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Review

The formation of the granuloma in tuberculosis infection

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ABSTRACT

The development of the granuloma and its subsequent degeneration and necrosis, is the hallmark of infection caused by *Mycobacterium tuberculosis*. These structures probably evolved as primitive particle responses, but in mammals they are facilitated by the emerging acquired immune response, in which cytokines and chemokines help control their formation and integrity. In this brief review we discuss the pathology of these lesions in the two most widely used animal models (mice and guinea pigs). In addition, we argue against the idea that there is a balance between host immunity and bacterial survival, and that the latter possess mechanisms that control this, as some currently believe, and moreover discuss newer information regarding the ability of bacilli to persist in these structures long enough to eventually escape and become retransmitted.

1. Introduction

The granuloma is the pathologic hallmark of the host response to infection with *Mycobacterium tuberculosis* [1]. It has an innate, inflammatory basis, but has evolved into a more complex and dynamic structure as a result of the evolution of acquired specific resistance that we now know is mediated by the various components of the TH1 T cell response.

In this review we discuss how our ideas regarding the specific role of granuloma formation has itself evolved, particularly over the past two decades, from a simple containment or physical barrier, to a more complex semi-symbiotic response in which host defenses and bacterial survival mechanisms interact. These more complicated concepts, we will argue here, have been somewhat overblown and over-interpreted.

For obvious reasons, we have had to mainly rely on animals to develop our understanding of the mechanisms, components, and structural basis of the granuloma. This of course complicates matters further, because these models, which range from zebrafish to macaques, all provide insights into these processes, but also differ in some ways both from humans and from the other animal models. As a result for instance we can investigate mechanisms of influx and organization of the cells forming granulomas in the mouse lung, but cannot investigate how this changes with increasing caseation and necrosis for the simple reason that mice (for the most part) do not exhibit these events [2].

Even now, there is no consensus on various aspects of granuloma formation and potential degeneration. As we will discuss here most reviews emphasize the role macrophages as central components of these processes [3–6], with little or no appreciation of the role of neutrophils, in our opinion a key villain [1,7–10]. In addition, the breakdown of necrotizing primary lesions are thought to be the primary reason cavities develop [3,4], the most serious form of tuberculosis, but this idea can be challenged [11], and for that matter may be more related to re-infection. Finally, new information directly challenges the concept of bacterial latency within granulomas, a favorite bête noire of our laboratory [7], with our recent development of a new model [8] based on bacteria surviving in an active rather than latent form in biofilms (in large numbers) in primary lesion residual necrosis, a model that solves several vexing issues in the field.

2. Developing concepts regarding the role of the granuloma

As our concepts of cell mediated immunity began to emerge half a century or so ago, it began to become apparent that the development of the granuloma (not just to tuberculosis infection, but other bacterial and fungal infections as well) was a defensive device designed to wall off and contain the pathogen. Formation of this structure in humans arose from the evolution of simple particle responses, seen even now in primitive animals, to a more complex response in which the simple act of surrounding the site of the infection became integrated and facilitated by the protective

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T cell response – which we now consider (primarily from the mouse model data) to be mediated by TH1 T cells in the specific context of tuberculosis [12].

A question that immediately arises from this is whether the granuloma is actually "protective" or just a form of pathology [5]? The simple answer is the first, but one can argue that the granuloma is, at least initially, an efficient response to wall off pathogens in situations in which there is not a lot the host can do otherwise. It is far from satisfactory, and in many cases these structures deteriorate into tissue destruction that can threaten the survival of the host, but it seems that the infected host not only has no choice, but the bacterium itself has also developed mechanisms not just to survive, but to persist under these circumstances to maximize its chances of transmission.

A further limitation is that very little data can be obtained from the target species itself. When human lungs become available for study they are either from deceased individuals or from lung resections, where the person is already seriously ill. Moreover, as Hunter has stressed [11] because we live in the antibiotic era, samples from people with untreated primary or post-primary tuberculosis are essentially unobtainable. As a result, we turn to animal models, particularly those most widely used in vaccine and drug studies – mice, guinea pigs, and nonhuman primates (NHP). This of course complicates matters considerably, because while each has some elements of disease pathology that we think is happening in humans, they differ both from this and from each other in various ways [13–15]. Moreover, even in the guinea pig model, which many of us think is a valuable model of the naturally occurring human disease process, the granulomatous response results in structures that are heterogeneous in nature [10], adding a further layer of complexity to an already complex process.

Much of the debate regarding early events in granuloma formation makes the assumption that these occur in the alveolar space. But is this so? Our own recent model [8] based on microscopic analysis of interstitial pneumonia in both the mouse and guinea pig, proposes instead that initial events occur in the interstitium. In our model, after escape from the alveolar macrophage that initially encountered it, the bacillus erodes through the lung surface using its ESAT/ESX molecules, establishing a site of implantation in the swelling interstitium. Here, macrophages and neutrophils quickly accumulate, initiating the host response – at this stage, innate.

