Mycobacterial Diseases
ISSN: 2161-1068

Harding and Sandor, J Mycobac Dis 2014, 4:5
DOI: 10.4172/2161-1068.1000167

Commentary Open Access

Protection Vs. Pathology in Tuberculosis: How Our Growing Understanding of the Molecular Regulators of Cell Recruitment Could Lead to New Therapies

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Key Points

1. The granuloma is a highly dynamic ecosystem, both in the activity of effector cells as well as the ongoing cellular recruitment required for granuloma formation and maintenance.

2. Granulomatous inflammation, which provides protection but also induces pathology, is likely in excess of what is needed for bacterial control.

3. Restricting cell traffic to the granuloma may be therapeutic for reducing granulomatous pathology during tuberculosis, and could allow a longer time window for treatment with antibiotics. Our growing understanding of the molecular regulators of cell traffic may offer new therapeutic targets.

The Granuloma as a Dynamic Environment

The granuloma is the hallmark pathological structure in patients infected with Mycobacterium tuberculosis (Mtb). It is a collection of mostly innate and adaptive immune cells organized around Mtb bacilli with a defined spatial arrangement and cellular composition [1-3]. Infection with Mtb begins after a few inhaled bacilli are phagocytosed by lung-resident macrophages. Infected macrophages release of TNFα, which initiates a cytokine storm and supports the release of other pro-inflammatory cytokines and chemokines like Il-1β, IL-6, Il-12, CCL2, and, CCL5, to name a few [4]. Eventually, dendritic cells from the granuloma transport bacterial antigen to the lymph node and activate Mtb-specific CD4 and CD8 T-cells, which then migrate to the granuloma and enhance macrophage anti-microbial activity with the release of IFNγ [5-8].

The granuloma is a highly active and dynamic ecosystem. This dynamism includes the processes that regulate intracellular bacterial killing, as well as the cell traffic to and from the granuloma that shapes adaptive immune priming, granuloma repopulation, and granuloma reformation. Describing the granuloma as a highly dynamic site is somewhat in contrast to the classical view, in which the initially active processes of cell signaling and recruitment subside into a relatively static structure that maintains a host-pathogen homeostasis. A description of this homeostatsis included the clinical observations that granulomas can undergo fibrosis and/or calcification. Multiple and converging data, however, now support a new granuloma paradigm that is much more complex and dynamic than previously appreciated. For instance, our group has used granuloma transplantation to show that nearly 30% of cells are replaced in acute granulomas within one week (representative data shown in Figure 1) [9,10]. Our same data show that chronic lesions, which have always been considered the least active, may have an even faster exchange rate. These data are fitting with the realization that granuloma effector cells are short-lived and must be continuously recruited from the periphery during acute and chronic infection to support continual bacterial containment in an ever-changing environment. While it is known that granulomas can disappear (resolution, fibrosis, calcification, etc), clinical observations show that even after the initiation of acute infection, new granulomas can appear in spaces that previously had none [11]. In the same report, and which is also in contract to the classical view, it was shown that protection and control of bacteria is a granuloma-specific phenomenon, and that sterile and non-sterile granulomas are present in both acute and chronically-infected animals. In this review, we argue that our growing understanding of granuloma dynamism, including the molecular regulators of cellular traffic, can identify therapeutic targets to help reduce pathology in tuberculosis.

Inflammation in Tuberculosis: Friend and Foe

Granuloma formation, as well as activation of granuloma cells, is required for host protection. This protection prevents uncontrolled Mtb growth, contains bacilli in the granuloma, and prevents bacterial

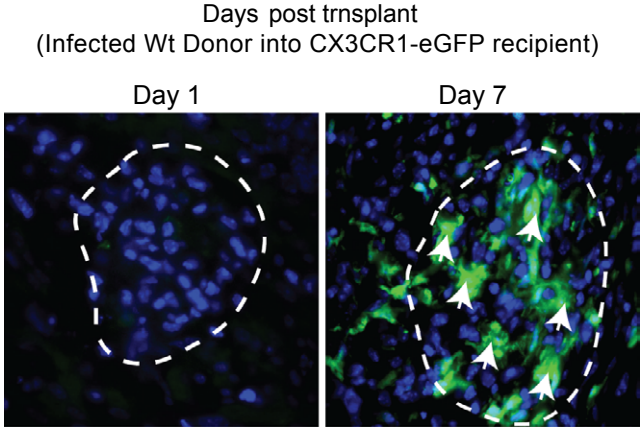

Figure 1: Repopulation of granuloma cells from the periphery.

