Revisiting the role of the granuloma in tuberculosis

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Abstract | The granuloma, which is a compact aggregate of immune cells, is the hallmark structure of tuberculosis. It is historically regarded as a host-protective structure that 'walls off' the infecting mycobacteria. This Review discusses surprising new discoveries — from imaging studies coupled with genetic manipulations — that implicate the innate immune mechanisms of the tuberculous granuloma in the expansion and dissemination of infection. It also covers why the granuloma can fail to eradicate infection even after adaptive immunity develops. An understanding of the mechanisms and impact of tuberculous granuloma formation can guide the development of therapies to modulate granuloma formation. Such therapies might be effective for tuberculosis as well as for other granulomatous diseases.

Necrosis
A common form of cell death that frequently results from toxic injury, hypoxia or stress. Necrosis involves cell swelling, dysregulation of cell-membrane ion and water fluxes, mitochondrial swelling and the eventual release of cell contents into the interstitium. This form of cell death usually occurs together with inflammation. Recent evidence suggests that necrosis can also represent a form of programmed cell death, in which case it is often referred to as programmed necrosis or necroptosis.

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Tuberculosis is the most frequent cause of granulomas, which are organized immune cell aggregates that form in response to persistent stimuli of an infectious or non-infectious nature[1] (BOX 1). Indeed, the granuloma as a discrete structure was first described in 1679 in tuberculous lungs and called a tubercle[2], preceding the discovery of the causative organism of tuberculosis (*Mycobacterium tuberculosis*) by more than 200 years[3]. It was even proposed at the time that tubercles cause tuberculosis, a conclusion that is presciently in line with new findings highlighted in this Review. There followed an accumulation of evidence for the association of tubercles with tuberculosis[4,5], which was named as such in 1839 in recognition of its inextricable connection with tubercles[6].

At its most basic level, a granuloma is a compact, organized aggregate of mature macrophages that arises in response to a persistent stimulus[7–9]. Mature macrophages are characterized by their increased cytoplasmic size and larger numbers of organelles, and by their ruffled cell membranes, which are thought to render them more phagocytic and microbicidal[10–13]. Granuloma macrophages can undergo additional changes: they can fuse into multinucleated giant cells[14] or differentiate into foam cells, which are characterized by lipid accumulation. The consequences of these changes are not well understood[14–18]. Particularly in tuberculous granulomas, the mature macrophages can undergo a distinct transformation into epithelioid cells (also known as epithelioid histiocytes), which have tightly interdigitated cell membranes in zipper-like arrays that link adjacent cells[10] (FIG. 1). Also particular to tuberculosis are characteristic regions of necrosis that form within granulomas owing to the death of participating cells, including macrophages; these necrotic areas are known as caseum when they are seen on gross pathology[19–22]. Many other cell types also populate the granuloma, such as neutrophils, dendritic cells (DCs), B and T cells, natural killer cells, fibroblasts and cells that secrete extracellular matrix components. Finally, the epithelial cells surrounding the granuloma are now thought to also participate in its formation[23].

The idea that such a concentrated immune response should sequester and eradicate the inciting pathogen is logical, and indeed many individuals who never showed signs of active tuberculosis have evidence of healed (and often sterile) granulomas[24–26]. However, the large number of tuberculosis cases worldwide indicates that many individuals fail to eradicate infection through granuloma formation. More people have died of tuberculosis than any other infectious disease throughout history, and more people have tuberculosis today than at any other time in history[27]. Why does the impressive arsenal of host defences in the granuloma not reliably eradicate infection? The answer lies in understanding the inner workings of the granuloma and what each step in its assembly accomplishes for host and pathogen. In this Review, I discuss recent work showing that mycobacteria exploit the early innate immune stages of granuloma formation to proliferate and disseminate throughout the host. I describe how the foothold they thus gain is often maintained through newly identified host–microorganism interactions that might

Box 1 | **Granulomatous diseases**

Granulomas are traditionally classified as either epithelioid or foreign-body granulomas. In epithelioid granulomas, macrophages respond to inflammatory stimuli, either known or unknown, and undergo morphological changes that make them appear similar to epithelial cells. By contrast, foreign-body granulomas arise in response to inert particles[7–9]. However, this terminology is confusing, as epithelioid granulomas can result from foreign bodies such as beryllium, which induces a strong hypersensitivity response[1,8]. An alternative granuloma classification scheme is based on the turnover of constituent macrophages[8]. In general, in epithelioid granulomas the invoking agent is toxic to participating macrophages, thus leading to their death and continual replenishment by newly arriving cells. By contrast, in foreign-body granulomas inert substances are surrounded by long-lived macrophages that are not replaced. High-turnover, epithelioid granulomas such as the tuberculous granuloma are the most common type and the most relevant medically. These can, in turn, be subdivided according to their aetiology — infectious or non-infectious — with the caveat that the latter might have an infectious aetiology that has not yet been discovered. For example, Whipple's disease and cat scratch disease were considered to be non-infectious granulomatous diseases until the recent discovery of their infectious agents (*Tropheryma whippeli*[163] and *Bartonella henselae*[164], respectively). Infectious granulomas are caused by diverse classes of infectious agents (bacteria, fungi, protozoa, trematodes and viruses) and represent important diseases worldwide.

Excessive granuloma formation can be detrimental in non-infectious granulomatous disorders (such as Crohn's disease, sarcoidosis and Wegener's granulomatosis), as it can be in tuberculosis. For schistosomiasis, the granulomatous response favours parasite extrusion and transmission[159].

Examples of granulomatous diseases

Non-infectious. Crohn's disease, sarcoidosis, Wegener's granulomatosis, primary biliary cirrhosis and berylliosis.

Infectious. Various bacterial, fungal, protozoal and viral agents can cause granulomas. Some important ones, in addition to those mentioned above, include leprosy (another mycobacterial disease); brucellosis and syphilis (bacterial); histoplasmosis and cryptococcosis (fungal); leishmaniasis and toxoplasmosis (protozoal); and infectious mononucleosis, mumps and measles (viral).

prevent mycobacterial eradication, even when adaptive immune elements are fully functioning in the granuloma. Modulating granuloma formation and function so as to convert these structures into unequivocally host-protective entities might provide new approaches to tuberculosis chemotherapy.

Granuloma formation and function: new concepts

A solely host-protective structure? It is important to try to understand the reasons behind the entrenched view that the granuloma is a crucial host-protective structure. The notion that granulomas restrict infection by 'walling off' bacteria dominated the literature, including in medical and immunology textbooks[28–33], until very recently[34]. Tuberculosis results from complex bacterium–host interactions. Inhaled bacteria are phagocytosed by macrophages in the lungs, wherein they can replicate by subverting phagocyte endocytic trafficking and resisting innate defence mechanisms[24,35]. Infected cells migrate into tissues and aggregate into granulomas. The reasonable extrapolation from the finding of healed fibrotic and calcified tuberculous granulomas in healthy individuals is that the granuloma must have tried but failed to curtail bacterial growth in active cases of tuberculosis. Therefore, it follows that without granuloma formation, pathogen proliferation and dissemination would be even greater.

Caseum

Necrotic material that has a cheesy white appearance on gross examination. Areas of caseum are a hallmark feature of human tuberculous granulomas that result from a distinctive type of necrotic breakdown known as caseous necrosis. Caseous necrosis seems to be a specialized form of coagulative necrosis in which cellular degradation (rather than rapid enzymatic digestion) dominates. On microscopic examination, caseum contains fragmented cells and their amorphous debris. Caseum can be hard or soft on gross examination; hard caseum is generally bacterium poor, whereas soft caseum is often laden with bacteria.

This hypothesis would seem to be supported by the association between poorly formed granulomas and hypersusceptibility to *M. tuberculosis* infection under various immunocompromising conditions — such as deficiency of tumour necrosis factor (TNF), interferon-γ (IFNγ) or interleukin-12 (IL-12), or of one of the signalling molecules signal transducer and activator of transcription 4 (STAT4) or myeloid differentiation primary-response protein 88 (MYD88)[36–47]. However, these conditions might result in hypersusceptibility to tuberculosis through non-granulomatous mechanisms, for example by decreasing the microbicidal capacity of infected macrophages[48]. The most prominent argument in support of the host-protective role of granulomas is the dogma that TNF — which is a key determinant of immunity in tuberculosis — is responsible for granuloma formation and maintenance. This model is founded on the well-known role of TNF in orchestrating macrophage trafficking and leukocyte movement during inflammation, together with the finding of disorganized granulomas in TNF-deficient mice[36,49–55].

