Immunology Letters 123 (2009) 185–188

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/

Short communication

IFN-γ plays a detrimental role in murine defense against nasal colonization of *Staphylococcus aureus*

Sara Elena Satorres[a], Lucía Esther Alcaráz[a], Ethelina Cargnelutti[a,b], Maria Silvia Di Genaro[a,b,*]

[a] *Laboratory of Microbiology, Chemistry, Biochemistry and Pharmacy Faculty, National University of San Luis, Argentina*
[b] *Immunopathology Laboratorio IMIBIO-SL, CONICET, 5700 San Luis, Argentina*

ARTICLE INFO

Article history:
Received 20 December 2008
Received in revised form 15 February 2009
Accepted 2 March 2009
Available online 19 March 2009

Keywords:
Staphylococcus aureus
Nasal colonization
Slime production
IFN-γ

ABSTRACT

The anterior nares are the major reservoir in humans of *Staphylococcus aureus* with the risk of developing endogenous infections or transmitting infections to susceptible persons. The mechanisms that mediate attachment of staphylococci to the nasal mucosa are little known. The purpose of the present work was to study some factors that could influence the nasal colonization in an animal model of mice. We investigated the possible role of IFN-γ. We used *S. aureus* ATCC 35556 (SA113) slime-producing and ATCC 25923 non-slime-producing strains. Male 6-week-old BALB/c, C57BL/6 (wild-type, WT), and gene-deficient IL-12p40 (IL-12p40−/−) or IL-4 (IL-4−/−) mice on C57BL/6 background were infected with a dose of *S. aureus* of 10^6 CFU in 10 μl of saline. The total number of *S. aureus* CFU per nose and lung, specific IgA response and IFN-γ levels were evaluated. Significant higher CFU were recovery from the narines of C57BL/6 compared with BALB/c mice either after ATCC 35556 ($p < 0.0001$) and ATCC 25923 ($p < 0.02$) strain infection. Low IgA response correlated with high bacterial counting in the C57BL/6 nasal region. Moreover, C57BL/6 mice showed major colonization of slime-producing *S. aureus* ATCC 35556 than non-slime-producing ATCC 25923 *S. aureus* strain ($p < 0.02$). IL-12p40−/−mice clarified the bacteria from their nose more efficiently that WT mice after slime-producing *S. aureus* ($p < 0.0001$). Accordingly, significant lower level of IFN-γ were detected in IL-12p40−/− compared with WT mice after infection with this strain ($p < 0.03$). The results suggested the influence of the slime production in nasal colonization of *S. aureus*, and indicate at first time that IFN-γ may play a detrimental role in this mucosal infection. These results could contribute to elucidate mucosal immune mechanisms involved in *S. aureus* colonization and then control infections in susceptible persons.

Staphylococcus aureus is known to be part of the normal skin and mucosal microflora. The anterior nares are the major reservoir in humans of this opportunistic pathogen with the risk of developing endogenous infections or transmitting infections to susceptible persons [1]. The mechanisms that mediate attachment of staphylococci to the nasal mucosa are little known. Colonization is a multifactorial process that requires a variety of adaptive mechanisms including nutrient acquisition, adherence to host tissues, and evasion of, or protection against host defenses [2,3]. *S. aureus* produces a myriad of virulence factors that contribute to its ability to cause disease. Cell wall components such as peptidoglycan, teichoic acids, and capsule, as well as adhesins, proteases, exoproteins, and exotoxins, all promote the virulence of this pathogen [4]. Certain *S. aureus* strains have the ability to develop highly consolidated structure: the biofilm, which is believed to make the organisms more resistant to antibiotics and host defenses [5]. Formation of the biofilm requires polysaccharidic intercellular adhesine (PIA), which is synthesized by enzymes encoded by the intercellular adhesion cluster (*ica*) [6]. Although numerous animal models have contributed to the knowledge of virulence factors involved in staphylococcal infection, only a few reported studies have examined the interactions of *S. aureus* with the nasal mucosa in an animal model [2,7].

An important aspect of the host innate immune response to *S. aureus* is opsono phagocytic killing by polymorphonuclear leukocytes (PMNs). The essential role of phagocytic killing in host clearance of *S. aureus* is apparent because patients who are neutropenic or have defects in PMN function suffer recurrent staphylococcal infections. In contrast, little information is available on how various T cell populations influence the outcome of staphylococcal infections. It is known that *S. aureus* and its products induce various cytokines that play beneficial and detrimental roles in hosts exposed to these bacterial infections [8,9]. The purpose of the present work was to study some factors that could influence the nasal colonization in an animal model in mice. We

* Corresponding author at: Laboratorio de Inmunopatología, Area Microbiología, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina. Tel.: +54 2652 423789; fax: +54 2652 431301.
E-mail address: sdigena@unsl.edu.ar (M.S. Di Genaro).

