain why B cells play little or no role in the control of phagosomal infections (97, 136–138). The dominance of Th1 cells during phagosomal infections could also be the result of the iterative stimulation of memory cells during persistent infection. Even if Tfh-like central memory cells are formed early after infection, they would be expected to yield mainly Th1 progeny after encountering the relevant p:MHCII ligand at a later time (34, 35, 69). Many rounds of this process would eventually lead to a Th1-dominated population.

Although the majority of effector cells induced by phagosomal infections are Th1 cells, some heterogeneity exists in this population. The *M. tuberculosis* p:MHCII-specific Th1 cell population consists of two subsets, one expressing killer cell lectin-like receptor G1 (KLRG1) and one lacking it (115). KLRG1<sup>−</sup> cells have a greater proliferative potential than KLRG1<sup>+</sup> cells, while KLRG1<sup>+</sup> cells are better IFN-γ and TNF producers than KLRG1<sup>−</sup> cells. KLRG1<sup>−</sup> cells may give rise to KLRG1<sup>+</sup> cells in response to

chronic TCR stimulation. The results suggest a conveyor belt model where KLRG1<sup>-</sup> Th1 cells periodically encounter their p:MHCII ligand on infected phagocytes, proliferate, and differentiate into KLRG1<sup>+</sup> cells.

Th1 cells are important for the control of phagosomal infections because their lymphokines enhance the microbe-killing functions of phagocytes, including the production of nitric oxide (9, 139, 140). A likely scenario is that dendritic cells from the site of infection migrate to the draining lymph nodes and present pathogen p:MHCII complexes and IL-12 to naive CD4<sup>+</sup> cells, which proliferate and become Th1 effector cells (Fig. 1). These cells then migrate to the site of infection and produce IFN- $\gamma$  after encountering pathogen p:MHCII complexes on infected phagocytes. IFN- $\gamma$  then binds the IFN- $\gamma$  receptor on the infected phagocyte, causing the production of inducible nitric oxide synthase (NOS2) and the generation of the microbicidal compound nitric oxide (9). The finding that NOS2-deficient mice cannot control phagosomal pathogens is evidence of the importance of this pathway (9, 141–144). Synergy between IFN- $\gamma$  and TNF for NOS2 induction could explain why T cells that produce both cytokines are more protective than T cells that produce IFN- $\gamma$  alone (59, 145). IFN- $\gamma$  and TNF also cause fusion of microbe-containing phagosomes with lysosomes, leading to microbial death due to exposure to acids, reactive oxygen species, and proteases (146, 147).

Srivastava and Ernst recently shed light on how the interaction of Th1 cells and infected phagocytes contains infection (148). They used chimeric mice that contained a mixture of MHCII-sufficient and MHCII-deficient macrophages and dendritic cells and found that the MHCII-sufficient cells controlled *M. tuberculosis* infection in a CD4<sup>+</sup> T cell-dependent fashion, while the MHCII-deficient cells in the same mouse did not. The fact that the MHCII-deficient cells could not control infection indicates that the IFN- $\gamma$  and TNF produced by CD4<sup>+</sup> T cells in response to MHCII-sufficient cells could not act in a bystander fashion on nearby MHCII-deficient cells. This phenomenon could be explained by focal secretion of the cytokines into the synaptic space formed by the opposed membranes of CD4<sup>+</sup> T cells and infected phagocytes displaying *M. tuberculosis* p:MHCII complexes (149).

Another way in which CD4<sup>+</sup> T cells could contain phagosomal infections is the direct killing of infected phagocytes. Although cytotoxicity is often considered to be the job of CD8<sup>+</sup> T cells, recent work (150) indicates that CD4<sup>+</sup> T cells can acquire this capacity under certain conditions. Naive CD4<sup>+</sup> T cells stimulated through the TCR, the TNF receptor family members CD134 (OX40) and CD137 (4-1BB), and the IL-2 receptor differentiate into effector cells that express the cytotoxic proteins perforin and granzyme B and kill cells displaying the relevant p:MHCII ligand (151, 152). This differentiation pathway depends on the eomesodermin transcription factor but not T-bet (151, 152). It will be of interest to determine if eomesodermin-dependent CD4<sup>+</sup> cytotoxic T cells play a role in the control of phagosomal infections.