Finally, evolutionary considerations might also favour the view that the tuberculous granuloma is a protective structure. Granulomas are evolutionarily primitive structures that form in response to persistent stimuli, which can be either living or inanimate[7]. Their most basic form — the foreign-body granuloma — probably functions to surround and digest inanimate foreign bodies that are too large to be engulfed by a single macrophage[7]. Infectious granulomas represent a more evolved form of this inflammatory response involving the epithelioid transformation of macrophages, which is thought to make the granulomas even more effective as 'containing' structures. For these reasons, the granuloma has traditionally been placed firmly at the centre of host protection.

Formed and shaped by innate immunity. Classically, the sequence of events involved in tuberculous granuloma formation has been inferred from histological and radiological studies of humans with tuberculosis, as well as from serial histological studies of animal models of tuberculosis (BOX 2). At the outset of human pulmonary tuberculosis, inhaled *M. tuberculosis* bacteria are thought to be taken up by macrophages and transported across the alveolar epithelium into the lung tissue, where granuloma formation is initiated[56]. Detailed analyses of mouse lungs and lymph nodes have shown that macrophages, neutrophils and DCs can be infected during the early stages of *M. tuberculosis* infection and have identified a surprising predominance of myeloid DCs over alveolar or recruited macrophages early in infection[57]. Although early infection of DCs might be predicted to promote host immunity, *M. tuberculosis* impairs DC-mediated antigen presentation and the migration of DCs to the draining lymph nodes, thereby limiting the development of an effective adaptive immune response[57,58].

In various animal models of tuberculosis, bacterial growth is rapid for the first 3 weeks of infection and reaches a plateau when adaptive immunity develops[59–61]. In the classical model, tuberculous granuloma formation requires adaptive immunity and is thought to be the

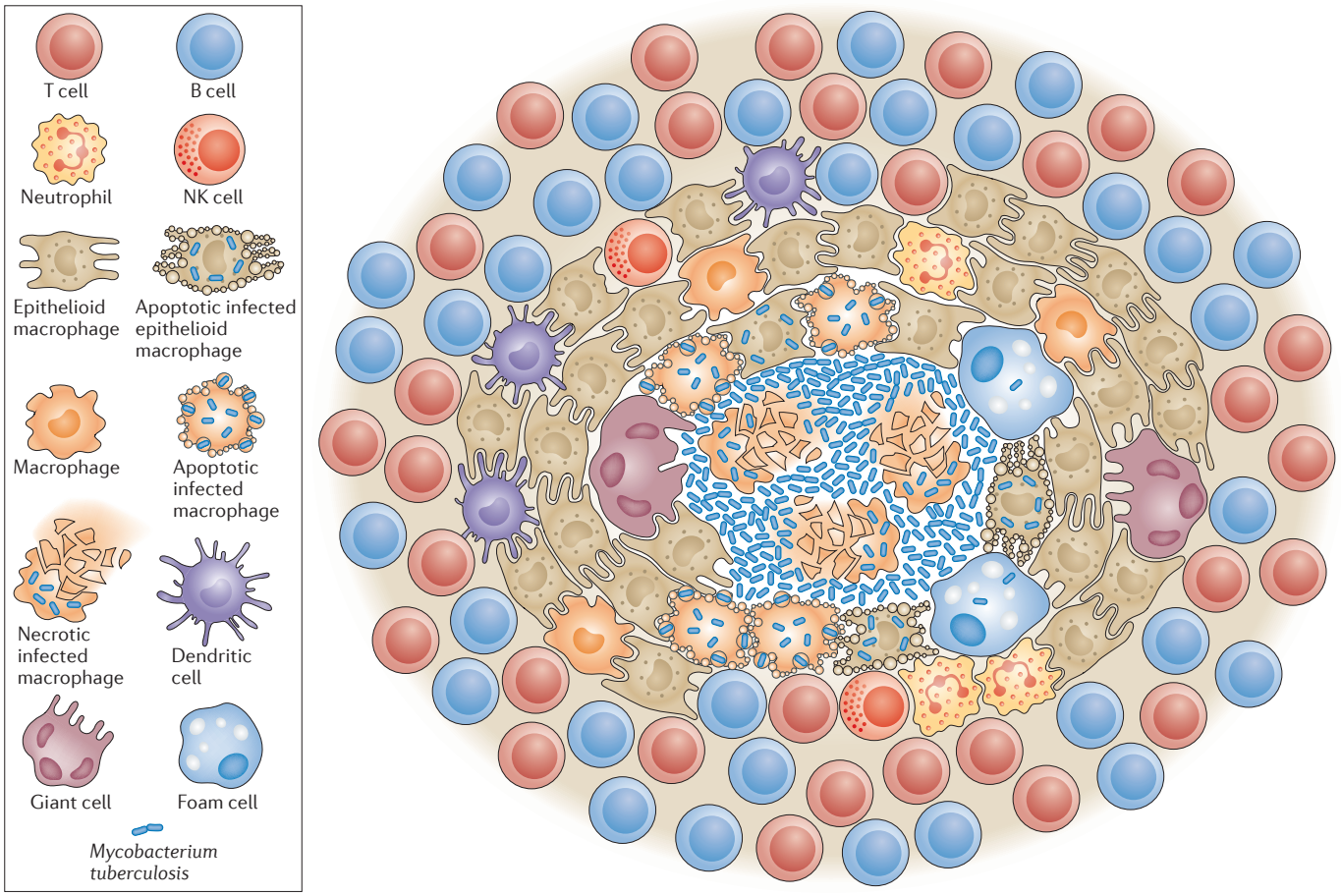

Figure 1 | **Structure and cellular constituents of the tuberculous granuloma.** The tuberculous granuloma at its most basic is a compact, organized aggregate of epithelioid cells — macrophages that have undergone a specialized transformation to have tightly interdigitated cell membranes that link adjacent cells. Epithelioid cells can be highly phagocytic but in some cases do not contain bacteria at all. Granuloma macrophages can also fuse into multinucleated giant cells or differentiate into foam cells, which are characterized by lipid accumulation. Foam cells have been noted to be most frequently located at the rim of the necrotic centre of a mature tuberculous granuloma. The consequences of these changes are not well understood, but in general foam cells and multinucleated giant cells have been reported to contain only a few bacteria, if any. Bacteria are most commonly present in the central necrotic areas in which dead and dying macrophages can be seen. Many other cell types also populate the granuloma, such as neutrophils, dendritic cells, B and T cells, natural killer (NK) cells, fibroblasts and cells that secrete extracellular matrix components. Finally, the epithelial cells surrounding the granuloma (not shown) are now thought to participate in its formation also.

RD1 locus
In virulent mycobacteria, this region encodes a type VII secretion system, namely ESX-1, an important substrate of which is early secreted antigen 6 (ESAT6). ESX-1-mediated secretion and ESAT6 are essential for mycobacterial virulence and have been implicated in phagosomal escape, cytolysis and pore formation, the induction of apoptosis and the recruitment of macrophages. The deletion of RD1 is thought to be the primary mutation responsible for attenuation of the *Mycobacterium bovis* vaccine strain bacillus Calmette–Guérin (BCG).

crucial event for restricting the expansion of bacterial populations[60,62,63]. However, the primary role of innate immunity in mediating bona fide tuberculous granuloma formation has been shown in the *Mycobacterium marinum*–larval zebrafish model (BOX 2), as these zebrafish are at a developmental stage at which adaptive immunity has not yet developed[52–54].

A highly dynamic structure promoting bacterial proliferation. The ability to visualize cellular events of granuloma formation at the whole-animal level in zebrafish (BOX 2) has provided new insight into the mechanisms and consequences of granuloma formation. Mycobacteria exploit granuloma formation for their proliferation and dissemination within the host[23,64–66]. The first clue regarding the complex role of granulomas came from the discovery that epithelioid granulomas can form in zebrafish larvae in the context of innate immunity only[66]. It was subsequently found in this model that granuloma formation coincided with the accelerated bacterial proliferation that was widely thought to precede it[65]. Moreover, mycobacteria lacking the ESX-1 secretion system, which is encoded by the RD1 locus and has a crucial role in virulence, caused an attenuated infection that was accompanied by poor granuloma formation[65]. These findings indicated that granuloma formation might actually aid bacterial proliferation.

