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Expression of CXCR1 (IL-8 receptor A) in splenic, peritoneal macrophages and resident bone marrow cells after acute live or heat killed *Staphylococcus aureus* stimulation in mice

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ABSTRACT

Literature reveals that interaction with live *Staphylococcus aureus* (*S. aureus*) or heat killed *S. aureus* (HKSA) promotes secretion of CXCL-8 or interleukin-8 (IL-8) from leukocytes, however, the expressions of CXCR1 in murine splenic (SPM), peritoneal macrophages (PM) and resident fresh bone marrow cells (FBMC) have not been identified. Currently, very few studies are available on the functional characterization of CXCR1 in mouse macrophage subtypes and its modulation in relation to acute *S. aureus* infection. SPM, PM and FBMCs were infected with viable *S. aureus* or stimulated with HKSA in presence and absence of anti-CXCR1 antibody in this study. We reported here that CXCR1 was not constitutively expressed by macrophage subtypes and the receptor was induced only after *S. aureus* stimulation. The CXCR1 band was found specific as we compared with human polymorphonuclear neutrophils (PMNs) as a positive control (data not shown). Although, we did not show that secreted IL-8 from *S. aureus*-infected macrophages promotes migration of PMNs. Blocking of cell surface CXCR1 decreases the macrophage's ability to clear staphylococcal infection, attenuates proinflammatory cytokine production and the increased catalase and decreased superoxide dismutase (SOD) enzymes of the bacteria might indicate their role in scavenging macrophage derived hydrogen peroxide (H_2O_2). The decreased levels of cytokines due to CXCR1 blockade before *S. aureus* infection appear to regulate the killing of bacteria by destroying H_2O_2 and nitric oxide (NO). Moreover, functional importance of macrophage subpopulation heterogeneity might be important in designing new effective approaches to limit *S. aureus* infection induced inflammation and cytotoxicity.

1. Introduction

Interleukin-8 (IL-8) or CXCL8 is a pro-inflammatory ELR^+ CXC chemokine, originally identified as a neutrophil chemoattractant [1]. CXCL8 makes an important contribution to the induction of innate immunity through its effect on neutrophil chemotaxis and activation. Accordingly, CXCL8 has been implicated in a number of inflammatory diseases [2,3]. CXCL8 mediates its effects via binding to two heterotrimeric G protein coupled receptors CXCR1 (IL-8RA) and CXCR2 (IL-8RB). These receptors have normally been found on the surface of human leukocytes (neutrophils, monocytes, macrophages, basophils, T lymphocytes) and endothelial cells [4]. In human, two high affinity receptors for CXCL8 designated CXCR1 and CXCR2 [5–7] have been reported. However, functional characterization of CXCR1 in mouse macrophage subtypes of Swiss albino mice and its modulation in relation to acute *S. aureus* infection has not been reported.

Recently, mice with targeted deletion of CXCR2 also known as murine CXCL8 homolog (CXCL8 RL) were constructed [8,9]. Murine CXCR1 share 64% and 89% homology at the amino acid level with the human CXCR1 and murine CXCR2 respectively [10]. Murine CXCR1 has been shown to bind many CXC chemokines, but it is unknown if the receptor is expressed in macrophages or bone marrow cells of Swiss albino mice [11]. CXCR1 expression in relation to mucosal and systemic candidiasis has been demonstrated in BALB/c and IL-8Rh−/− mice [12]. Although chemokines and chemokine receptors probably evolved to coordinate leukocyte recruitment that supports an antimicrobial response, many have

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List of abbreviation

CPCSEA	Committee for the purpose of control and supervision of experiments on animal
CXC-chemokine	which have a single amino acid residue interposed between the first two canonical cysteines
CXCL-8	interleukin-8
CXCR1-CXC	chemokine receptor-1
EDTA	ethylenediaminetetraacetic acid
ELR^+	glutamate–leucine–arginine (ELR) motif near the N terminal
FBS	fetal bovine serum
HBSS	Hank's balanced salt solution
IL-8RA	interleukin-8 receptor A
iNOS	inducible nitric oxide synthase
JNK	c jun N-terminal kinase
MAPK	mitogen activated protein kinase
MHC-II	major histocompatibility complex-II
$NaNO_3$	sodium nitrate
NaOH	sodium hydroxide
NF-κB	nuclear transcription factor kappa beta
NOS2	nitric oxide synthase −2
PBMC	peripheral blood mononuclear cells
PIP3	phospho inositol trisphosphate
PMSF	phenyl methyl sulfonyl fluoride
RIPA	radio immune precipitation assay buffer
SDS	sodium dodecyl sulphate

been exploited by infectious agents, like *Staphylococcus aureus (S. aureus)* to facilitate infection [13].

