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Beyond the traditional immune response: bacterial interaction with phagocytic cells

Anna Norrby-Teglund*, Linda Johansson

Karolinska Institutet, Center for Infectious Medicine, Karolinska University Hospital, S-141 86 Stockholm, Sweden

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ABSTRACT

The human immune response is well equipped to sense and react to infectious agents in order to achieve efficient immune defence in most instances. A major event in the early control of infection is phagocytic killing of the pathogen. However, important human pathogens have evolved sophisticated mechanisms to modulate the immune response and thereby promote their own survival. In this review, we focus on pathogen-mediated modulation and/or exploitation of phagocytic cells. In particular, the mechanisms employed by *Streptococcus pyogenes* are described, with special attention given to intracellular persistence and stimulation of phagocytic cells leading to disease severity. The crucial role of these mechanisms in the pathogenesis of severe invasive streptococcal infections, such as necrotising fasciitis and streptococcal toxic shock syndrome, is described.

1. Introduction

Streptococcus pyogenes (group A *Streptococcus*) is a Gram-positive bacterium that is responsible for a wide range of human infections, ranging from uncomplicated infections of the throat and skin to severe and potentially life-threatening invasive infections such as streptococcal toxic shock syndrome (STSS) and necrotising fasciitis (NF). This is a pathogen that causes disease primarily through its ability to modulate and exploit the host's immune system. The pathogenesis of streptococcal infections is complex and multifactorial, involving a wide range of bacterial virulence factors, both cell-surface attached and secreted. A large proportion of these virulence factors are of great importance for the bacterium when initialising attachment and invasion into the tissue; others are of importance in order to evade the host phagocytic cell defences. Expression of streptococcal virulence factors is tightly controlled by a number of regulatory genes. These comprise two-component sensor kinase/response regulators (TCSs) [1,2] as well as stand-alone response regulators that respond to one or more environmental signals such as changes in ion concentration, osmotic pressure or pH [3]. One of the most well-described TCSs is the control of virulence (CovR/S) system, also known as CsrR/S, which affects ca. 15% of the bacterial genome and acts primarily by repressing transcription of virulence [4,5]. CovR/S has received considerable attention due to the emergence of highly disseminated hypervirulent *S. pyogenes* strains associated with in vivo selection of mutations in *covS* [6,7]. For the purpose of this review, we have divided the virulence factors into three categories based on their main functions: adhesion/invasion; immunomodulation; and immunostimulation (Fig. 1). The immunomodulatory properties include a variety of strategies aimed at neutralising the effector molecules of immune defence, such as proteolytic cleavage of antimicrobial peptides and antibodies, binding the Fc portion of antibodies, complement inhibition, DNase-mediated disruption of neutrophil extracellular traps, as well as lysis of immune cells [9,10]. Below we describe in further detail intracellular survival strategies in phagocytic cells and immunostimulatory events contributing to disease pathology.

2. Intracellular bacterial persistence and replication contribute to a bacterial reservoir at the infected tissue site

Although *S. pyogenes* is usually considered an extracellular organism, it has been shown to reside intracellularly in a variety of cell types [11–15]. Of particular relevance to this review is the finding of bacteria residing within neutrophils and macrophages [11,12,14]. In an analysis based on snap-frozen tissue biopsies from patients with severe soft-tissue infections caused by *S. pyogenes*, Thulin et al. [11] demonstrated the presence of viable *S. pyogenes* in tissue collected from the epicentre of infection as well as in distal, seemingly unaffected, tissue. The viable streptococci were found to reside predominantly in macrophages in the infected soft tissue, although some neutrophils harbouring bacteria could also be detected (Fig. 2) [11]). The bacterial load was often high, especially in severely inflamed epicentre tissue, and even a substantial

* Corresponding author. Present address: Karolinska Institutet, Center for Infectious Medicine F59, Karolinska University Hospital, S-141 86 Stockholm, Sweden. Tel.: +46 8 585 83297; fax: +46 8 746 7637.
E-mail address: anna.norrby-teglund@ki.se (A. Norrby-Teglund).