Dynamic imaging studies in zebrafish larvae have revealed several unexpected findings about the mechanisms of tuberculous granuloma formation that contradict long-held views based on static images[64,66] (FIG. 2). Macrophages arriving at forming granulomas continue to move rapidly within these structures. These macrophages have similar morphological features to leukocytes undergoing chemotaxis and move at speeds comparable to those of leukocytes responding to a

Box 2 | **Modelling granuloma formation**

Animal models of *Mycobacterium tuberculosis*

Tuberculosis animal models date back to 1868 when the French physician Jean Antoine Villemin showed that human tuberculous tissue could produce disease in rabbits that was transmissible to other rabbits[165]. In 1881, Koch refined this study, using bacteria isolated on solid media from patients with tuberculosis to produce disease in guinea pigs[3]. Historically, the diagnosis of tuberculosis was confirmed by inoculating suspect tissue or sputum into guinea pigs. Modern microbiology has supplanted this use, but guinea pigs are still commonly used for tuberculosis research, together with mice, rabbits and nonhuman primates[166]. Mice are widely used to study tuberculosis immunology because abundant immunological tools and reagents, and inbred strains, enable classical genetics and adoptive transfer experiments. Protective determinants of tuberculosis have been discovered in mice, and these have been brought to the fore of granuloma research by elegant studies that use intravital microscopy to document granuloma dynamics[72,73]. Granulomas in most mouse strains comprise loose non-necrotic aggregates, but a mouse strain that develops necrotic granulomas (which are a hallmark of human tuberculosis) has been identified[167]. Guinea pigs and rabbits produce necrotic granulomas and are becoming more tractable with the development of immunological reagents[166]. Guinea pig caseum generally remains hard, whereas rabbit granulomas can undergo the characteristic softening or liquefaction of human granulomas that is associated with increased bacterial proliferation. Nonhuman primates (rhesus monkeys and macaques) have the additional advantage of presenting varied outcomes to infection, similarly to humans[166]; by contrast, other species uniformly develop progressive disease. However, cost, paucity of reagents and ethical considerations preclude the widespread use of nonhuman primates.

***Mycobacterium marinum* granuloma models**

M. marinum is a close genetic relative of *M. tuberculosis*. Owing to its lower optimal growth temperature, it is a natural pathogen of ectotherms (fish, amphibians and reptiles), in which it produces a systemic tuberculosis-like disease[168]. In humans, it produces a granulomatous infection (known as fish tank, aquarium tank or swimmer's granuloma) that is histologically similar to tuberculosis but restricted to the cooler surfaces of the body, such as the extremities. *M. marinum* provides the advantage of modelling tuberculosis in a natural host–pathogen pair. Leopard frogs, medaka, goldfish and zebrafish have been developed as laboratory models and, like the different *M. tuberculosis* animal models, they have different granuloma pathologies and clinical outcomes. Leopard frogs develop highly organized, non-necrotic granulomas and harbour lifelong asymptomatic infection unless immunocompromised[59]. Goldfish and zebrafish develop organized, necrotic granulomas. The zebrafish has unique advantages as a tuberculosis model because of its genetic tractability and optical transparency in early life, when only innate immunity is present. It is thus possible to dissect the contributions of innate and adaptive immunity to pathogenesis and protection and to use genetic mutants in conjunction with whole-animal microscopy to understand granuloma dynamics and the host and bacterial determinants involved.

chemokine gradient[64]. The chemotactic morphology and movements of macrophages are dictated by the presence of the mycobacterial RD1 virulence locus; if a granuloma is initiated by RD1-deficient mycobacteria, the few arriving macrophages have a more rounded shape and move slowly, thereby covering much less territory[64]. This RD1-mediated chemotactic motility of cells arriving at granulomas is closely linked to an increased infection rate.

The rapid and continuous migration of the newly arriving cells allows them quick access to the numerous dying infected cells in the granuloma. Multiple roving macrophages rapidly phagocytose the contents of a given dying infected macrophage, thereby increasing the number of infected cells. Interestingly, the macrophages move randomly (which suggests that they are migrating in response to a uniformly distributed signal) until they are near a dead infected cell, when they acquire a distinct morphology and move towards that cell to mediate its phagocytosis[64].

Thus, a mycobacterial RD1-dependent signal induces macrophage migration to and random movement within the granuloma. Ultimately, a second signal, generated by dying cells, directs nearby macrophages to them for phagocytosis. Both signals seem to be necessary for efficient phagocytosis in granulomas. These events suggest that pathogenic (RD1$^+$) mycobacteria have turned the granuloma to their advantage. The granuloma probably evolved for situations in which recruiting more macrophages to dying cells might be beneficial (for example, in the case of non-replicating foreign bodies). Indeed, the slower kinetics of macrophage recruitment, death and phagocytosis induced by RD1-mutant *M. tuberculosis* seem to inhibit bacterial proliferation.

The continuous rapid movement of macrophages throughout nascent zebrafish granulomas mimics the behaviour of lymphocytes in mouse lymph nodes[67,68]. Recent work has described tertiary lymphoid structures in tuberculous granulomas that have characteristic features such as follicular B cell aggregates, high endothelial venules and follicular dendritic cells[69,70]. Hence, it is all the more intriguing that the roving movements of macrophages and T cells (see below) in granulomas are reminiscent, both in form and in speed, of those described for B and T cells in lymph nodes as they seek out their cognate antigens on other leukocytes[67,71]. Indeed, the chemokines CCL19 (CC-chemokine ligand 19) and CCL21, which are characteristically expressed in secondary lymphoid organs, are induced in the lungs of mice infected with *M. tuberculosis*, and deficiency of CCR7 (CC-chemokine receptor 7; a receptor for both chemokines) is associated with aberrations in the formation of granuloma-associated lymphoid structures[69]. Together, these results indicate that mechanisms involved in homeostatic immune cell migration and in the homeostasis of lymphoid structures are invoked during granuloma formation.

Tertiary lymphoid structures
Ectopic lymphoid aggregates that are generated during the process of chronic immune stimulation and that have the structural characteristics of secondary lymphoid organs.

Another approach to imaging granuloma dynamics has involved three-dimensional time-lapse microscopy of mouse liver granulomas 2–3 weeks after intravenous injection of the attenuated vaccine strain *Mycobacterium bovis* bacillus Calmette–Guérin (BCG), which lacks RD1 (REF. 72). These granulomas consist of local and recruited Kupffer cells, which are liver-resident macrophages, and monocyte-derived macrophages. In contrast to observations in the zebrafish experiments, these myeloid cells are relatively static, although their membranes do seem to be in constant flux. The reason for this lack of movement remains to be resolved, but possible explanations include: the absence of RD1, as would be predicted from the zebrafish studies; the fact that macrophages move more slowly during later adaptive phases of granuloma formation; or intrinsic differences between the