Some Th17 effector cells are generated during *M. tuberculosis* and *Salmonella* infections (153–155). Th17 cells might be expected to participate in the control of phagosomal infections by recruiting neutrophils. Indeed, it was reported that mice lacking the IL-17 receptor were defective in the long-term control of *M. tuberculosis* infection (156). On the other hand, *M. tuberculosis* and *Salmonella* infections are controlled normally in IL-23-deficient mice, in which Th17 generation does not occur (157, 158), and neutralization of IL-17 had no effect on the control of *L. major*

infection (159). Furthermore, people with mutations that affect the expression of IL-17 or IL-17 receptor are susceptible to *Candida* and *Staphylococcus aureus* skin infections (160) but do not appear to be susceptible to the phagosomal pathogens that afflict people lacking CD4<sup>+</sup> T cells or IL-12 receptor (27, 28, 161). Thus, the bulk of the evidence to date suggests that Th1 cells, and not Th17 cells, are critical for controlling phagosomal infections.

**The curious case of herpes simplex virus.** One microbe that afflicts CD4<sup>+</sup> T cell-deficient hosts but is not thought to persist in phagocytes is herpes simplex virus (HSV). This virus produces a lytic infection of epithelial cells in the skin and then spreads to nearby neurons, where it can persist for the life of the host (162). Local dendritic cells are also infected, at least early on (163–167). T cells are thought to play a role in keeping the infection in a latent state in neurons, although this state can sporadically break down, resulting in viral replication and a skin lesion (162). CD8<sup>+</sup> T cells likely contribute to the control of infection by killing epithelial cells that become infected after the virus emerges from persistently infected neurons (162). The susceptibility of AIDS patients to HSV infection (27) suggests that CD4<sup>+</sup> T cells also play a role in the control of this infection. This role may be forced by HSV shutting down the expression of MHCII molecules in dendritic cells using a protein called vhs (168, 169), thereby limiting the capacity of infected dendritic cells to stimulate CD8<sup>+</sup> T cells. Nearby uninfected dendritic cells of the CD8 $\alpha$ <sup>+</sup> subtype, however, engulf dying infected cells and produce HSV peptide:MHCII complexes (163). It is therefore possible that CD4<sup>+</sup> T cells are critical for the control of HSV infection by producing the antiviral cytokine IFN- $\gamma$  in response to HSV peptide:MHCII complexes generated by CD8 $\alpha$ <sup>+</sup> dendritic cells that engulf dying infected cells or their debris. Thus, even though HSV is not a phagosomal infection *per se*, CD4<sup>+</sup> T cells may be critical for its control because of the special role that phagosomal antigen presentation plays in this infection.

**Phagosomal pathogen resistance to Th1-mediated control.** Although it is clear that CD4<sup>+</sup> T cells control phagosomal infections, it is equally clear that infection is rarely eliminated (86, 170). Granulomas, which form in the lungs, skin, and liver in *M. tuberculosis*, *L. major*, and *S. enterica* infections, respectively, may be involved in this process (85, 120, 171–173). Granulomas consist of tight clusters of macrophages, some of which are infected and many of which fuse to form multinucleated giant cells (174). The macrophage clusters are surrounded by lymphocytes and interspersed with CD4<sup>+</sup> T cells, collagen fibers, and epithelial cells. It can be argued that granulomas provide an advantage to the host by walling off the microbe in a site full of activated T cells and phagocytes (175). *M. tuberculosis* organisms, however, produce substances that cause host epithelial cells to secrete matrix metalloproteinase 9 (MMP9), which is critical for granuloma formation (176, 177). The fact that the pathogen appears to foster granuloma formation suggests that granulomas provide an advantage to the pathogen. Although Th1-mediated killing of infected macrophages and their microbes likely restrains the infection within granulomas, new phagocytes, which are actively recruited to granulomas, quickly engulf the dying macrophages and their microbes and become infected in the process (85, 117) (Fig. 2). Thus, granulomas are sites where *M. tuberculosis* organisms obtain access to a constant supply of new cells to infect. Another point in favor of the microbe is that infected phagocytes within granulomas display very small numbers of microbe p:MHCII complexes, thereby lim-