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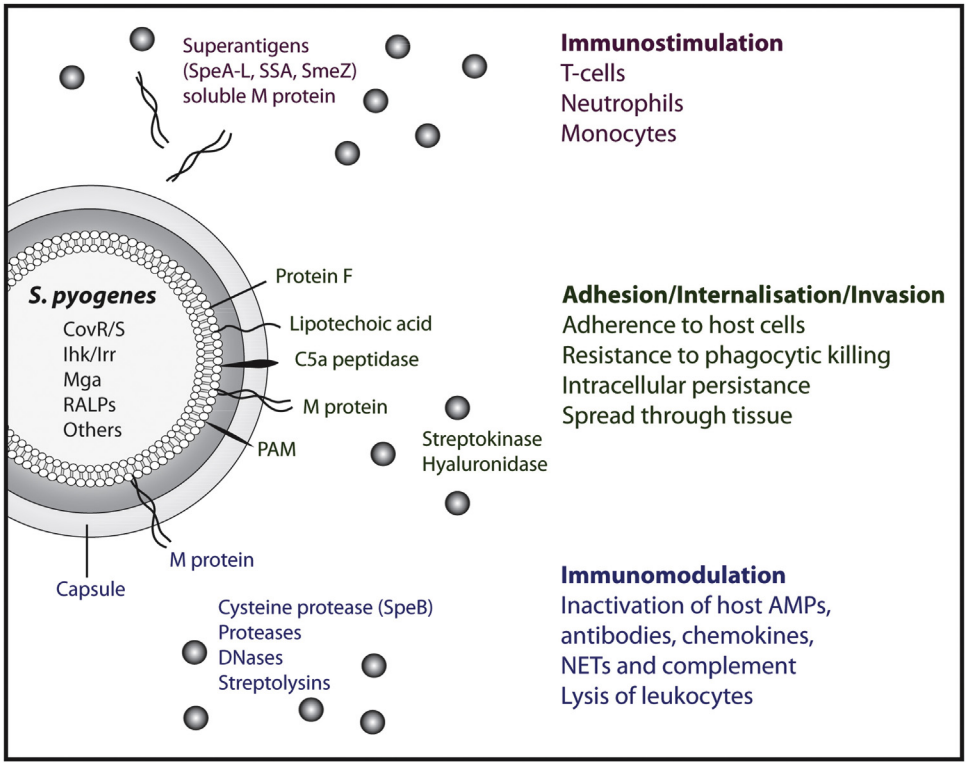

Fig. 1. Streptococcal virulence factors. *Streptococcus pyogenes* has evolved an impressive selection of immune evasion strategies and expresses a wide range of membrane-bound and extracellular virulence factors. Summarised in this figure are factors that stand of great importance for bacterial adhesion and invasion, as well as immunomodulatory and immunostimulatory factors. AMPs, antimicrobial peptides; CovR/S, control of virulence R/S; Mga, multiple gene regulator; NETs, neutrophil extracellular traps; PAM, plasminogen-binding group A streptococcal M-like protein; RALPs, RofA-like proteins; SmeZ, streptococcal mitogenic exotoxin Z; Spe, streptococcal pyrogenic exotoxin; SSA, streptococcal superantigen. The figure is based on the following reviews and original papers [8,10,18–20].

proportion of biopsies collected late after onset of disease (up to 20 days) had a high bacterial load (Fig. 2A). This is of clinical concern considering that the patients had received prolonged intravenous antimicrobial therapy, usually a β-lactam in combination with clindamycin. Taken together, the results suggested that intracellular persistence represents a mechanism by which the bacteria can avoid antibiotic clearance in infected soft tissue, similar to what was suggested in recurrent tonsillitis where intracellular cocci were identified in tonsillar epithelium [16].

Further studies revealed that *S. pyogenes* reside within phagocytic vacuoles in the macrophage, and intracellular survival both in macrophages and neutrophils is associated with an M1 protein-dependent impaired fusion with lysosomes [12,17,18]. The results also indicated a skewing towards a suppressed inflammatory

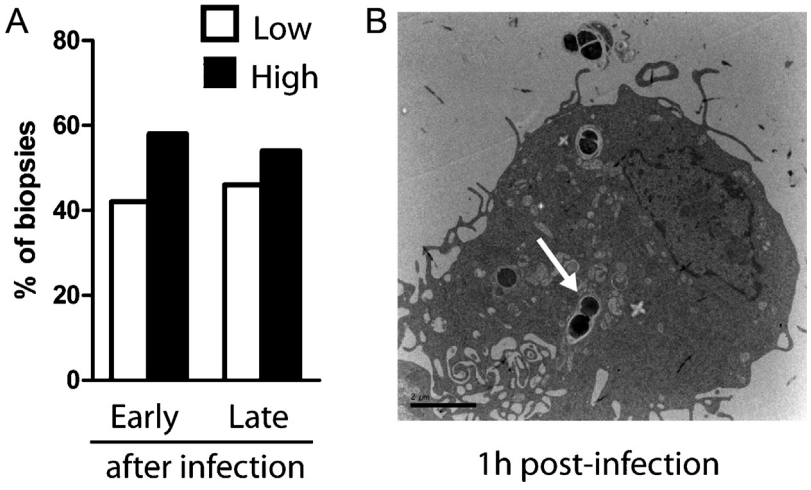

Fig. 2. Massive bacterial burden in tissue biopsies from patients with *Streptococcus pyogenes* infection. (A) Cryosectioned tissue biopsies from patients with necrotizing fasciitis (NF) or cellulitis caused by *S. pyogenes* were immunostained for bacteria. Percentage of biopsies with low or high bacterial load defined by their in situ imaging value, in groups of tissue biopsies collected within 3 days after diagnosis (early) or tissue obtained >3 days after diagnosis (late). Original data have been presented previously [11]. (B) Transmission electron microscopy image of human monocyte-derived macrophages infected with a clinical M1T1 isolate at 1 h post infection (Images: Dr Matthias Mörgelin, Sweden). The arrow indicates bacteria residing in intracellular vacuoles.