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Short communication

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

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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 nonslime-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 10⁶ 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. *gureus*, 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.

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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

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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 retroorbital 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 nonslime-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. aureusspecific 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-y 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.

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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.

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