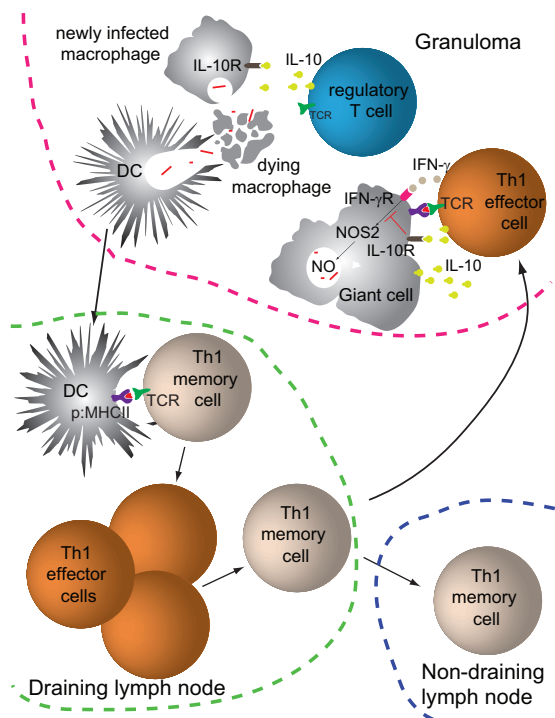


FIG 2 Events during the persistent phase of a phagosomal infection. The schematic illustrates events at the initial site of infection and a draining lymph node, months after the initial infection.

iting IFN- $\gamma$  and TNF production by Th1 cells (178, 179). It is also possible that the abnormal multinucleated giant cells found in granulomas (174) are impaired with respect to antigen processing and p:MHCII ligand production.

Another factor that limits pathogen clearance is that Th1 cells at sites of persistent phagosomal infection acquire the capacity to produce the suppressive cytokine IL-10 (111, 180) (Fig. 2). The differentiation of Th1 cells to an IL-10-producing state may depend on very high levels of IL-12 receptor signaling (181–184), which may be most likely to occur at sites of persistent infection. Importantly, IL-10 receptor signaling in macrophages inhibits MHCII expression (185, 186), thereby potentially limiting the display of microbial p:MHCII complexes by infected phagocytes in granulomas. IL-10 receptor signaling also suppresses IFN- $\gamma$  and TNF production (187) and nitric oxide generation (188), which could blunt the capacity of infected macrophages to kill their microbes. This model is supported by the observation that IL-10 neutralization increases killing of phagosomal pathogens (102, 189, 190).

Regulatory T cells provide another brake on the killing of phagosomal pathogens in granulomas (Fig. 2). *L. major*-induced skin lesions contain abundant regulatory T cells, which are required for persistent infection (191). Some (192) but not all (111) of these regulatory T cells express TCRs that are specific for *L. major* p:MHCII complexes and accumulate in skin lesions through the expression of the E-cadherin binding integrin CD103 (193). These observations suggest that *L. major* p:MHCII-specific regulatory T cells suppress the microbe-killing functions of *L. major* p:MHCII-specific Th1 cells in the same lesions. *M. tuberculosis*-induced granulomas also contain abundant regulatory T cells (194). The

TCR specificity of these cells is unclear, however, since *M. tuberculosis* p:MHCII-specific regulatory T cells are lost at later times after infection (114). In addition, the relevance of regulatory T cells in granulomas is uncertain, since late depletion of regulatory T cells had no effect on the number of *M. tuberculosis* organisms in this location (195).

