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IMMUNOLOGICAL ASPECTS

Neutrophils exacerbate tuberculosis infection in genetically susceptible mice

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SUMMARY

Mice of the I/St inbred strain genetically hyper-susceptible to TB infection and prone to form neutrophil-abundant necrotic lung lesions and relatively resistant mice of the C57BL/6 (B6) strain were infected with 100 CFU of *M. tuberculosis* H37Rv. To verify the role of neutrophils in TB immunity, we selectively depleted neutrophils from infected mice with highly specific 1A8 anti-Ly6G antibodies at day 2 and 6 post-challenge. Depletion of neutrophils resulted in reduced lung tissue pathology, mycobacterial CFU counts and an increase of the survival time in genetically susceptible I/St, but not in B6 mice. Furthermore, we demonstrated that *in vivo* neutrophil depletion at the onset of TB infection results in a significant increase in numbers of mycobacteria-specific IFN-γ-producing T-cells at the time point when the acquired immunity to mycobacteria is fully developed. These results suggest antagonistic activity of neutrophils and immune T-cells in the course of TB infection and provide further evidence of deleterious rather than protective role of the former.

1. Introduction

Neutrophils had in the past been treated as the outcast when studying complex interrelated networks of natural and adaptive immunity. Their prominent features include extremely short life span, rapid replacement of their pool in the periphery from the bone marrow and almost indiscriminative capacity to rapidly engulf and kill different bacteria without obvious cooperation with other cells of the immune system. This implied the conclusion that these cells are important element of bacterial clearance and acute inflammation, often accompanied by significant tissue damage, but have little to do with sophisticated immune responses during chronic inflammatory diseases (reviewed in [1–3]). Not the least, inherent difficulties in working with these fragile cells delayed acquisition of a critical amount of data required for a more comprehensive interpretation of the neutrophil physiology. However, during the last decade this traditional simplified view practically vanished.

Two lines of evidence changed diametrically our perception of the role of neutrophils in immunity. Firstly, an increasing amount of data indicates that neutrophils are actively involved in chronic inflammatory conditions, such as chronic obstructive pulmonary disease [4], pulmonary tuberculosis [5,6], arthritis [7] and inflammatory bowel disease [8]. Secondly, and more importantly, a series of recent publications directly demonstrated that neutrophils are competent participants in the cross-talk between virtually all cells of the immune system. Thus, both in mice and humans, neutrophils regulate mononuclear cell recruitment to the site of inflammation (reviewed in [9,10]) and development and function of NK cells [11]. We have shown that in mice B-lymphocytes delayed neutrophil recruitment to the site of BCG injection, the type of interaction crucial for development of efficient vaccination against tuberculosis (TB) infection [12]; more recently, data were obtained indicating the involvement of IL-17A in this regulatory pathway [13]. On the other hand, in a non-infectious experimental system neutrophils inhibited B-cell response to protein antigens [14]. Even broader influence of neutrophils on innate and adaptive immunity, involving regulation of B and T lymphocytes activation, monocyte/dendritic cell infiltration and the balance between type 1 *vs.* type 2 immune responses, was demonstrated in the mouse models of chronic *Brucella abortus* infection [15].

An interesting observation regarding capacity of neutrophils to shape phenotypes of other leukocytes was published by D'Avila et al. [16]. In their model of BCG-induced pleurisy in B6 mice, neutrophils rapidly engulfed BCG, underwent apoptosis and were phagocytosed by macrophages. The latter increased lipid body

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formation, as well as PGE_2 and TGF-β syntheses. These results imply an interesting link between two important features of mycobacterial pathogenesis. On the one hand, foamy macrophages containing huge amounts of lipid vacuoles are considered as a niche for mycobacterial survival and latency development, rather than effector antimycobacterial or antigen-presenting cells [17]. On the other hand, TGF-β and PGE_2 are potent inhibitors of T-cell immune responses and their increased production may down-regulate protective anti-mycobacterial immunity [18–20]. Thus, mycobacteria-containing neutrophils might have a dual deleterious effect on the host response to mycobacteria, stimulating foamy macrophage accumulation and inhibiting $CD4^+$ T-cell activation.

In the present work, we further verified the role of neutrophils in TB immunity. To this end, we infected mice of I/St inbred strain, which are genetically hyper-susceptible to TB infection and prone to neutrophil-abundant necrotic lung lesions [21], with virulent *Mycobacterium tuberculosis* and demonstrated that selective neutrophil depletion *in vivo* reduced lung tissue pathology and mycobacterial CFU counts, resulting in an increase of the survival time. These results are in line with the data obtained in other TB-susceptible mouse strains [22,23]. Furthermore, we demonstrated that *in vivo* neutrophil depletion at the onset of TB infection results in a significant increase in numbers of mycobacteria-specific IFN-γ-producing T-cells at the time point when the acquired immunity to mycobacteria is fully developed. These results suggest antagonistic activity of neutrophils and immune T-cells in the course of TB infection and provide further evidence of deleterious rather than protective role of the former.