0165-2478/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2009.03.003

investigated the possible role of IFN-γ in the nasal colonization by *S. aureus*.

We used *S. aureus* ATCC 35556 (SA113) slime-producing strain (kindly provided by Dr. Andreas Peschel, Universidad de Tübingen, Alemania), and *S. aureus* ATCC 25923 non-slime-producing strain. Bacteria were grown at 37 °C for 24 h in mannitol salt agar. Detection of slime production was corroborated by qualitative Congo red Agar (CRA) technique as previously described by Freeman et al. [10]. Moreover, the *icaA* and *icaD* sequences were detected by PCR [11].

We used male 6-week-old BALB/c, C57BL/6 (wild-type, WT), and gene-deficient IL-12p40 (IL-12p40−/−) or IL-4 (IL-4−/−) mice on C57BL/6 background. The mice were infected with a dose of *S. aureus* of 10^6 CFU in 10 μl of saline, which was pipetted slowly onto the nares of the mice without actually touching the pipette tip to the nose. Eight days after inoculation, extraction of blood from retro-orbital plexus was realized and then the animals were euthanized by intraperitoneal injection of potassium chloride in conjunction with general anesthesia. Nasal region and the lung were aseptically removed, and organ homogenates were prepared. The total number of *S. aureus* CFU per nose and lung was evaluated by plating a 50 μl aliquot of the homogenates in mannitol salt agar incubated for 24–48 h at 37 °C. Antibiotype and slime production were evaluated in recovery colonies. Anti-*S. aureus* IgA was determined in sera by ELISA as described previously [12]. Uninfected mice were included as controls. Samples were considered positive if they had an OD exceeding the mean + 2S.D. of the control group values. IFN-γ concentration in the nasal homogenates was determined by capture ELISA with a commercial kit according to the manufacturer's instructions (kit Fento HS ELISA RSG, eBioscience, San Diego, USA). Statistical analysis was performed using Student *t* test. A value of $p < 0.05$ was considered to indicate statistical significance.

We corroborated that only *S. aureus* ATCC 35556 (SA113) strain was slime-producing by method of CRA and presented *ica* genes (data not shown). No bacteria were detected in blood and in the lung of infected mice, indicating local nasal bacterial colonization. Significant higher CFU were recovery from the narines of C57BL/6 compared with BALB/c mice either after ATCC 35556 ($p < 0.0001$) and ATCC 25923 ($p < 0.02$) strain infection. Since it is known that C57BL/6 is a T helper (Th) 1 prototype mouse strain, and BALB/c a Th2 mouse strain [13], this result suggest that Th1 could influence *S. aureus* nasal colonization. Moreover, C57BL/6 mice showed major colonization of slime-producing *S. aureus* ATCC 35556 than non-slime-producing ATCC 25923 *S. aureus* strain ($p < 0.02$) (Fig. 1A), suggesting that the slime production participates in *S. aureus* nasal colonization in susceptible host. We observed higher *S. aureus*-specific IgA antibody response in *S. aureus* infected BALB/c than C57BL/6 mice ($p < 0.001$) without significant differences between both bacterial strains (Fig. 1B). The low IgA response correlated with high bacterial counting in the C57BL/6 nasal region (Fig. 1A and B). This result emphasizes the influence of host and the capacity of response of IgA in the protection of colonization by *S. aureus*. However, specific IgA response was similar among C57BL/6 WT, IL-12−/− and IL-4−/− mice (data not shown) even when IL-12p40−/− mice clarified the bacteria from their nose more efficiently that WT mice after slime-producing *S. aureus* infection ($p < 0.0001$) (Fig. 2A). Since IL-12p40 is a subunit of IL-12, a cytokine involved in Th1 response, this result could indicate that lower Th1 cytokines could increments the capacity of the host to clarify *S. aureus* from nasal mucosa. With this purpose, we evaluated the levels of IFN-γ, a Th1 cytokine, in IL-12p40−/− and WT C57BL/6 mice and detected significant lower level of this cytokine in IL-12p40−/− mice infected with slime-producing *S. aureus* ATCC 35556 strain ($p < 0.03$) (Fig. 2B). Accordingly, IL-4−/− mice showed similar narine CFU and IFN-γ level to WT mice (Fig. 2A and B), these results suggest that IL-4 deficiency did not influence *S. aureus* nasal colonization. In contrast, IFN-γ may play a detrimental role in this murine mucosal infection