Phagosomal pathogens also have intrinsic mechanisms to resist the attempts of Th1 cells to activate their phagocyte host. As mentioned above, many phagosomal microbes produce molecules that interfere with the IFN- $\gamma$ -enhanced process of phagosome fusion with lysosomes (146, 171). For example, *M. tuberculosis* has many enzymes that detoxify microbicidal compounds, such as superoxide anion, hydrogen peroxide, and nitric oxide, that are produced in large amounts in the phagolysosomes of IFN- $\gamma$ -activated phagocytes. *M. tuberculosis*, *L. major*, and *S. enterica* organisms also cause phagocytes to produce IL-10 (196–199) (Fig. 2), which, in addition to its T cell-suppressive effects, inhibits phagosome-lysosome fusion (128, 200, 201).

Thus, phagosomal pathogens persist by resisting phagosome toxification, quickly jumping from dying phagocytes to newly recruited phagocytes, and promoting regulatory T cells and IL-10 that restrain the microbicidal functions of Th1 cells.

**Concomitant immunity.** Remarkably, healthy hosts that harbor *Leishmania* parasites in phagocytes at a site of persistent infection are resistant to superinfection in other parts of the body (202). This situation is advantageous to the host, since unlike persistent granuloma infection, which is usually asymptomatic, systemic superinfections can be fatal. This odd state of affairs raises the question, how does the immune system eliminate infections at secondary sites but leave the initial infection intact? The answer may lie in the fact that superinfecting organisms are taken up by phagocytes that are not in the immunosuppressive environment of the granuloma and are thus susceptible to complete killing by circulating memory T cells.

The concept that a localized infection is required for protection from systemic infection is called concomitant immunity and has been studied primarily with *Leishmania* infection. *L. major* parasites are deposited in the skin by a sand fly bite and are taken up by dendritic cells and macrophages, some of which migrate to the draining lymph nodes (203). The infected phagocytes process proteins from the parasite and present parasite p:MHCII ligands to naive CD4<sup>+</sup> T cells, which expand and produce Th1 effector cells (Fig. 1). The Th1 cells migrate to the skin infection site and limit the replication of the parasites within phagocytes by stimulating nitric oxide production, as described above (Fig. 1). However, Treg cell- and IL-10-rich granuloma-like structures quickly form at this site to protect the microbes from complete elimination (102, 188, 191, 204), allowing the parasites to persist long-term (205) (Fig. 2). Th1 cells also circulate to other body sites, including non-draining lymph nodes and uninfected skin (111) (Fig. 2). Occasionally, some of the Th1 cells return to the site of infection and respond to parasite p:MHCII molecules, keeping the infection in check (Fig. 2) and maintaining the population of parasite p:MHCII-specific CD4<sup>+</sup> T cells. In the event of a later sand fly bite, Th1 cells at the new bite site, or in the spleen if the parasites reach the blood, produce IFN- $\gamma$  and TNF and kill all the parasites in these locations before new granuloma-like organoids can form to protect the parasites (204, 206) (Fig. 3). Because CD4<sup>+</sup> memory T cells do not persist indefinitely without periodic stimulation by the relevant p:MHCII ligand (34, 35, 46, 66), the decrease in the

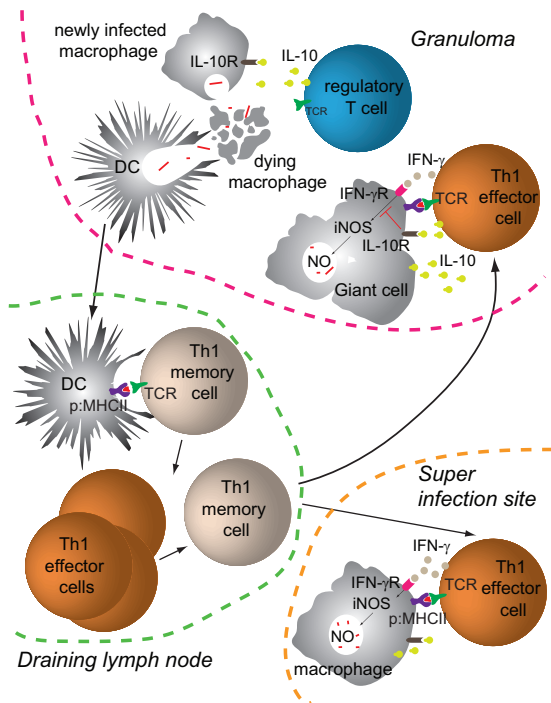


FIG 3 Events during superinfection of a host with a persistent phagosomal infection. The schematic illustrates events at the indicated sites of a persistently infected host, 1 week after a new infection.