Granulomas were transplanted from BCG-infected tissue into uninfected CX3CR1-eGFP recipients (monocyte reporter strain). Repopulation of transplanted granulomas with recipient-derived cells (right panel, white arrows) was measured 1 and 7 days later. Depending on the cell type, up to 30% of cells are replaced in acute granulomas within 7 days.

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Received July 30, 2014; **Accepted** August 25, 2014; **Published** September 10, 2014

Citation: Harding J, Sandor M (2014) Protection Vs. Pathology in Tuberculosis: How Our Growing Understanding of the Molecular Regulators of Cell Recruitment Could Lead to New Therapies. J Mycobac Dis 4: 167. doi:10.4172/2161-1068.1000167

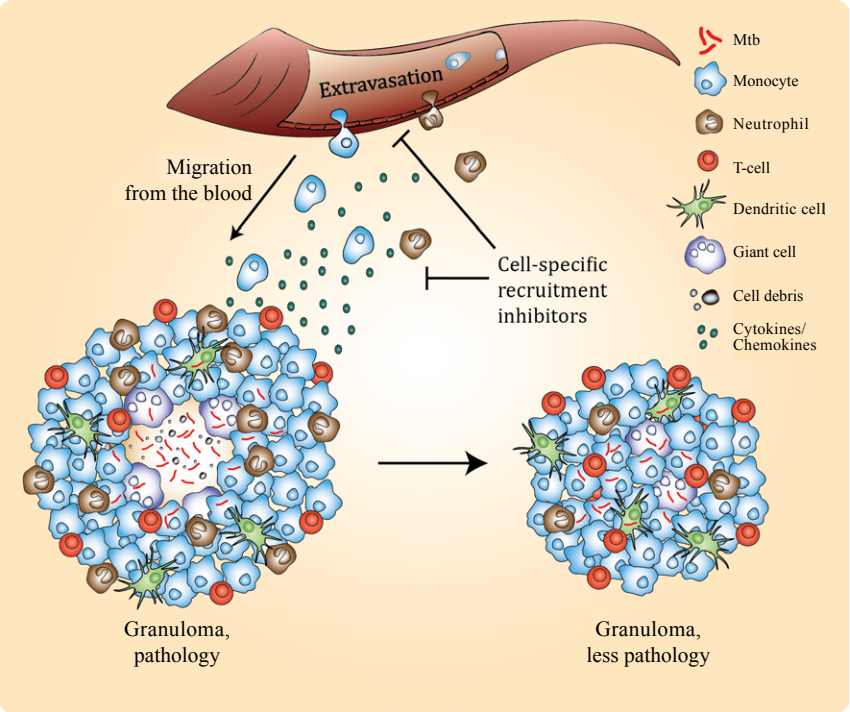

Figure 2: Model of Immunomodulatory therapy.

Immunomodulatory therapies that target molecular regulators of cell recruitment could reduce the pathological character of granulomatous inflammation during active tuberculosis disease.

dissemination. The risk of AIDS patients to tuberculosis disease results from the breakdown of protective granulomas. Animal models with impairments in cellular recruitment, organization, or activation of granuloma cells have severe susceptibility to mycobacterial infection. TNFα KO mice have deficient granuloma formation and die shortly after virulent and non-virulent mycobacterial infection with massive bacterial burden and disseminated bacilli [12,13]. When ICAM-1 is mutated to prevent monocyte binding and transmigration across the endothelium, delays in granuloma formation result in similar susceptibilities to disease [14]. Mice deficient in Myd88, an intracellular adapter for TLRs that activates pro-inflammatory NFkB, die after Mtb infection with high bacterial burden and necrosis in the lung [15].

However, it is also true that leukocyte accumulation in the lungs induces a cost on the host by limiting oxygen exchange through the clogging of alveoli and destruction of lung cells directly. Inflammation is accompanied by reactive oxygen and nitrogen species, which are required for bacterial killing, but are also toxic for cells. Tuberculosis was historically called "consumption" from the observation that patients' health and weight deteriorated as cell accumulation made breathing laborious and inefficient. These clinical observations define inflammation itself as a key aspect of disease and mortality during pulmonary tuberculosis, which is born of the molecular machinery that forms granulomas. This fact is reflected in the many molecular regulators that restrain granulomatous inflammation, even during active disease. Anti-inflammatory molecules IL-10 [16,17], TGF-β [18-20], and NRF2 are released in low levels during acute infection to constrain inflammation, but also in higher levels during chronic infection for the same reason: granulomatous inflammation is inherently pathological. The importance of restraining immunity is demonstrated in mice with deletion of PD-1, a molecule that downregulates T and B-cell responses. Mtb-infected PD-1 KO mice have excessive inflammation, increased necrosis, increased neutrophilic infiltration, and uncontrolled bacterial growth coupled to early mortality [21]. One common phenotype in hosts unable to form a protective response is extensive and disorganized granulocytic and monocytic infiltration [22]. Though it may be a compensatory mechanism, massive neutrophil influx after loss of protection almost always correlates with extensive pathology, necrosis, and a moribund state in humans and animal models. Patients with this type of inflammation require antibiotics for survival.

