Seminars in Immunology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

Macrophage immunoregulatory pathways in tuberculosis

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ARTICLE INFO

Keywords:
Macrophages
Innate immunity
Pattern recognition receptors
MicroRNAs
Lung

ABSTRACT

Macrophages, the major host cells harboring *Mycobacterium tuberculosis* (*M.tb*), are a heterogeneous cell type depending on their tissue of origin and host they are derived from. Significant discord in macrophage responses to *M.tb* exists due to differences in *M.tb* strains and the various types of macrophages used to study tuberculosis (TB). This review will summarize current concepts regarding macrophage responses to *M.tb* infection, while pointing out relevant differences in experimental outcomes due to the use of divergent model systems. A brief description of the lung environment is included since there is increasing evidence that the alveolar macrophage (AM) has immunoregulatory properties that can delay optimal protective host immune responses. In this context, this review focuses on selected macrophage immunoregulatory pattern recognition receptors (PRRs), cytokines, negative regulators of inflammation, lipid mediators and microRNAs (miRNAs).

1. Introduction

Macrophages serve as the major host cell niche for intracellular growth and persistence of *M.tb* during all phases of TB, from primary infection with bacillary dissemination, through latency (with bacterial persistence within granulomas) and reactivation TB. In addition, macrophages are responsible for activation of protective immune responses, both innate and acquired, thus playing a critical role in the ongoing cross-talk that is necessary to control or eliminate the infection [1–6].

Our understanding of the great heterogeneity and plasticity of macrophages residing in diverse tissues of different mammals continues to evolve [7–10]. If our goal of finding relevant tissue bio-signatures and therapeutic targets to combat human *M.tb* is to be achieved, we must pay particular attention to the stage of TB infection being modeled (e.g. primary infection, latency with bacterial persistence, reactivation), source of the macrophage (e.g. primary or cell line, human, or other mammal), and experimental conditions, particularly as they apply to the specific tissue microenvironment being modeled (e.g. media for *in vitro* studies, organ for *in vivo* studies, granuloma).

In this review, we will summarize current concepts in macrophage biology as they pertain to *M.tb* pathogenesis. We place the discussion in the context of lung biology and alveolar macrophages (AMs), given their prominent role in airborne TB. AMs are unique mucosal immunoregulatory cells and there is increasing evidence that they are important in allowing *M.tb* to replicate for an extended period of time prior to complete activation of protective immune responses [11–16]. We have coined the time period necessary for optimal responses to occur in the lung "the switching time" [11] and provide evidence that *M.tb* itself can further drive the immunoregulatory activation state of macrophages to enhance its survival [12]. Thus we highlight emerging key macrophage immunoregulatory determinants for *M.tb*.

2. Airborne *M.tb* infection and the lung

M.tb nearly always infects humans via inhalation of airborne droplets released by individuals with active TB that are deposited in the lung alveolus, where as few as one to five bacteria can result in infection. Deposited *M.tb* are engulfed by the resident AMs, which are less able to kill and clear all of the bacteria, ultimately becoming the microbe's home and allowing for dissemination to occur. Primary *M.tb* infection is generally self-limited (subclinical), most often resulting in latency and containment of the bacteria that were

Abbreviations: IM, interstitial macrophage; DPPC, dipalmitoylphosphatidylcholine; CRD, carbohydrate recognition domain; CAM, classically activated macrophage; AAM, alternatively activated macrophage; SP, surfactant protein; ManLAM, mannosylated lipoarabinomannan; PIM, phosphatidyl inositol mannoside; LM, lipomannan; P–L fusion, phagosome–lysosome fusion; AM, alveolar macrophage; BAL, bronchoalveolar lavage; *M.tb*, *Mycobacterium tuberculosis*; PG, prostaglandin; LX, lipoxin; LT, leukotriene; miRNA, microRNA.

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http://dx.doi.org/10.1016/j.smim.2014.09.010
1044-5323/

not eradicated, although complete clearance is possible. During primary infection bacteremia can occur, resulting in bacterial deposition in other organs which serve as a nidus for extrapulmonary reactivation later [17].

Fourteen thousand liters of inhaled air passes through the nose, mouth and trachea each day, where mechanical defenses clear particulates and microbes ≥5 μm in diameter. Smaller inhaled items may pass through the bronchioles and settle in the alveoli where they encounter AMs. The alveoli are delicate grape-like clusters which exchange gases with the surrounding capillary meshwork [18]. Due to the fragility of alveoli and the need for gas exchange, clearance of pathogenic matter without excessive and destructive inflammation is extremely important in this locale.

2.1. Alveolar physiology and cell types

A thin lining of epithelial type I and type II cells surrounds the alveolus. Type I cells are thin, flat and cover 93–97% of the alveolar surface, allowing for efficient gas exchange. Type II epithelial cells are cuboidal with apical microvilli and cytoplasmic lamellar bodies. They produce and secrete pulmonary surfactant lipids and proteins as well as other soluble components of the innate immune system [19]. These substances have widespread immune activity, functioning as opsonins and/or microbial aggregating agents, signaling molecules that shape immune cell phenotypes and microbicides that destroy or destabilize microbial cell walls [20–32]. In addition to AMs, and the epithelium and interstitium which contain capillaries and venules, there are several other innate immune cells including intravascular and interstitial macrophages (IMs), dendritic cells (DCs) and scattered neutrophils [33,34].