level of cognate p:MHCII that would accompany a sterile cure of *Leishmania* infection would likely lead to a gradual decrease in the number of CD4<sup>+</sup> T cells and eventual loss of immunity. Indeed, protection from superinfection is lost if the original infection is cleared (191, 207, 208).

While concomitant immunity is best understood in the context of *Leishmania* infection, it may operate for all phagosomal infections. Concomitant immunity could be responsible for containing *M. tuberculosis* and *C. neoformans* infections to lung granulomas, limiting their spread and preventing superinfection. Concomitant immunity may have evolved because it meets the needs of the microbe and the host: the microbe gets a niche to live in, and the host becomes resistant to more serious systemic forms of the infection.

**Implications for vaccines.** Most vaccines consist of attenuated microbes, dead microbes, or components of microbes that are given to subjects a limited number of times. This regimen produces a situation where vaccine antigens are present in an extracellular form and are eliminated from the body shortly after administration. Transient exposure to extracellular antigen is sufficient to stimulate the production of Tfh cells, which help B cells become long-lived plasma cells that produce neutralizing antibodies for the life of the host, even after the eliciting antigens have been cleared from the body (41). Vaccines of this kind are therefore very effective for extracellular infections. Given what we now know about the immune response to phagosomal infections, it is easy to see why this vaccination strategy has failed for this class of microbes. Phagosomal pathogens are not susceptible to antibodies, because they reside in phagosomes and are often transmitted to new phagocytes that engulf an infected phagocyte. In addition, transient exposure to antigen is not an effective way to induce a

numerically stable population of CD4<sup>+</sup> memory T cells over long periods of time (113). Indeed, the fact that the bacillus Calmette-Guérin (BCG) vaccine, an attenuated strain of *Mycobacterium bovis*, induces partial immunity to *M. tuberculosis* infection that fades with time (209) may be explained by the decline in the number of relevant CD4<sup>+</sup> memory T cells as BCG antigens disappear.

The practice of “leishmanization” provides a clue as to how a vaccine for a phagosomal pathogen could work (202). People in areas where the disease is endemic have long recognized that individuals with a *Leishmania*-infected skin lesion rarely contract the often-fatal visceral form of the infection. This knowledge has led to leishmanization, a process whereby people inoculate themselves in an inconspicuous skin site with material from the skin lesion of an infected person. It is reasonable to suspect that this practice produces a localized persistent infection and concomitant immunity.

Although it may be effective, leishmanization is a dangerous practice in the AIDS era (210). An AIDS-related drop in the number of CD4<sup>+</sup> T cells could lead to a loss of control of the *Leishmania* parasites in the skin lesion, resulting in systemic infection and death. Thus, the question becomes how to deliver antigens from phagosomal pathogens safely for a long period of time and in small amounts from a localized site, perhaps with features of a granuloma. Perhaps, it will be possible to build a better BCG vaccines, that is, more persistent vaccine strains containing suicide molecules that could be triggered in the event of AIDS. Alternatively, peptides from phagosomal pathogens could be incorporated into next-generation polymers designed to release their cargo over very long periods of time. Peptides could be incorporated to limit the formation of antibodies that would speed the clearance of the vaccine antigen. These formulations could be injected with MMP9 in an attempt to recreate a granuloma-like structure at the injection site. Success could depend on abandoning approaches aimed at elicitation of neutralizing antibodies in favor of approaches aimed at generating large, intermittently stimulated populations of Th1 cells.

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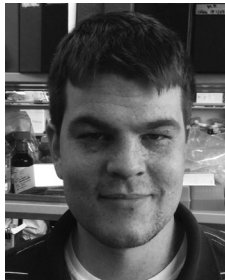


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