In view of these data, an important observation has come from mouse models used to study specific inflammatory regulators during Mtb infection: Even protective granulomatous inflammation may be more than is needed. For instance, CCR2 KO mice recruit significantly fewer monocytes to the lungs, but have no changes in bacterial burden after infection with a lose-dose inoculum of Mtb [23]. The author's interpretation of the data was that the wild-type immune response is "more vigorous than necessary." Mtb proteins and lipids are known to exert counter-regulatory effects over the immune response [24], and could support excessive inflammation as a way of supporting granuloma necrosis, which precedes and is required for Mtb escape and transmission. Or excessive neutrophilic/monocytic inflammation could simply be an innate inflammatory response generated whenever loss of protection in infected tissue occurs.

Restraining Pathological Inflammation during Tuberculosis

The studies and clinical observations highlighted here suggest that restraining pathological inflammation during active Mtb infection could be an efficacious therapy. The potential for this approach was recently demonstrated in mice lacking CXCL5, which, despite having

no fewer bacilli, had reduced mortality after Mtb infection due to fewer granulomatous neutrophils [25]. CXCL5 KO mice had similar levels of TNF-α, number of CD4 and CD8 T-cells, and IFN-γ compared to non-transgenic controls. These data suggest that one mode of therapy could involve targeting and/or ablation of specific cell subsets. More generally, any reduction in the number of lung-infiltrating cells could be beneficial for health outcomes as long as it does not abrogate protection. There is already clinical support for this hypothesis–a recent review of 41 human trials between 1955 and 2012 showed that adjunctive corticosteroid treatment reduced morality in patients by nearly 17%, and this efficacy was present in every included tuberculosis organ system [26]. Fitting with the hypothesis that Mtb-induced inflammation is more than needed is the fact that corticosteroids reduced mortality in patients with a hyperinflammatory phenotype, but not those with a hypoinflammatory one.

New tuberculosis therapies are desperately needed. Mtb still results in 8.6 million cases of active disease and 1.3 million deaths every year [27]. The HIV pandemic coupled with increasing drug-resistance is bringing the threat of tuberculosis back into first world. Tuberculosis as a global crisis persists in part due to the weak and variable efficacy of the Bacillus Calmette-Guerin (BCG) vaccine. Though widely administered and able to prevent tuberculosis meningitis in children, BCG immunization rarely prevents infection or the development of pulmonary disease in adults, which still accounts for most Mtb deaths. Exciting new approaches will result in next-generation vaccines, but global implementation is still decades away. One limitation to vaccine approaches is that HIV eliminates the very cells needed to generate protective immunity after vaccination. It is this same immune-deficiency that favors reactivation of Mtb during latency. Additionally, the fact that cured patients can be re-infected with Mtb shows the difficulty that new vaccine approaches face. On another front, the evolution of Mtb resistance is reducing the efficacy of multiple first and second line antibiotics. Only a single new drug, Bedaquiline, has been approved for tuberculosis in the last 40 years [28]. New drugs are in the pipeline, and getting through clinical trials and approval for any individual one is surely needed. Meanwhile, the number of tuberculosis deaths increases every year.

Immunomodulatory therapy could be especially useful for patients with multi-drug resistant Mtb (XDR-Mtb). These patients often require multiple rounds of antibiotics to find one that works. Many times none can be found or patients succumb to disease beforehand. Patients with a hyperinflammatory phenotype, even if they do not have drug-resistant Mtb, could also benefit from reductions in acute symptoms. Immunomodulatory therapy could decrease the severity of pathological granulomatous inflammation and give clinicians more time to treat active disease with antibiotic therapies. Since necrosis of the granuloma center is driven in part by local and destructive immunopathology, immunomodulatory therapy may also mitigate bacterial dissemination to peripheral organs, as well as disease transmission. We expect this mode of treatment to be most efficacious when used with antibiotics, which would mitigate the potential risks associate with any immune-dampening approach.