It has been reported that staphylococcal surface protein -A mediated CXCL8 secretion plays a central role in initiating the neutrophilic response against *S. aureus* infections. It is mediated by several signalling pathways which ultimately trigger NF-κB and other signalling molecules that induce proinflammatory cytokines and chemokines to promote neutrophil trafficking from the circulation into the infected tissue [14]. Thus, there has been intense interest in understanding how its production is triggered during acute *S. aureus* infection. Investigation on the functional expression of CXCR1 in macrophage and its regulation in respect to long term *S. aureus* infection in peritoneal macrophages of Swiss albino mice have been reported [15]. Studies evaluating the role of acute *S. aureus* infection induced CXCR1 expression in murine splenic, peritoneal macrophages and resident bone marrow cells of Swiss albino mice have not been described.

Previously, bacterial infection was also shown to augment CXC chemokine receptor CXCR1 and CXCR2 expression by human epithelial cell lines, and anti-CXCR1 antibodies were shown to impair transmigration [16]. Based on their affinity for CXCL8 and on internalization and recycling characteristics, CXCR1 is thought to mediate CXCL8-induced chemotaxis at sites of inflammation (where CXCL8 concentration is high) [17]. Blockade of CXCR1/2 or blockade of chemokines, which act on these receptors, such as CXCL8 [interleukin-8 (IL-8)], have been shown to prevent neutrophil influx and tissue injury in several models of acute and chronic inflammation [18,19]. A blockade of CXCR1 or CXCR2 could inhibit excessive infiltration or activation of neutrophils during acute inflammatory processes [20]. There is an abundance of evidence supporting the validity of targeting CXCL8/CXCR1/2 signalling in cancer. Targeting of CXCR1 or CXCR2 receptors may be attempted using neutralizing antibodies, small molecule antagonists or peptide derived inhibitors [21,22]. Neutralizing antibodies may also be used to target CXCR1 and CXCR2 preventing ligand binding at the extracellular domain. Blockade of CXCR1 via neutralizing antibody has been shown to inhibit CXCL8 induced proliferation of cancer cells. There was an extensive body of evidence to support the use of CXCR1/2 targeted therapy in the treatment of cancer [23,24]. However, it has also been demonstrated that targeting of CXCR1 was likely to be more efficacious than neutralizing IL-8 alone [23]. Blockage of CXCR1 was considered a potential therapeutic approach for inflammation related malignancies [25–27]. It was reported that normal mice cleared infection within 3–7 days, but the bacterial numbers increased in the CXCR1 knockout mice, which also developed symptoms of bacteremia [16,28] suggesting the involvement of CXCR1 in bacterial clearance. It was reported that CXCR2 is essential for protective innate host response in murine model [29]. Currently there is no study on the functional characterization of CXCR1 in mouse peritoneal, splenic macrophages and resident fresh bone marrow cells and its modulation in relation to acute *S. aureus* infection of wild type Swiss albino mice. The present study was performed in order to investigate the functional expression of CXCR1 and its regulation in respect to acute viable *S. aureus* infection and heat killed *S. aureus* stimulation in peritoneal, splenic macrophages and resident fresh bone marrow cells of Swiss albino mice.

CXCR1 expression in different subtypes of macrophage in Swiss albino mice is not clearly known. Macrophages are remarkably versatile in their ability to recognize and respond to a wide range of stimuli, expressing a variety of surface and intracellular receptors, multiple signal transduction pathways and complex, adaptable arrays of gene expression [30]. For the present, the questions arise, how is the phenotype of macrophages influenced by different tissue environments, and what are the effects of macrophage activation on the particular tissue in which they reside? A further issue is whether organ-specific differences persist after macrophage activation by inflammation, infection, and malignancy [30]. In addition, macrophages have heterogeneous phenotypes and complex functions within both innate and adaptive immune responses [31]. Despite the toxic effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) of host macrophages *S. aureus* can survive and grow within macrophages. Upon stimulation by *S. aureus* and its products macrophages have been known to synthesize and release proinflammatory cytokines [32,33]. ROS and RNS are produced by macrophages as part of their antimicrobial response [34], whereas, several bacterial gene products have been associated with the detoxification of host derived ROS and RNS [35–37]. However, the role of CXCR1 in the intracellular survival of *S. aureus* and involvement of cytokines particularly in the peritoneal, splenic macrophages and resident fresh bone marrow cells of Swiss albino mice during acute bacterial infection was still unclear. So, this study was undertaken in Swiss Albino mice model to investigate the differential modulation of ROS and cytokines due to neutralization CXCR1 after acute infection with either live *S. aureus* (LSA) or heat killed *S. aureus* (HKSA) stimulation in splenic (SPM), peritoneal (PM) macrophages and fresh bone marrow cells (FBMC).