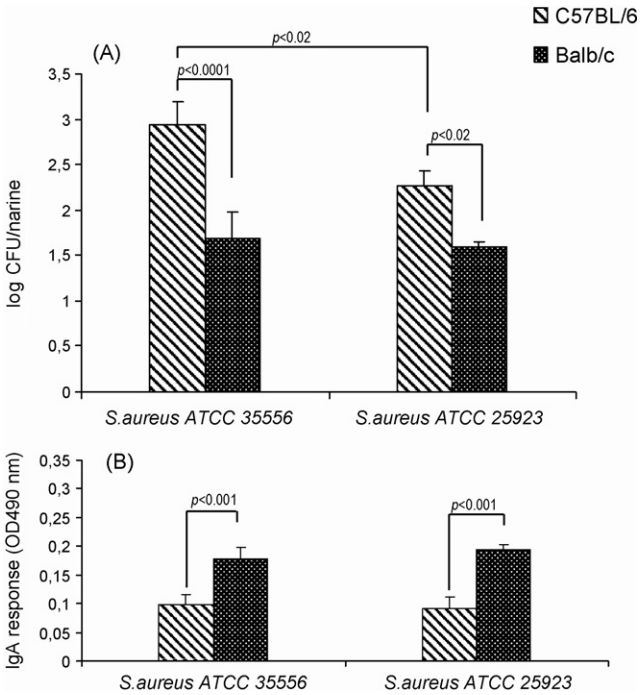

Fig. 1. Bacterial load in narine of *S. aureus* infected C57BL/6 and BALB/c mice. (A) The number of bacteria in narine at day 8 after nasal infection in C57BL/6 and BALB/c mice. (B) *S. aureus*-specific IgA antibodies in narine homogenates of infected C57BL/6 and BALB/c mice. In (A) and (B), the data are representative from two independent experiments and they are the results of four mice per group.

model. IFN-γ plays both beneficial and detrimental roles in hosts exposed to bacterial infections [14–16]. The production and role of endogenous IFN-γ in the nasal colonization by *S. aureus* have not been still studied. This is the first report on the role of IFN-γ in the nasal colonization by *S. aureus*. On the other hand, the IL-12p40−/− mice used in the present study lack not only IL-12 but also IL-23 since both cytokines share the subunit p40 [17]. IL-23 is required for IL-17-producing T cell proliferation and maintenance [18]; moreover, IL-17 is a cytokine associated with mucosal host defense [19]. However, IL-17 may not play a protective role in our study since lower CFU in narine was detected in IL-12/IL-23 deficient mice. In addition, it has been reported that intact Gram-positive bacteria such as *S. aureus* stimulate more IL-12 than IL-23 [20].

Further studies could clarify the mechanisms by which this IFN-γ regulates nasal immune response. However, we might hypothesize that this cytokine contributes to nasal *S. aureus* colonization by modulating neutrophil trafficking and activation. Thus, IFN-γ-dependent neutrophil recruitment has been reported by other authors [9,21]. Accordingly, McLoughlin et al. demonstrated, in a *S. aureus* surgical wound infection model, lower level of bacteria associated with neutrophil deficiency in the wound of C57BL/6 IFN-γ−/− mice compared with C57BL/6 WT mice [9]. As a result, T cell-derived IFN-γ generates a neutrophil-rich environment that can potentiate *S. aureus* pathogenesis by facilitating bacterial survival within neutrophil [9]. On the other hand, since IFN-γ participates in mucosal injure [22] and induces neutrophil activation [23] that cause tissue damage [24], IFN-γ can facilitate nasal *S. aureus* colonization inducing nasal mucosal injure by neutrophils. Therefore, our results are in line with previous systemic *S. aureus* infection model and report at first time the detrimental role of endogenous IFN-γ in nasal colonization. These results could contribute to elucidate mucosal immune mechanisms involved in *S. aureus* colonization and then control both endogenous infections and those transmitted to susceptible persons.