2.2. Surfactant Proteins and hydrolases

Pulmonary surfactant is a lipid and protein complex which forms a thin film at the air–liquid alveolar interface for the purpose of reducing surface tension and preventing alveolar collapse during expiration. AMs are bathed in surfactant which is primarily composed of phospholipids such as dipalmitoyl phosphatidylcholine (DPPC) with lesser concentrations of other lipids and cholesterol [35]. There are four surfactant associated proteins, Surfactant protein -A (SP-A), SP-B, SP-C and SP-D. Surfactant lipids adsorb to the air–liquid interface with the assistance of SP-B and SP-C (reviewed in [36,37]). SP-A and SP-D are large, multimeric and relatively hydrophilic collagenous lectins (collectins) with carbohydrate recognition domains (CRDs) that are important in the Ca^{2+}-dependent recognition of microbes (reviewed in [37]). SP-A and SP-D are key regulators in the pulmonary innate immune response through several mechanisms including microbe binding, agglutination, and direct effects on immune cells [38].

SP-A enhances macrophage phagocytosis of apoptotic cells and various pathogens including *M.tb* [39–43] through direct interaction with macrophages [44] as well as binding to the bacterial cell wall proteins and lipoglycans [45,46]. SP-A is a major regulator of macrophage phenotype and function with effects on PRRs, the oxidative burst, and negative regulators of inflammation [21,47–49]. By interacting with mannosylated lipoarabinomannan (ManLAM) found on virulent *M.tb* (and some other pathogenic mycobacteria) [22], SP-D agglutinates *M.tb* and decreases macrophage phagocytosis while enhancing phagolysosomal fusion and killing of the bacilli that are phagocytosed [22,23,50]. Type II cells produce lamellar bodies which are packed with surfactant phospholipids, hydrolytic enzymes and hydrolases in the extracellular lining of the lung [51,52]. Hydrolases can alter the outer cell wall of *M.tb* with the potential to change the macrophage–microbe interaction and host immune response [20].

3. Macrophage phenotype and functional diversity

Macrophages are phenotypically heterogeneous, having diverse functions in different tissues. Some studies indicate that the initial interaction of macrophages with specific cytokines determines their functional phenotype, while others have shown that macrophages can be continuously altered as the environment changes [53–55]. Macrophage heterogeneity has a direct impact on *M.tb* interactions in different tissue environments. Macrophages have been categorized into two major groups: the pro-inflammatory, "classically" activated M1 type macrophage and the immunoregulatory, "alternatively" activated M2 type macrophage [56,57]. However, it is increasingly clear that macrophage function represents a spectrum, with biological activities varying greatly among mammalian species and experimental stimuli [7,58–65]. For example, mouse macrophages express unique phenotypic markers for M1/M2, not expressed by human macrophages [57,61].

3.1. Classically activated macrophages (CAMs)

Once primed by IFNγ, followed by a second signal such as TNFα or LPS, CAMs mediate more efficient antigen presentation, increased synthesis and release of pro-inflammatory mediators and more efficient phagocytosis [57]. One of the most reliable markers of mouse CAMs is robust nitric oxide (NO) production; however, human macrophages produce limited NO even after activation [66], an observation which holds relevance for TB research, discussed below.

3.2. Alternatively activated macrophages (AAMs)

AAMs are generated by IL-4, and IL-13, which are produced by T helper 2 (Th2) cells, and partially share receptor complexes [67]. AAMs generally mediate Th2 type immune responses [57]. In antithesis to CAMs, AAMs are generally anti-inflammatory, producing high levels of IL-10 and TGF-β and are less efficient antigen presenting cells due to reduced MHC class II expression [56,57,60]. AAMs also demonstrate impaired killing of intracellular pathogens, but play an important role in controlling extracellular parasites [68]. In general, AAMs are believed to promote resolution of inflammation and wound healing. The impact of these types of macrophages in TB pathogenesis is being increasingly realized [11–16,69].

3.3. AMs

AMs reside beneath the surfactant monolayer on the luminal side of the alveolar epithelial cells and are a first line of immune cell defense in the alveolus (Fig. 1). It is estimated that there are 8–12 AMs per alveolus [70–72], which through irradiation studies, were determined to originate from blood monocyte precursors [73]. Following differentiation in the lung, resident AMs are relatively long lived, with a turnover rate of around 40% of the population per year [74]. However, there is also evidence in mice for local production of macrophages from stem cells [75].

AMs are uniquely adapted to functioning in the alveolar environment, where they act as sentinels against invading organisms but also serve to limit inflammation and minimize lung injury to preserve alveolar function. AM activation is tightly regulated and involves a complex balancing act between activating and repressing signals. On the one hand, PRRs like Toll-like receptors (TLRs) recognize pathogen associated molecular patterns (PAMPs) and initiate inflammatory responses, while receptors for inflammatory cytokines such as TNFα, IL-1β and IFN-γ perpetuate inflammation [7]. On the other hand, signaling through IL-10 and TGFβ, as well

Please cite this article in press as: Rajaram MVS, et al. Macrophage immunoregulatory pathways in tuberculosis. Semin Immunol (2014), http://dx.doi.org/10.1016/j.smim.2014.09.010