2. Materials and methods

Mice of inbred strains I/StSnEgYCit (I/St) and C57BL/6JCit (B6) were bred and maintained under conventional, non-SPF conditions at the Animal Facilities of the Central Institute for Tuberculosis (CIT, Moscow, Russia) in accordance with guidelines from the Russian

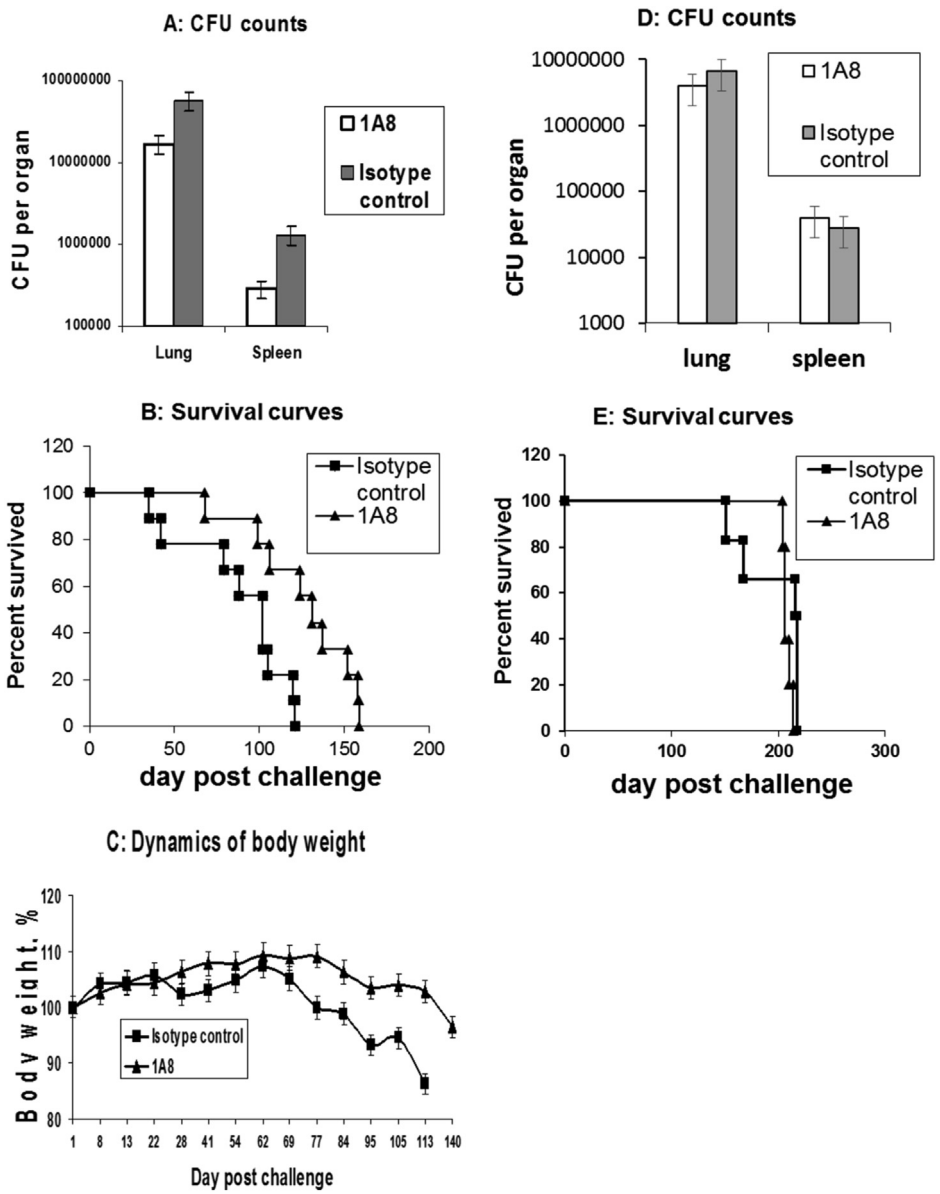

Figure 1. Administration of antibodies selectively depleting neutrophils attenuates the course of TB infection in I/St but not in B6 mice. Compared to isotype-matched control, I/St recipients of 1A8 antibodies displayed lower CFU counts in lungs and spleens (A, $P < 0.05$, ANOVA), longer survival time (B, $P < 0.01$, Gohen's criterion) and less pronounced cachexia (C, $P < 0.01$ starting week 10 post infection, ANOVA). No differences in CFU counts (D) and survival curves (E) were observed in B6 mice.

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