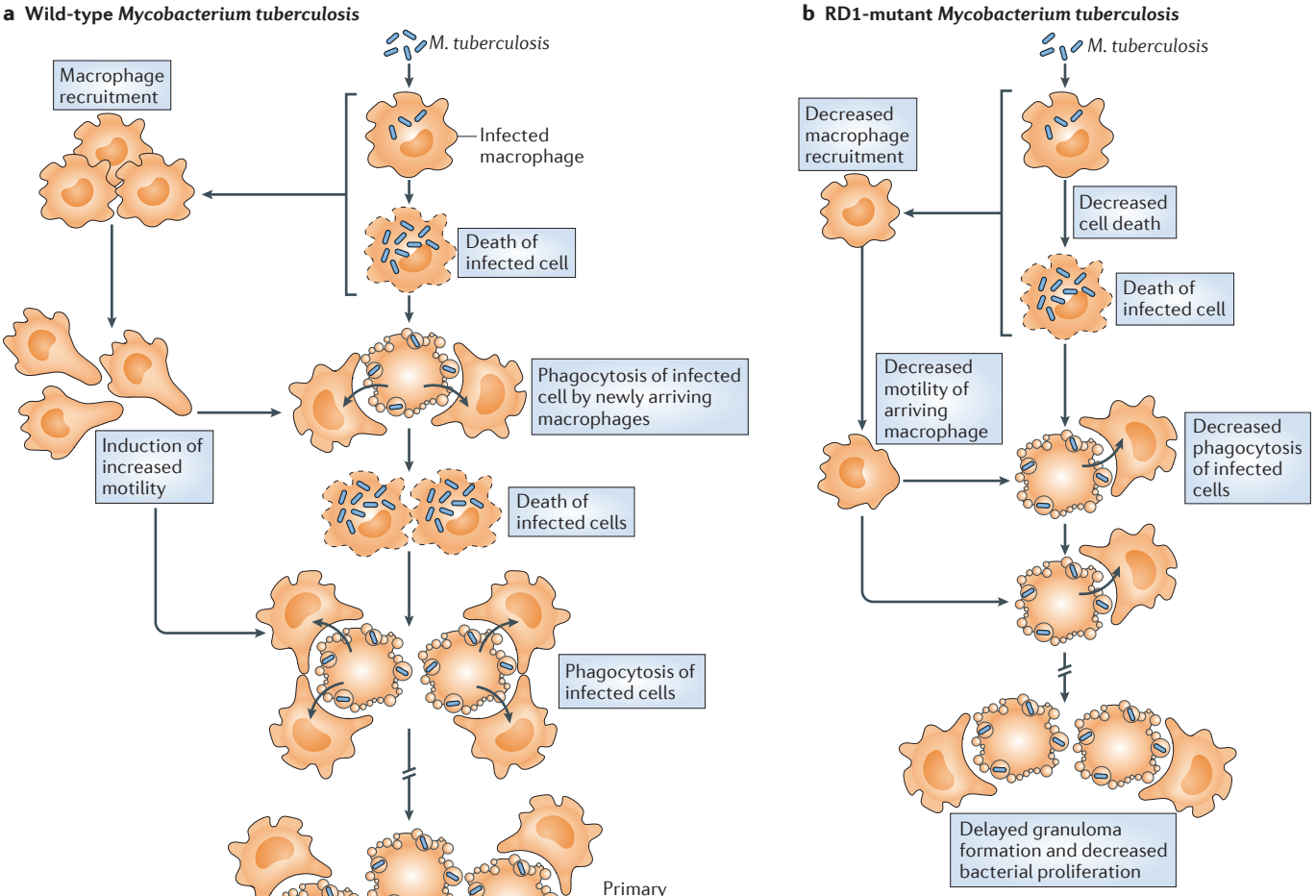

Figure 2 | **Proliferation and dissemination of bacteria through granulomas. a** | A macrophage infected with wild-type *Mycobacterium tuberculosis* recruits new macrophages and induces their rapid movement. Following the apoptosis of the infected cell, it is phagocytosed by the recruited cells. After more bacterial growth, these infected cells also die and are phagocytosed by additional recruited macrophages. Infected cells egress from the primary granuloma to initiate secondary granulomas. **b** | The same events are altered during infection with RD1-mutant *M. tuberculosis*. The intracellular growth of these mutant mycobacteria within an infected macrophage occurs in a similar manner to that of wild-type *M. tuberculosis*. However, the death of this macrophage is delayed compared with wild-type infection, and the recruited macrophages are fewer in number and lack the rapid movement observed during wild-type infection. The slower death of infected macrophages and the delayed phagocytosis of the dead cells combine to produce small, delayed granulomas with better containment of infection. Image is modified, with permission, from REF. 64 © (2009) Cell Press.

behaviour of fish and mouse macrophages. In a subsequent study using virulent (RD1$^+$) *M. tuberculosis* in mice, only a limited amount of macrophage movement was visualized, but some of the arriving macrophages moved quickly, whereas others moved slowly[73]. As the bacteria were not simultaneously visualized in this study, it is possible that the motile macrophages were uninfected and that the non-motile macrophages were heavily infected, similarly to those that move slowly in the early zebrafish granulomas[64]. Adult zebrafish and mice infected with RD1-deficient mycobacteria both have attenuated infections and loose, poorly structured granulomas[61,74], which indicates that this virulence determinant might be crucial for macrophage motility throughout granuloma formation. Moreover, even the necrotic core of the granuloma, which was previously regarded as being isolated from immune interactions, is permeated by both infected and uninfected macrophages[75,76].

The study of mouse liver granulomas described above also used fluorescent transgenic mice to track the movement and behaviour of T cells in granulomas[72]. Activated polyclonal T cells arrive at the granulomas within days of infection and are in constant motion throughout the lesion, moving at similar speeds to the macrophages in zebrafish granulomas[64]. Each T cell wanders through the entire granuloma, probably making direct contact with most macrophages. Intriguingly, the T cells do not leave the granuloma. Imaging suggests that, rather than resulting from a physical barrier to departure, this T cell retention is due to the macrophages functioning as a scaffold on which the lymphocytes crawl, analogous to T cell migration in lymph nodes[71]. This emerging picture of the granuloma as a tight accumulation of highly motile macrophages that supports T cell migration and interactions (with macrophages, other T cells and perhaps other cell types populating the granuloma) changes our view of the granuloma from a fixed barricade to a more dynamic structure. Most importantly, the granuloma actually functions as a tool for bacterial proliferation: mycobacteria accelerate normally protective host processes (apoptosis, macrophage chemotaxis and the phagocytosis of dead macrophages[77,78]) to increase their numbers, at least in part through products of the RD1 locus.

Macrophage chemotaxis to granulomas

Role of TNF. In the traditional model of the granuloma as a host-protective structure, TNF was thought to have a beneficial effect by inducing granuloma formation[36,49–55]. This hypothesis stems from the finding that TNF-deficient mice are hypersusceptible to tuberculosis and contain numerous bacteria in poorly formed granulomas[52]. However, temporal monitoring of granuloma formation in zebrafish larvae has shown that in fact granulomas can form in the absence of TNF signalling[48]. Moreover, without TNF, granuloma formation is accelerated, probably because TNF deficiency causes a reduction in the microbicidal capacity of the infected macrophages, leading to increased bacterial numbers and a net increase in RD1 expression[48]. The overladen macrophages then undergo necrosis so that ultimately granuloma structure is not preserved[48], which might account for the observation of poorly formed granulomas[52]. In the extracellular milieu, the bacteria grow exuberantly, corroborating findings in macrophage-depleted zebrafish that the extracellular environment is highly permissive for mycobacterial growth[48,79].

TNF might have additional effects on granuloma structure in the context of adaptive immunity by influencing processes such as T cell trafficking and activation. These changes might independently alter cell recruitment and structural organization in mature granulomas. Studies of mouse granulomas induced by *M. bovis* BCG showed that TNF is essential for T cell recruitment to granulomas and for the retention of uninfected, but not infected, macrophages[72]. The selective loss of uninfected macrophages as a consequence of TNF blockade has several possible explanations that have yet to be tested, including decreased migration of new, uninfected macrophages to the lesion, or differential effects on the survival of infected versus uninfected macrophages.

The findings in zebrafish are supported by several other lines of evidence. First, computational modelling studies have suggested that TNF deficiency does not impair granuloma formation[80]. Second, in a nonhuman primate model of TNF blockade, hypersusceptibility to tuberculosis was associated with normal granuloma formation and structure[81]. And, most importantly, there have been reports that patients given TNF antagonists can develop tuberculosis with organized granulomas and areas of caseum[82,83].

Role of host epithelial cells and MMP9. Recent work in zebrafish reveals an unexpected mechanism for the acceleration of granuloma formation by an RD1 determinant[23]. The chemotactic effects of the mycobacterial RD1 locus are mediated through the induction of host matrix metalloproteinase 9 (MMP9) production by epithelial cells surrounding the growing granuloma (FIG. 3). MMP9 secretion promotes the recruitment of new macrophages to the granuloma, where they become infected and expand the granuloma[23]. MMP9 is probably induced through a direct interaction between the mycobacterial virulence protein ESAT6 — which is secreted through the RD1-encoded ESX-1 secretion system — and epithelial cells, as induction does not require macrophages. ESAT6 can be released either from dead infected macrophages, or from live ones through its pore-forming activity[84].