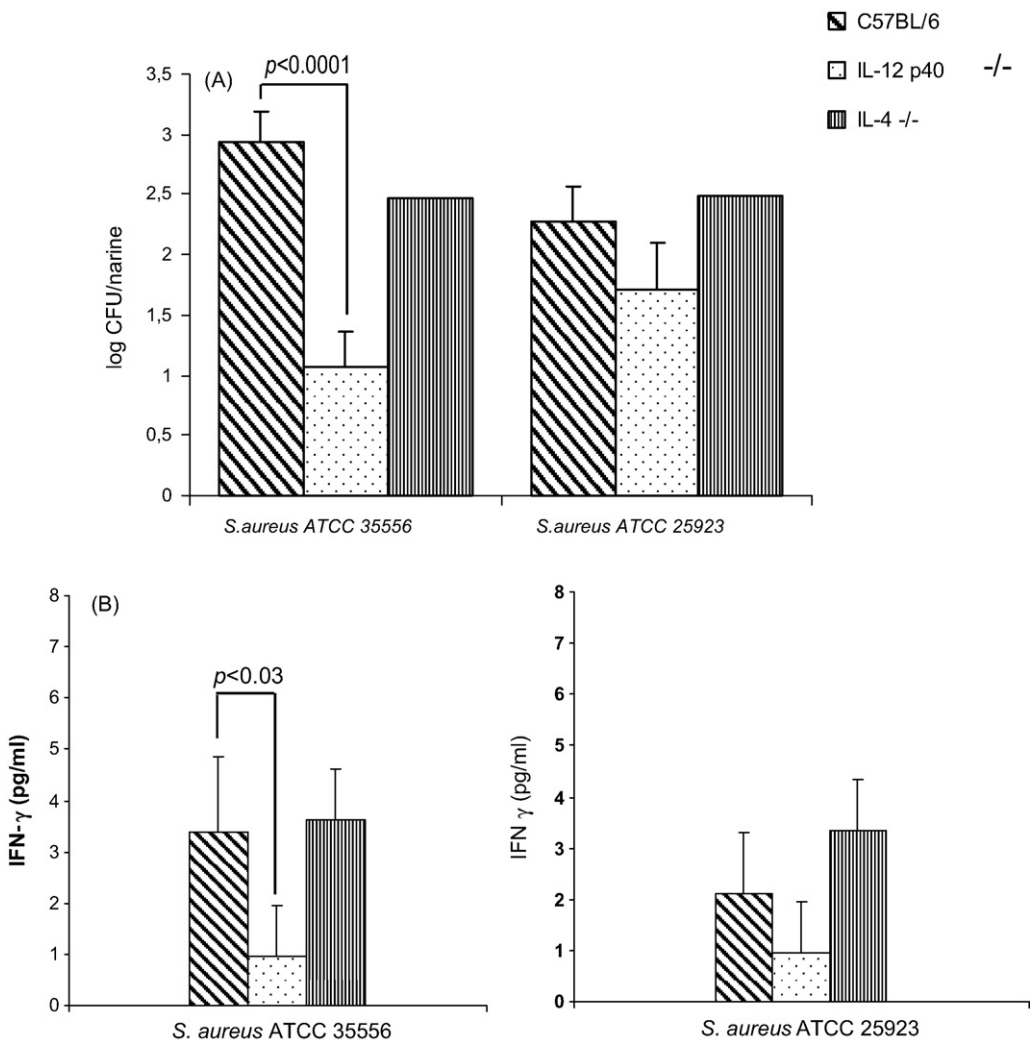

Fig. 2. Bacterial load in narine of *S. aureus* infected C57BL/6 WT, IL-12p40−/− and IL-4−/− mice. (A) The number of bacteria in narine at day 8 after oral infection in the mice. (B) IFN-γ levels in nasal homogenates of infected mice. In (A) and (B), the data are representative from two independent experiments and they are the results of four mice per group.

Acknowledgements

This study was supported by Universidad Nacional de San Luis (Proj. 8802 and 0401) and Fondo para la Investigación Científica y Tecnológica (FONCYT) (Proj PICT 2006 N° 838). M.S. Di Genaro is member career of CONICET, and E. Cargnelutti fellow of FONCYT.

