Institut Pasteur
Microbes and Infection 19 (2017) 157–165
www.elsevier.com/locate/micinf

The infectious hypoxia: occurrence and causes during *Shigella* infection

Ellen T. Arena [a,b,1], Jean-Yves Tinevez [c,1], Giulia Nigro [a,b], Philippe J. Sansonetti [a,b,d], Benoit S. Marteyn [a,b,e,*]

[a] *Institut Pasteur, Unité de Pathogénie Microbienne Moléculaire, 28 rue du Dr Roux, 75724 Paris Cedex 15, France*
[b] *INSERM Unité 1202, 28 rue du Dr Roux, 75724 Paris Cedex 15, France*
[c] *Institut Pasteur, Citech, Imagopole, 28 rue du Dr Roux, 75724 Paris Cedex 15, France*
[d] *Collège de France, 11 Place Marcellin Berthelot, F-75231, Paris Cedex 05, France*
[e] *Gustave Roussy Cancer Campus, Laboratoire de Thérapie Cellulaire, 114 Rue Edouard Vaillant, 94800 Villejuif, France*

Received 18 July 2016; accepted 31 October 2016
Available online 21 November 2016

Abstract

Hypoxia is defined as a tissue oxygenation status below physiological needs. During *Shigella* infection, an infectious hypoxia is induced within foci of infection. In this review, we discuss how *Shigella* physiology and virulence are modulated and how the main recruited immune cells, the neutrophils, adapt to this environment.

Keywords: *Shigella*; Infectious hypoxia; Neutrophils

1. Introduction

Shigella are a Gram-negative, facultative anaerobic bacteria of the Enterobacteriaceae family and are the etiological agents of bacillary dysentery or shigellosis. Each year, 165 millions shigellosis cases are reported worldwide, leading to one million deaths, mainly in the developing world. Shigellosis is marked by fever and leads to hemorrhagic diarrhea; diagnosis relies on the detection of erythrocytes, polymorphonuclear neutrophils (neutrophils), and mucus in the stools.

Shigella encompasse four subgroups (*Shigella flexneri*, *Shigella sonnei*, *Shigella dysenteriae*, and *Shigella boydii*) (reviewed in Refs. [1] and [2]): *S. dysenteriae* includes 15 serotypes, *S. flexneri*, 14 serotypes, *S. boydii*, 20 serotypes, and *S. sonnei* a single serotype. The endemic form of the disease is caused essentially by *S. flexneri* 2a and *S. sonnei* [2].

Shigella colonize and invade the colon, preferentially targeting colonic crypts [3], and is associated with an acute inflammation [4]. The main steps of colonic infection by *Shigella* are the adhesion, invasion, intracellular replication, and cell-to-cell spreading. Each of these steps is likely to be regulated by environmental cues, including the oxygen tension, pH, temperature, and salts concentration. *Shigella* invasion of the host colonic epithelium is dependent of the Type Three Secretion Apparatus (T3SA). *Shigella* induce a controlled inflammatory response, which includes release of both inflammatory (IL6, IL-8, IL1β, TNFα and β) and anti-inflammatory cytokines (IL-10 and TGF-β) [15].

Within a few hours of *Shigella* invasion in the colonic mucosa, an important influx of inflammatory cells is observed, mainly driven by IL-8 released by infected epithelial cells. Neutrophils represent the predominant population of recruited cells, monocytes are also detected [5]. Lymphoid cells (mainly T cells) have also been observed within the rectal mucosa of infected patients [6]. The dissemination of *Shigella* within the colonic mucosa requires an efficient manipulation of the immune response (as reviewed elsewhere [7,8]). It has previously

* Corresponding author. Institut Pasteur, Unité PMM, 28, rue du Dr. Roux, 75724, Paris Cedex 15, France. Fax: +33 1 45488953.
E-mail address: marteyn@pasteur.fr (B.S. Marteyn).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.micinf.2016.10.011
1286-4579/

been shown that *Shigella* induce monocyte [9], macrophage [10], and B cell [11] apoptosis and inhibits T cell migration [12].

Neutrophils are believed to play a key role in the *Shigella* clearance process through phagocytosis, intracellular killing, as well as the extracellular release of antimicrobial molecules. However, the respective survival of *Shigella* and neutrophils upon interaction remains controversial. It has been shown that Shigella induce neutrophil necrosis in a T3SA-dependent manner [13]. Conversely, it has been shown that neutrophils efficiently kill *Shigella* upon phagocytosis [14]. More recently, it has been shown in Zebrafish larvae infected with *Shigella* that neutrophils and macrophages engulf *Shigella*; although in this model, the impact of the microenvironment is not controlled and does not reflect the colonic lumen and mucosa low oxygen conditions [15].