Macrophages express MMP9 in many inflammatory conditions[84], so the co-option of epithelial cells for MMP9 production during tuberculous granuloma formation is intriguing. Perhaps epithelial cells offer mycobacteria a means of amplifying MMP9 secretion in the vicinity of a single infected macrophage, so as to accelerate granuloma formation at that site. Given the evidence that mycobacteria dampen macrophage inflammatory responses to promote bacterial growth[85–88], mycobacteria might induce distinct programmes in macrophages and epithelial cells. The differential cellular responses could generate a hospitable growth niche for mycobacteria in macrophages while harnessing epithelial cells to facilitate the chemotaxis of additional macrophages for niche expansion.

Apoptosis
A common form of cell death that is also known as intrinsic or programmed cell death. Apoptosis involves cell shrinkage, chromatin condensation in the periphery of the nucleus, cell-membrane blebbing and DNA fragmentation into multiples of ~180 base pairs. Eventually, the cell breaks up into many membrane-bound apoptotic bodies, which are phagocytosed by neighbouring cells.

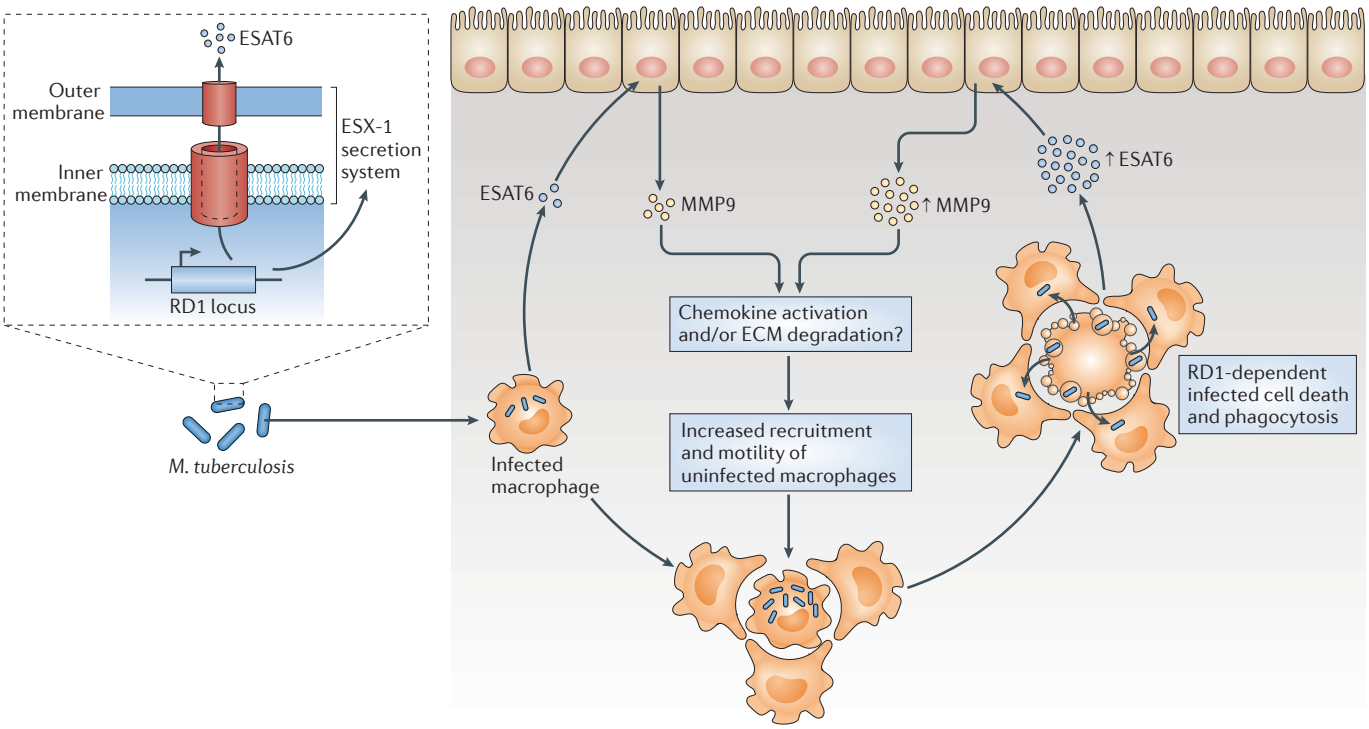

Figure 3 | **Mechanism of cell recruitment to granulomas through MMP9 induction in epithelial cells.** This figure shows the steps by which matrix metalloproteinase 9 (MMP9) can be induced in the epithelial cells surrounding a forming granuloma. The *Mycobacterium tuberculosis* protein ESAT6 can be released from infected macrophages through their apoptotic death (which it causes) and/or through its pore-forming activity. It then causes the transcriptional induction of *Mmp9* in neighbouring epithelial cells; increased levels of MMP9 production in turn result in the enhanced chemotactic recruitment of uninfected macrophages to the granuloma, by as yet unknown mechanisms. Possible mechanisms include the activation of a macrophage-specific chemokine, and chemokine release from the extracellular matrix (ECM). Image is modified, with permission, from REF. 23 © (2010) The American Association for the Advancement of Science.

Consistent with this idea, ESAT6-mediated induction of MMP9 expression in epithelial cells is independent of pro-inflammatory MYD88 and TNF signalling and probably involves a pathway distinct from pro-inflammatory responses[23]. This MMP9 induction pathway might be unique to mycobacterial granulomatous infection; infection of zebrafish larvae with *Salmonella* spp. induces MMP9 production in a MYD88-dependent manner, although the cell types in which induction occurred were not reported[89]. Zebrafish mutants that are defective in granuloma formation have been identified in a forward genetic screen and should yield further information about this pathway[90].

These findings from innate granulomas in zebrafish larvae are probably relevant for advanced tuberculous granulomas. *Mmp9*-knockout mice infected with *M. tuberculosis* have decreased macrophage recruitment to the lungs and poor granuloma development that is associated with decreased bacterial loads[91]. Importantly, human studies show that MMP9 expression is induced in lung epithelia surrounding tuberculous granulomas, and implicate MMP9 as a pathogenic factor in tuberculous meningitis[92–95]. The zebrafish model provides a mechanistic explanation for the involvement of MMP9 in human tuberculosis — that is, MMP9 enhances granuloma formation through macrophage recruitment and therefore increases bacterial proliferation. Thus, inhibition of MMP9 expression or activity could represent a new therapy for tuberculosis and potentially also for non-infectious granulomatous diseases. The zebrafish studies could also explain the longstanding observation that, although tuberculosis can affect most organs, tuberculosis of skeletal or cardiac muscle is extremely rare[22]. In zebrafish, if a granuloma begins in muscle, the closest epithelium expressing MMP9 could be at some distance from the infected macrophages[23]. Perhaps as a consequence of this imposed distance, granulomas forming in muscle generally fail to grow[96].

Promoting dissemination of infection. Serial monitoring of infection in whole zebrafish larvae shows that some infected macrophages leave the primary granuloma and participate in the establishment of secondary granulomas at other sites[64]. Indeed, seeding of macrophages from the primary granuloma is the main, if not only, means of dissemination during infection. Again, these observations are incompatible with the view of granulomas as static barriers and are consistent with the increasing appreciation that human tuberculosis is a disseminated infection[97].

Mycobacteria have been suggested to disseminate haematogenously from the primary granuloma early in infection[98,99]. Zebrafish studies have confirmed this model and shown that haematogenous dissemination occurs through infected macrophages departing established, enlarging granulomas. Thus, phagocytes are required for the initial transport of inhaled bacteria to deeper tissues for the establishment of infection[56,57,79] and then repeat their role as bacterial transporters by disseminating infection from the primary granuloma. It is interesting in this context that recent transplantation experiments have shown that inflammatory DCs rapidly traffic in and out of both acute and more-chronic granulomas induced by *M. bovis* BCG in mice[100]. The exiting DCs have a widespread dissemination pattern and seem to efficiently prime $CD4^+$ T cells. It is tempting to speculate that the macrophage egress that disseminates infection might be another example of mycobacteria turning a host-beneficial process (in this case, a process involved in generating adaptive immunity) to their own benefit. In this light, it is intriguing that macrophages in human granulomas can express DC markers[101]; it is possible that similar signals induce both macrophages and DCs to egress from granulomas, whereby the macrophages disseminate infection.