References

[1] Lecomte F, Nouvellon M, Levesque H. Nasal carriage of *Staphylococcus aureus*. N Eng J Med 2001;344:1399–400.
[2] Kevin B, Kiser JM, Cantey-Kiser, Lee JC. Development and characterization of a *Staphylococcus aureus* nasal colonization model in mice. Infect Immun 1999;67:5001–6.
[3] Sanford BA, Thomas VL, Ramsay MA. Binding of staphylococci to mucus *in vivo* and *in vitro*. Infect Immun 1989;57:3735–42.
[4] Cheung AL, Bayer AS, Zhang G, Gresham H, Xiong YQ. Regulation of virulence determinants *in vitro* and *in vivo* in *Staphylococcus*. FEMS Immunol Med Microbiol 2004;40:1–9.
[5] Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167–93.
[6] Götz F. *Staphylococcus* and biofilms. Mol Microbiol 2002;43:1367–78.
[7] Bruno Gonzalez Z, Senna JPM, Fiette L, Shorte S, Testard A, Chignard M, et al. Bacterial and host factors implicated in nasal carriage of methicillin-resistant *Staphylococcus aureus* in mice. Infect Immun 2005;73:1847–51.
[8] Nakane A, Okamoto M, Asano M, Kohanawa M, Minagawa T. Endogenous gamma interferon, tumor necrosis factor, and interleukin-6 in *Staphylococcus aureus* infection in mice. Infect Immun 1995;63:1165–72.
[9] McLoughlin RM, Lee JC, Kasper DL, Tzianabos AO. IFN-γ regulated chemokine production determines the outcome of *Staphylococcus aureus* infection. J Immunol 2008;181:1323–32.
[10] Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase-negative staphylococci. J Clin Pathol 1989;42:872–4.
[11] Arciola CR, Baldassari L, Montanaro L. In catherer infections by *Staphylococcus epidermidis* the intercellular adhesion (*ica*) locus is a molecular marker of the virulent slime-producing strains. J Biomed Mater Res 2002;59:557–62.
[12] Satorres SE, Alcaraz L, Di Genaro MS. Association between high IL-8 levels and *Staphylococcus aureus*-specific IgA antibodies in subjects with type I diabetes mellitus from Argentina. Diab Res Clin Pract 2007;77:489–91.
[13] Autenrieth IB, Beer M, Bohn E, Kauffman SH, Heesemann J. Immune response to *Yersinia enterocolitica* in susceptible BALB/c and resistant C57BL/6 mice: an essential role for gamma interferon. Infect Immun 1994;62:2590–9.
[14] Zhao YX, Nilsson LM, Tarkowski. The dual role of interferon-γ in experimental *Staphylococcus aureus* septicaemia versus arthritis. Immunology 1998;93:80–5.
[15] Boelens JJ, Poll T, Dankert J, Zaat SA. Interferon-γ protects against biomaterial-associated *Staphylococcus epidermidis* infection in mice. J Infect Dis 2000;181:1167–71.
[16] Nasaki S, Nishikawa S, Miuria T, Mizuki M, Yomada K, Madarame H, et al. Interleukin-4 and interleukin-10 are involved in host resistance to *Staphylococcus aureus* infection through regulation of gamma interferon. Infect Immun 2000;68:2424–30.
[17] Hoeve MA, Savage ND, de Boer T, Langenberg DM, de Waal Malefyt R, Ottenhoff TH, et al. Divergent effects of IL-12 and IL-23 on the production of IL-17 by human T cells. Eur J Immunol 2006;36:661–70.
[18] Layh-Schmit G, Colbert RA. The interlukin-23/interlukin-17 axis in spondyloarthritis. Curr Opin Rheumatol 2008;20:392–7.
[19] Aujla SJ, Dubin PJ, Kolls JK. Th17 cells and mucosal host defense. Sem Immunol 2007;19:377–82.

[20] Smits HH, van Beelen AJ, Hessle C, Westland R, de Jong E, Soeteman E, et al. Commensal Gram-negative bacteria prime human dendritic cells for enhance Th1 development. Eur J Immunol 2004;34:1371–80.
[21] Sun K, Salmon SL, Lotz SA, Metzger DW. Interleukin-12 promotes γ interferon-dependent neutrophil recruitment in the lung and improves protection against respiratory *Streptococcus pneumoniae* infection. Infect Immun 2007;75:1196–202.
[22] Kugathasan S, Saubermann LJ, Smith L, Kou D, Itoh J, Binion DG, et al. Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. Gut 2007;56:1696–705.
[23] Ellis TN, Beaman BL. Interferon-γ activation of polymorphonuclear neutrophil function. Immunology 2004;112:2–12.
[24] Lee WL, Downey GP. Neutrophil activation and acute lung injury. Curr Opin Crit Care 2001;7:1–7.