Oxygen, among other environmental cues plays a critical role in host-pathogen interactions (also reviewed elsewhere [1]). Hypoxia induction during bacterial infection had been observed more than 50 years ago, although the cause remains elusive [2,16,17]. For technical reasons, the level of oxygen at bacterial, viral, or fungal infection sites could never been quantified *in situ* or in intact organs using non-disruptive techniques; the exception being the blood circulation compartment, which is the most accessible body compartment. However, the consequences of its modulation on pathogen physiology and virulence or potentially on immune response efficiency constitute a fundamental biological question.

In this review, we will define hypoxia in the context of *Shigella* infection, introducing the concept of the "Infectious Hypoxia" before describing how neutrophils and *Shigella* are physiologically adapted to low oxygen environments focusing on the pathogen metabolism and virulence modulation and the regulation of neutrophils survival and antimicrobial activity. In conclusion, we will discuss the potential causes and pathophysiological consequences of hypoxia induction during *Shigella* infection.

2. 1-Pathophysiological hypoxia(s)

A prerequisite for the characterization of a pathophysiological oxygen level in an infected tissue is the definition of the physiological oxygen level, named normoxia. The normoxic tissue oxygen level varies between organs, depending on their perfusion efficiency (input) and oxygen consumption, associated with their metabolic activity (output). As mentioned above, for technical reasons, the precise measure of physiological oxygen in most organs remains difficult and is still unknown. Most oxygen pressures (pO_2 organ) reported in animal models were determined using a Clark electrode, which is a disruptive method [3,16,17].

In pathophysiological conditions, hypoxia is defined as a tissue oxygenation status that stands below normoxia and results from an inefficient oxygen supply or increased oxygen consumption. Accordingly, the oxygen level associated with hypoxia will vary between and within organs. Organ hypoxia induction may be associated with extrinsic or intrinsic factors. Extrinsic causes are associated with a reduced oxygen supply to an organ, perhaps due to a low red blood cell haemoglobin concentration (anemic hypoxia), a limited capillary perfusion (geographic hypoxia), a reduced local blood flow (ischemic hypoxia), insufficient cardiac activity (stagnant hypoxia), abnormal pulmonary function (hypoxic hypoxia) [4,18], or a reduced environmental oxygen pressure (at the sea level, pO_2 atm = 160 mmHg; at 8848 m, pO_2 atm = 53 mmHg), causing a drop in arterial pressure of oxygen associated with a lowered fraction of oxygen-saturated haemoglobin (oxygen saturation), defining the environmental hypoxia.

Intrinsic causes of hypoxia are characterized by a transient or permanent increase of the organ oxygen consumption. It might be physiological or pathophysiological. Physiological hypoxia induction can be illustrated by muscle contraction, which is associated with an increased oxygen consumption rate by myocytes, leading to a transient hypoxia induction. As a physiological response, the resulting and combining increase of the partial pressure of carbon dioxide (pCO_2), pH decrease, and temperature increase leads to a local reduction of the haemoglobin affinity for oxygen: the so-called "Bohr effect" (first described by Dr. Christian Bohr in 1904). This physiological adaptation leads to an increased oxygen supply to the organ, counteracting the causes of hypoxia and restoring normoxic conditions. Pathophysiological hypoxia induction may be associated with the infiltration of "newcomers", such as immune cells in an organ, associated with increase oxygen consumption. This phenomenon has been recently described in the context of sterile inflammation and was named 'inflammatory hypoxia'. The recruitment of neutrophils was identified as the main cause of hypoxia induction, mainly due to the NADPH oxidase-dependent oxygen consumption, inhibited by Diphenyleneiodonium (DPI) [5–8,19–21].

In this review we will define a new type of hypoxia, named "Infectious Hypoxia", associated with pathogen invasion, in particular bacterial pathogens. During infectious processes, the organ cell composition is dramatically modified; in this model, the "newcomers" are invading pathogens and recruited immune cells. The metabolic activity of the "newcomers" is hypothesized to modify the overall organ oxygen consumption. Hence, hypoxia can be associated with an increased consumption of the available oxygen by bacteria if they are able to consume oxygen as a final electron acceptor for their respiration (aerobic and facultative anaerobic bacteria). It may also be associated with immune cell respiration (mitochondria activity) or Reactive Oxygen Species (ROS) production (NADPH oxidase activity). Both "newcomers"' oxygen consumption would represent potential intrinsic causes of hypoxia induction. Until now, no report has addressed this issue, although hypoxia has been described as a critical factor modulation neutrophil antimicrobial functions, notably through a limitation of the oxidative burst mediated by the NADPH oxidase, during *Staphylococcus aureus* infection [22]. The main immune cell population recruited at a site of bacterial infection are neutrophils; their role in the infectious hypoxia induction has not yet been characterized. It has to be