Regardless of the mechanism by which macrophages exit the granuloma, this strategy seems to be clinically important even in the context of tuberculosis treatment. Recent work has identified antibiotic-tolerant (but not genetically resistant) mycobacteria that arise through the expression of bacterial efflux pumps that are induced in response to the macrophage environment[96]. Zebrafish granulomas expand and disseminate these antibiotic-tolerant bacteria even in the face of antimicrobial chemotherapy[96]. This finding may explain the longstanding clinical observation that lesions containing genetically drug-sensitive bacteria appear in new locations during tuberculosis treatment[19,102,103].

Cell death: complexities and consequences

Both necrosis and apoptosis of macrophages are observed in human tuberculous granulomas, independently of one another[19,104–106]. Mycobacterial and host factors contribute to both types of cell death, and observational and mechanistic studies indicate that both forms of cell death can promote bacterial growth and proliferation under some circumstances, but inhibit them under other conditions.

Necrosis. Caseating granulomas — which are thought to result from the necrotic breakdown of participating cells, especially macrophages — are a signature of active tuberculosis in immunocompetent hosts and are implicated in tuberculosis transmission. The sequence of events leading to caseation was gleaned from histological studies of tuberculous organs obtained from autopsies in the pre-chemotherapy era[19,22]. Observations correlating bacterial numbers with the presence of caseum showed that areas of caseation in early granulomas were associated with marked increases in bacterial numbers as compared with noncaseating early lesions[19,22]. Enhanced bacterial growth was particularly noted in macrophage-rich areas where the bacterium-rich exudate harboured cellular debris, suggesting the recent release of bacteria from necrotic macrophages[19]. In mature granulomas, areas of 'hard' caseum had sparse bacteria, whereas areas of 'soft' caseum were associated with the greatest numbers of bacteria, thus linking the organization and hardening of the caseum with bacterial killing, both of which are attributed to the onset of adaptive immunity[19].

Recent work showing that the onset of caseation is also associated with increased bacterial proliferation in zebrafish[61] corroborates the findings in humans that the early caseum is growth promoting (FIG. 4). Despite being adapted to intracellular growth, mycobacteria grow even more exuberantly in an extracellular milieu created by an absence of macrophages or through macrophage necrosis (caused either directly or indirectly by a deficiency or excess of TNF)[48,90,107]. In addition, both mouse and zebrafish studies show that virulent mycobacteria induce the production of host lipoxins, which are anti-inflammatory eicosanoids that induce macrophage necrosis through TNF suppression[90,108].

Apoptosis. A model developed mainly from *in vitro* studies proposes that apoptosis is detrimental to mycobacteria, in contrast to necrosis, which favours bacterial growth[109–113]. However, recent live imaging studies in zebrafish indicate that bacterially induced apoptosis can promote bacterial proliferation during granuloma formation[64]. Granuloma macrophages infected with virulent mycobacteria undergo bona fide apoptosis with the characteristic morphological hallmarks of nuclear collapse and cellular fragmentation that results in spherical remnants[64]. In contrast to the case of necrosis[48,90,107], the bacteria remain encased within the intact membranes of apoptotic macrophages. How then might the apoptosis of infected macrophages promote bacterial proliferation?

The bacterial contents of the apoptotic macrophages are phagocytosed by newly arriving macrophages, which are recruited to the granuloma through the ESAT6–MMP9 signalling mechanism described above[64,65]. These observations suggest a model in which migrating macrophages engulf infected apoptotic macrophages, leading to subsequent bacterial proliferation within the newly infected macrophages. Mathematical modelling of these events indicates that 70–100% of all bacterial proliferation in the early granuloma can be attributed to macrophage apoptosis and the reuptake of the bacteria through phagocytosis[64]. In addition to inducing macrophage migration to the granuloma[23,65], expression of the mycobacterial RD1 locus simultaneously enhances the apoptosis of infected granuloma macrophages in zebrafish[64,65], as it does infected human and mouse macrophages *in vitro*[114,115]. Macrophage apoptosis is probably mediated through ESAT6, which can induce multiple cell death programmes in cultured cells[116–118].

What might account for the discrepancy between the *in vitro* and *in vivo* studies in terms of the effects of apoptosis on mycobacteria? First, many of the *in vitro* studies used additional agents to induce specific

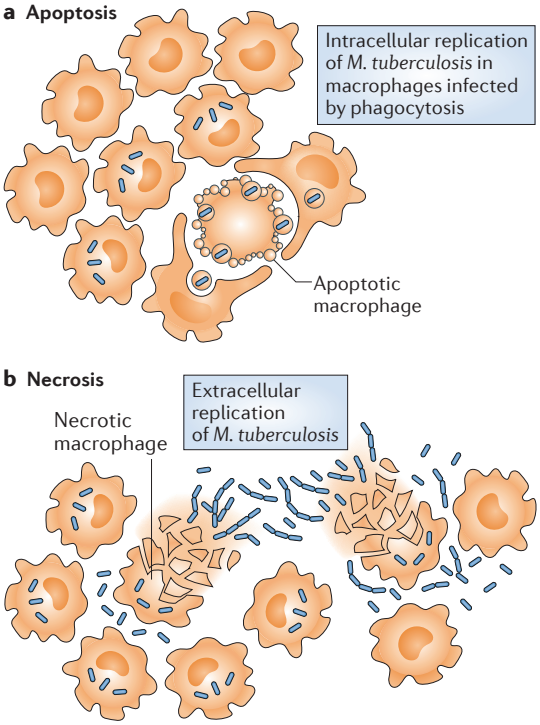

Figure 4 | **Macrophage necrosis promotes more bacterial growth than macrophage apoptosis.** This figure illustrates how the apoptosis and necrosis of granuloma macrophages can promote bacterial growth. **a** | The bacterial contents of an apoptotic macrophage are distributed into multiple uninfected macrophages through phagocytosis (as shown in FIG. 2). This provides the bacteria with new niches for bacterial replication. **b** | A necrotic macrophage releases bacteria into the extracellular environment, which is highly permissive for mycobacterial growth. The exuberant extracellular proliferation of mycobacteria surpasses that possible through the death and phagocytosis of apoptotic macrophages. It is not clear to what extent the extracellular bacteria released from necrotic macrophages are rephagocytosed by new macrophages.

apoptotic death pathways that might override those induced by *M. tuberculosis*[110,111,113,119]. For example, apoptosis induced by ATP kills intracellular mycobacteria, whereas apoptosis induced by FAS ligand (also known as CD95L) does not[120]. Similarly, ESAT6-induced apoptotic cell death *in vivo* may not be harmful to mycobacteria. Alternatively, or in addition, the rapid phagocytosis of the dead macrophages may nullify any bactericidal effects of apoptosis.

Complicating matters, there are mycobacterial virulence determinants that have anti-apoptotic effects in tissue culture models[121–124]. However, the anti-apoptotic function of these determinants has not been demonstrated *in vivo*, so their relationship to virulence remains unknown, particularly as these genes probably influence multiple cellular functions (*nuoG* encodes a subunit of the type I NADH dehydrogenase, *secA2* encodes a mycobacterial secretion system and *pKnE* encodes a serine/threonine kinase[122–124]). For example, NuoG has been found to inhibit TNF-mediated apoptosis in cultured macrophages[125]. However, *in vivo*, a reduction in TNF signalling through knockdown of TNF receptor 1 expression in zebrafish does not alter apoptosis; instead, it independently promotes necrosis[48].

In summary, *in vivo* studies indicate that, at least in the early granuloma, both apoptosis and necrosis benefit bacterial proliferation (FIGS 2,4). The magnitude of the effect from necrosis seems to be greater, as intracellular bacterial proliferation promoted by apoptotic death and rephagocytosis does not match the exuberance of extracellular growth[48,79]. Necrosis might also benefit bacteria in the adaptive immune phase, in which *M. tuberculosis* is proposed to decrease DC-mediated cross-priming of T cells by shifting the balance from apoptosis to necrosis of infected cells[126]. The induction of both apoptosis and necrosis can be linked to bacterial factors, but it remains unclear whether these processes represent mutually independent programmes[48] or alternative decisions in a common pathway[126,127]. In an additional twist, RD1 promotes the formation of necrotic caseum in mature granulomas through post-apoptotic necrosis or a mechanism distinct from its pro-apoptotic function[61,65]. A better understanding of the relationship between the necrotic and apoptotic pathways in tuberculosis could open up additional therapeutic avenues. For example, the interception of apoptosis in expanding granulomas could allow for more effective immune control provided that it does not promote necrosis.