In this regard, the great majority of recent reviews on this topic either ignore the possible role of neutrophils, or only mention them in passing. In our model [8] not only do they significantly contribute to the pathogenesis of the disease process, but may well be the initial trigger of the necrosis that is characteristic of the core of the granuloma. This can be seen directly in early guinea pig lung lesions [9], and seems more likely than models based on "softening and breakdown of the macrophage derived caseum" some reviews continue to promote.

One central mechanism of the granuloma is thought to be the prevention of bacterial dissemination, but this is in fact not actually the case. Very early in the process in the mouse model both dendritic cells (DC, already present in lung tissues in large numbers), macrophages (including interstitial macrophages), and monocytes (from the blood) arrive in the developing lesion. It has now been clearly shown that DC engulf bacteria and then leave this site, entering the lymphatics and taking them to the draining lymph nodes [16] where acquired immunity will then be triggered, but also possibly via the blood to lymphoid tissue in the spleen, thus establishing sites of dissemination in which CFU levels begin to rise about 10–15 days later. Macrophages have also been implicated in this carriage process, but this conclusion can be questioned. For obvious reasons, whether these mechanisms are equally central in humans is not known.

Much of this confusion arises from studies in zebrafish embryos, which many recent reviews cite as an example of the bacilli using the granuloma to their own advantage, by seeding macrophages and then releasing them. These studies provide important evidence [17] that the bacteria (in that specific case, *Mycobacterium marinum*) uses ESAT not only to escape engulfing macrophages, but can also stimulate the production of host metalloproteinases (MMP). As a result, in this model macrophages become freed from the particle response and can disseminate to other sites. What is lost in the argument here is that these events are occurring in the embryo hemocoel, so it is hardly surprising that macrophages can easily float away since there is nothing to prevent it. In mammals, these events are taking place in a solid structure, the lungs. It seems more likely therefore that the ESAT/MMP mechanism is designed to create space in the parenchyma around the interstitial site so that macrophages can come *in* – and act as host cells – rather than go *out*.

A further concept that prevails in the literature is that once the bacteria are trapped inside a developing granuloma, and finding themselves under conditions of hypoxia, and nutrient starvation, as well as pressure from acquired immunity, they become latent [3,5,6,18,19]. The multiple holes in this argument have been pointed out before [8,20,21], despite its popularity, but there is now no doubt that bacilli persist in the necrotizing granuloma core [22,23], and this is probably the root source of reactivation disease, as will be argued in more detail below.

It is also noticeable that reviews that tend to support the notion of latency also propose that from within the granuloma these latent bacilli can still modulate the immune response, despite no evidence for this, and also propose that the stability of the granuloma is dependent on a balance between pro-inflammatory and anti-inflammatory mechanisms, often then invoking the TH1/TH2 paradigm to explain this, or that the progressive degeneration of the granuloma is due to dysregulation of immunity [6]. Such concepts, which imply some sort of symbiotic relationship between the host and the bacillus, seem to significantly overcomplicate observable events. Indeed, it is very hard to understand the concept that bacteria (whether active or latent) trapped in the centers of caseating lesions can somehow control the progression and fate of the granuloma, or for that matter exploit it. While one can posit that it is the remaining antigens not the bacteria that drive this, a much simpler concept is that the immune response to the infection, for instance in the well-studied guinea pig model, is taking place in intact tissue layers within the granuloma, but can do nothing about (and have no influence on for that matter) the damage taking place in the central core, leaving the host needing to use other mechanisms, such as calcification [1] to try to control and then heal it.

For cells to arrive to start to build the granuloma, three things have to happen. First, we have proposed that the bacillus has to reach the interstitium to trigger local inflammatory signals so that the site can swell and allow a few cells in (this seems more logical than models in which events remain at the alveolar surface, where the alveolar macrophage is embedded in the surfactant layer – moreover, cells falling into the alveolus itself would rapidly dessicate). As shown by zebrafish and mouse models [17,24,25], this probably is further facilitated by MMPs. The local signals will attract lung DC and macrophages, but for an amplified response these will also have to trigger the adjacent blood capillaries to express the adhesion/integrin molecules that can attract cells out of the blood. The third process involves directing these cells, and this involves chemokine production and some cytokine production (TNFα, IL-17), an event that can involve multiple cell types including macrophages, neutrophils, and γδ T cells.

For these reasons the granuloma is a dynamic process at first, as more and more cells move in and the structure grows in size. In mice intravital imaging [26] shows T cell movement within the