Molecular Regulators of Inflammation as Targets for Therapy

The molecular regulators that govern granuloma dynamism and cell recruitment will be the interface where immunomodulatory therapies are approached. Our understanding of these regulators has advanced significantly since the time of corticosteroid trials. The goal will be to design reagents that target specific molecules or cell populations while preserving critical elements of bactericidal immunity. In fact, potential drugs with the required properties are already in clinical use for other human diseases. We have shown that vascular endothelial growth factor (VEGF) is upregulated in the granuloma and supports monocyte recruitment into BCG and Mtb-induced murine granulomas. We also have shown that blockade of VEGF attenuates granulomatous inflammation without compromising host protection or control of bacteria. VEGF blockers have already been developed in a variety of forms and are currently used to treat certain cancers as well as age-related macular degeneration.

Our knowledge of how complex chemokine networks regulate the movement of different cell populations is rapidly expanding, and the data generated in this space will highlight important and relevant molecular targets. TNF-α has emerged as a master inducer of chemokines like CCL2, CCL3, CCL4, and CCL5, and the receptors that mediate these molecules' role is starting to be equally well-understood [29]. Of course, these are only a few chemokines in the overlapping and larger network, much of which has been described in detail in many comprehensive reviews [4,30-33]. Of note is that all of the reviews highlight our growing understanding of the molecular details of cell migration and infiltration into infected tissue. Given the known role of neutrophils in tissue pathology and destruction, the molecular regulators over their recruitment, like CXCL5 and IL-23, will be especially relevant. For instance, Cooper et al. showed that anti-IL-17 antibodies could reduce the number of granulocytes in the granuloma. Therapies could also target neutrophils directly for destruction, or target the molecular regulators of survival. Granulocyte depletion using anti-GR-1 or anti-GCSF antibodies improved survival after Mtb challenge of mice lacking CARD9, an adapter molecule that regulates signals from PRRs and TLRs [34]. Lowering the availability of this cell type would decrease their accumulation in granulomatous tissue. Neutrophil depletion using monoclonal antibody NIMP-R14 resulted in reduced CFU in BALB/c mice [35]. Given that monocytes are the dominant granuloma cells, the molecular regulators of their recruitment will also be important targets, including CCL2 and CCL5.

If ubiquitous or cell-specific attenuation of cell accumulation can be therapeutic, then regulators of cell extravasation from the blood will also important. Leukocyte migration across endothelium requires several key steps, including upregulation of adhesion molecules like ICAM-1, ICAM-2, VCAM-1, PECAM-1, L-selectin, and P-selectin, which support the arrest of fast-moving cells in circulation at the site of inflammation [36]. Permeabilization of the endothelium itself is another key step, and factors that regulate this response include VEGF, histamine, and IL-8, to name a few. Supporting the relevance of our murine work, VEGF has already been identified in Mtb lung granulomas [37], and high VEGF levels have been measured in the serum of patients with active pulmonary disease [38,39] as well as those with neurotuberculosis [40]. Immunomodulatory treatments could also target host mediators of tissue destruction and remodeling. MMP-1 is expressed as a function of TNF-α, and IL-1β, and plays a role in the tissue remodeling needed for granuloma formation [41]. Transgenic mice that overexpress MMP-1 during Mtb infection were having increased damage to the lungs and dissemination of bacilli [42]. Ultimately, ablating specific cell populations, or reducing cell traffic to the granuloma by targeting specific chemokines or adhesions molecules, could alleviate Mtb-induced pathology and give clinicians more time to find the right antibiotics (Figure 2).

Concluding Remarks

Our understanding of the granulomas and its role as the host-pathogen interface has been transformed by the recognition that this ecosystem is much more dynamic than previously appreciated. Much of this dynamism includes the role of continuous cell traffic to and from the granuloma-both to replace effector cells, as well as transport bacterial antigen to the lymph and how cellular traffic supports granuloma formation, function, and maintenance. Underlying these facts is that granulomatous inflammation, while necessary for protection, can be the source of pathology and mortality. There is an increasing understanding of the factors that regulate cell traffic and the list of available agents that inhibit traffic is also increasing. Most of this knowledge has been generated in animal models and there is clearly a need to extend these studies to humans. Immunomodulatory therapy would be especially useful in those patients where loss of protection has lead to aberrant, disorganized, and massive cell accumulation in the infected tissues. This commentary has focused on mycobacterial granulomas, but it is important to remember that many autoimmune and infectious diseases are characterized by pathological granulomatous inflammation [43,44], including leprosy, schistosomiasis, histoplasmosis, sarcoidosis, and Crohn's, among others [45-50]. Given many of the similar molecular requirements for granulomatous cell recruitment, it is possible that therapies developed in this space will have usefulness among many disease types. Ultimately granuloma dynamism is the underlying context from which to approach these therapies, and it is the molecular regulators of the continuous cell recruitment during inflammation that will serve as the targets.

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