Failure modes of the mature granuloma

The new understanding that granuloma formation favours bacterial growth still begs the question of why sterilizing immunity often fails to develop even after the granuloma matures to fully involve adaptive immune elements (TABLE 1). That $CD4^+$ T cells protect against tuberculosis is not in question and is highlighted by the increased susceptibility of HIV-infected individuals to tuberculosis even during the early stages of HIV infection, when their $CD4^+$ T cell numbers are only somewhat decreased[32–34]. In a mouse model also, a lack of $CD4^+$ T cells leads to hypersusceptibility to tuberculosis[128]. However, apparently immunocompetent humans can harbour the infection indefinitely and, in animal models, granuloma maturation induces bacteriostasis but not eradication, despite the influx of effector T cells into the granuloma resulting in increased production of TNF and IFNγ, which mediate macrophage microbicidal activity[129]. Thus, the paradox of tuberculosis is that despite the concentration of vigorous host immune responses in granulomas, reinfection and bacterial persistence can occur[130–132].

Accordingly, the live attenuated BCG vaccine has been largely ineffective in preventing tuberculosis in adults even when engineered for improved antigenicity[133]. Reinfection was long attributed to bacterial avoidance of the concentrated host immune responses in granulomas through the establishment of infection either outside them, or within their necrotic caseum, which was proposed to provide a secluded niche. However, work in zebrafish and mice has shown that

Table 1 | **The failure of adaptive immunity to eradicate infection in mature tuberculous granulomas**

Host defence strategy	Failure mode in tuberculous granuloma	Possible mycobacterial counterstrategy contributing to host failure
Concentrate host defences in an organized immune structure: the granuloma	Granulomas are 'safe havens' for mycobacteria; newly infecting mycobacteria traffic preferentially to, and flourish within, established granulomas[75,134]	Activate specific genes in response to the granuloma environment that might allow the bacteria to resist the granuloma environment[169]
Mount a swift and strong adaptive immune response by recruiting effector T cells to the granuloma	Delayed recruitment of adaptive immune elements as compared with infections that are cleared[58,129,135]	Delay DC migration to lymph nodes[58], where adaptive immunity is initiated[99] Rapidly induce antigen-specific T_{Reg} cells that delay the priming of effector T cells and their subsequent recruitment to the granuloma[137], thus prolonging the period of unrestricted bacterial proliferation in the granuloma through macrophage death and rephagocytosis[64]
Rapidly activate adaptive effector T cells in the granuloma	Delayed activation of effector T cells that reach the granuloma	Render infected cells 'invisible' to pathogen-specific effector $CD4^+$ T cells either through the downregulation of key mycobacterial antigens[12,143] or through the sequestration of mycobacteria within suboptimal antigen-presenting cells[73,135,142,143]

DC, dendritic cell; T_{Reg}, regulatory T.

superinfecting mycobacteria traffic rapidly into pre-existing mature granulomas, including their necrotic centres, through specific mycobacterium-directed, host cell-mediated processes, and adapt quickly to persist long term therein[75,134]. These findings demonstrate the failure of established granulomas to eradicate even newly deposited mycobacteria that are 'naive' to adaptive host immune responses. A growing body of work in mice broadly explains the failure of the granuloma to eradicate infection (or ward off new infection) as follows: the initiation of adaptive immunity is delayed in tuberculosis compared with infections for which sterilizing immunity is achieved[58,129,135], and this delay allows exponential mycobacterial growth before it is slowed by the onset of adaptive immunity[136]. However, this delay in itself does not explain the failure to eradicate infection once effector T cells have arrived; T cell activation is also impaired.

Delayed arrival of effector T cells. The appearance of *M. tuberculosis*-specific T cells in the lungs is correlated with control of infection[129]. The adaptive immune response to *M. tuberculosis* is initiated not in the forming lung granuloma but in local lymph nodes, thus following the norm for other infections[99]. The lag in the initiation of an adaptive immune response is attributed first to the delayed arrival of infected DCs from primary granulomas at the lymph nodes, which may simply be due to delayed acquisition of bacteria by DCs[58].

There is also a challenge in recruiting effector T cells to the primary lesion, and this defect can be attributed, in large part, to forkhead box P3 $(FOXP3)^+$ regulatory T (T_{Reg}) cells[137]. T_{Reg} cells can counteract host mechanisms that are in place to recruit effector T cells to sites of *M. tuberculosis* infection — namely, the induction of T helper 17 (T_H17) cells that would otherwise promote the recruitment of T_H1 effector cells to the tuberculous lungs[138]. T_{Reg} cells are present in lymphoid areas of tuberculous granulomas, and their depletion results in a decreased bacterial burden, although it fails to eradicate infection[139,140]. T_{Reg} cells are present both in the primary granuloma and in the draining mediastinal lymph nodes where secondary granulomas form[137,139]. Importantly, pathogen-specific T_{Reg} cell populations expand preferentially from a pre-existing population of natural T_{Reg} cells, and even small numbers of these natural T_{Reg} cells are sufficient to delay the appearance of specific effector T cells in the lungs[137]. They do so by preventing the proliferation of effector T cells in the lymph nodes — which are crucial sites for T cell priming (despite the formation of tertiary lymphoid structures in the primary granuloma)[58,99] — and by inhibiting the subsequent arrival of these effector T cells in the primary granuloma[137]. Consistent with this role of T_{Reg} cells in dampening immunity to tuberculosis, IL-10 depletion decreases bacterial burdens and increases the influx of IFNγ-producing T cells to the site of lung infection[141].

Delayed activation of effector T cells. A second, independent bottleneck to pathogen eradication by the adaptive immune system lies in the delayed and poor responsiveness of mycobacterium-infected macrophages to T cell help. This was brought into focus by experiments showing that although ESAT6-specific T_H1 cells that were transferred into syngeneic mice before aerosol infection could enhance protection by overcoming the delay in the arrival of antigen-specific T cells in the lungs, they could not control bacterial replication until 7 days after infection[135]. The delayed effect of pre-administering a large number of antigen-specific T cells strongly indicates that infected macrophages are either 'not seen' by the pathogen-specific effector $CD4^+$ T cells or are functionally incapable of being stimulated by them to increase bactericidal activity in the early stages of granuloma formation.

Multiple mechanisms might be responsible. *In vitro*, infected macrophages have a selective block in the presentation of mycobacterial antigens to $CD4^+$ T cells[142]. Two recent mouse studies address this problem in a complementary way. One study found that, in infected mouse lungs, T cell receptor-transgenic $CD4^+$

T cells specific for an immunodominant *M. tuberculosis* antigen (Ag85B peptide 25) were activated only at low frequencies, which were not significantly higher than the frequencies of polyclonal $CD4^+$ T cells responding to *M. tuberculosis* infection[143]. This phenomenon can partly be explained by the fact that Ag85B is downregulated during infection: introducing exogenous Ag85B peptide 25 or using organisms that are engineered to continuously express Ag85B resulted in a higher frequency of cognate $CD4^+$ T cell activation, accompanied by decreased bacterial burdens and prolonged survival of the infected mice. However, the bacteria still grew and the mice still died, showing that there are other, undetermined mechanisms for this failure of otherwise competent effector T cells to become activated.

Mechanistic insights for this phenomenon come from another recent study that visualized, using intravital multiphoton imaging, the migration within mouse liver granulomas of adoptively transferred pre-activated or naive $CD4^+$ T cells that were either specific or nonspecific for Ag85B peptide 25 (REF. 73). Both *M. tuberculosis*-specific and -nonspecific T cells underwent continuous migration, with hardly any of the *M. tuberculosis*-specific T cells displaying the migration arrest that is a hallmark of cognate antigen recognition. Although the *M. tuberculosis*-specific cells did increase their expression of the activation marker CD69, which is indicative of some recognition of their cognate antigen, very few of them produced IFNγ.

A second possible mechanism for the failure of effector T cells to increase macrophage microbicidal capacity comes from *in vitro* experiments indicating that *M. tuberculosis* inhibits IFNγ signalling in infected macrophages. Mycobacterial cell wall components and lipoproteins mediate this unresponsiveness to IFNγ by as yet incompletely understood mechanisms downstream of Janus kinase (JAK)–STAT interactions[85–88]. As IFNγ is a key host-protective cytokine in a wide range of conditions, the inhibition must only be partial and might be explained by the transcriptional inhibition of only a subset of IFNγ-responsive genes[87]. Relevant to the granuloma environment with its clustered macrophages, the inhibition of IFNγ responsiveness extends to adjacent uninfected macrophages, perhaps owing to the secretion by infected cells of IL-6, which can transcriptionally inhibit the same subset of IFNγ-responsive genes[144]. Alternatively, bacterial products from infected macrophages might make their way into surrounding macrophages to mediate these inhibitory effects[23,145]. However, recent work has shown that transferred T_H1 cells can control *M. tuberculosis* *in vivo* in an IFNγ-independent manner, and such an IFNγ-independent pathway for mycobacterial growth restriction brings into question the relevance of mycobacterial effects on IFNγ signalling (see next section). In summary, in addition to inducing the formation of granulomas by the innate immune system, mycobacteria seem to have an elaborately choreographed programme to block a sterilizing adaptive immune response (TABLE 1).

A revised view of IFNγ immunomodulation. An unexpected role for IFNγ function in tuberculosis came from experiments using bone marrow chimaeras. These studies uncovered a role for IFNγ-mediated responses in non-haematopoietic cells in the control of tuberculosis during the later stages of infection[146]. Indeed, the effect of a deficiency in IFNγ signalling in non-haematopoietic cells was biphasic. An initial transient decrease in bacterial burdens associated with decreased recruitment of myeloid cells was consistent with the idea from zebrafish studies that increased macrophage recruitment to granulomas is deleterious to the host[64]. But, thereafter, the bacterial burdens of IFNγ signalling-deficient mice were higher than those of normal mice and were accompanied by necrotic lesions that were devoid of macrophages but associated with an influx of neutrophils; these phenotypes were similar to the case of total IFNγ deficiency[40,147,148]. However, these studies could not determine whether the neutrophil influx was secondary to defective macrophage microbicidal mechanisms and resultant macrophage necrosis[40,147,148].

Two recent studies implicate neutrophils as mediators of pathology and increased bacterial growth[146,149]. One study[146] found that IFNγ induces the lung epithelium and/or endothelium to produce the enzyme indoleamine 2,3-dioxygenase (IDO), which converts tryptophan into its kynurenine metabolites[146]. Although kynurenines are known to have broad-spectrum bactericidal properties[150], their protective role in tuberculosis is attributed to IL-23 inhibition, which limits the proliferation of IL-17-producing cells that induce neutrophil migration[146,151]. This model fits with previous work showing that the number of infected neutrophils peaks 21 days after infection and declines sharply thereafter; this peak coincides with the induction of adaptive immune responses, including the generation of T_H17 cells[57]. The causal relationship between IL-17, neutrophil influx and pathogenesis remains to be determined, although a correlation between neutrophilic granulomas and increased pathology is emerging from multiple studies. IL-18 deficiency is associated with neutrophil-rich granulomas, as well as with decreased IFNγ and increased IL-17 levels in both the serum and the lungs and decreased levels of *Ido* mRNA in the lungs[152]. Increased IL-17 levels — resulting from IL-10 deficiency or from repeated BCG vaccination in the face of mycobacterial infection — also induce neutrophilic granulomas, as does a deficiency of caspase recruitment domain-containing protein 9 (CARD9)[141,153,154]. How neutrophils affect the pathogenesis of granulomas is unclear; neutrophil depletion in CARD9 deficiency was associated with increased survival without affecting bacterial burdens[154]. In the context of IL-10 deficiency, decreasing neutrophilic infiltrates has been proposed to reduce the dissemination of tuberculosis to secondary organs without affecting bacterial burdens at the local site[141].

Summary and implications

For decades, the tuberculous granuloma was widely regarded as a barrier to bacterial proliferation and dissemination that favoured mycobacteria only in as much as its necrotic breakdown in the lung enhanced aerosol

Box 3 | **Potential therapeutics to render granulomas protective**

Modulation of granuloma expansion and/or maintenance through macrophage recruitment, death and phagocytosis

- Decrease macrophage recruitment to granulomas: decrease bacterial production of ESAT6 using drugs or neutralizing antibodies; decrease ESAT6-induced production of matrix metalloproteinase 9 (MMP9) by epithelial cells; decrease MMP9 activity; block MMP9-induced macrophage chemotaxis to granulomas
- Decrease ESAT6-induced apoptotic cell death of macrophages
- Decrease the phagocytosis of apoptotic macrophages by blocking 'eat me' signals
- Decrease the necrosis of infected macrophages that occurs under certain conditions mediated by specific host genotypes

Inhibition of bacterial determinants that facilitate resistance to adaptive immune elements in the mature granuloma environment

- Prevent the downregulation of mycobacterial antigens in chronic granulomas
- Enhance the stimulatory capacity of antigen-presenting cells infected with *Mycobacterium tuberculosis*
- Inhibit the induction of antigen-specific regulatory T cells

transmission to other hosts[155]. Work in the past decade presents an opposing view of the role of the granuloma that has profound implications for tuberculosis treatment and prevention. Recognition of the granuloma as an important site of bacterial proliferation should lead to new therapeutic strategies (BOX 3).

Host pathways — such as the induction of MMP9 — that are exploited by bacteria to induce granulomas could be targeted pharmacologically. As they target the host, such therapies should be useful in both drug-sensitive and drug-resistant tuberculosis. But there is probably an argument to be made for promoting nuanced granuloma formation to optimally resist mycobacteria rather than completely inhibiting the process[64]. As argued earlier, slower kinetics of macrophage recruitment, death and phagocytosis, as seen with RD1-mutant *M. tuberculosis*, could result in a gradual decrease in mycobacterial numbers. It is conceivable that these low levels of infected cell death and rephagocytosis of the bacteria might be necessary for the control of infection; for example, if an apoptotic macrophage is never phagocytosed, it could undergo post-apoptotic necrosis, which would release the bacteria into the extracellular environment[48,79,90]. Also important might be a careful modulation of the type and extent of cell death and a balance between the dual effects of IL-17 (which has beneficial effects mediated through the recruitment of IFNγ-producing $CD4^+$ effector T cells[138] but is deleterious in excess owing to increased neutrophil recruitment)[146,149]. Epithelial cells — which have only recently been recognized as being central to the granuloma — also have a dual role in granuloma pathogenesis. They are detrimental because they increase macrophage recruitment through MMP9 production, but they are also protective owing to their inhibition of neutrophil recruitment through IFNγ-mediated signalling[23,146]. Discerning the balance between functional and pathological consequences of the granuloma will be instrumental to the success of new therapeutic strategies directed at the hallmark structure of tuberculosis.

During the adaptive immune phases of granuloma formation, the bacteria seem to take the opposite approach of keeping host immune elements away and disarming them when they do reach the granuloma. Modulation of T_{Reg} cells during infection may offer one approach to mitigating this problem[137,139]. These exciting discoveries of the past decade provide stepping stones for the development of effective therapeutic and preventive strategies.

Finally, granuloma-inhibiting therapies for tuberculosis could provide opportunities for therapeutic intervention in other granulomatous diseases. Schistosomiasis — another important granulomatous disease, caused by helminth parasites — might also illustrate the double-edged nature of granulomas. In schistosomiasis, granulomas form around the helminth eggs, and the subsequent fibrosis and scarring are responsible for disease pathology. Although the granulomatous response seems to be host protective by containing infection[156–158], it also directly benefits the parasite by facilitating the extrusion of eggs lodged in tissues into the gut lumen, from where they are capable of transmission[159]. Thus, there is an argument for limiting granuloma formation in schistosomiasis. For other granulomatous diseases, increased expression of MMP9 has been associated with susceptibility to Crohn's disease, sarcoidosis and Wegener's granulomatosis[160–162], so therapies that block MMP9 might also find use in these diseases.

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