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Journal

Journal of Allergy and Clinical Immunology, 144(1)

ISSN

0091-6749

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Publication Date

2019-07-01

DOI

10.1016/j.jaci.2019.02.033

Peer reviewed

HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2020 November 22.

Published in final edited form as:

J Allergy Clin Immunol. 2019 July ; 144(1): 330–333.e6. doi:10.1016/j.jaci.2019.02.033.

Mast cell recruitment is modulated by hairless skin microbiome

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Capsule Summary

Hairless mice have countless mast cells (MCs) in the skin and a more gram positive microbiome (GPM). MC number normalizes when TLR2, a GPM sensor, is deleted, proving a connection between hair-loss, GPM and MCs.

Keywords

Skin microbiome; mast cell hyperplasia; SCF; TLR2; hairless mice; alopecia areata

To the Editor:

MCs are abundant in skin that interfaces with the outside environment. Mast cell (MC) precursors migrate to the skin during embryogenesis and they develop under the influence of tissue specific factors [1]. While, it is well-established MC role in allergy, their relationship with hair is less understood. In fact, MCs are present in lesions of alopecia areata (AA) [2] and patients with AA frequently report itching during the activity phase of the disease. Interestingly, MC hyperplasia is a striking feature of hairless mice discovered by Fujii et al. [3]. However, the mechanisms of this MC hyperplasia in hairless mice were not investigated further. Genetically, hairless mice are characterized by the absence of the *Hr* gene.

The hairless gene (*Hr*) is highly expressed in both human and mouse skin. Degrading *Hr* mRNA results in the transformation from hairy skin into the hairless skin. However, MC hyperplasia observed in the skin of hairless mice is not a distinctive feature of *Hr* mutant mice. In fact, an increased number of MCs in the skin was reported in different strains of hairless mice including Balb/c nude mice, a strain of mice with the absence of thymus but normal *Hr* gene [4]. Moreover, a previous study noted that MC numbers increased during the period when hair regeneration enters late anagen, suggesting that MC numbers may oscillate with environmental changes during the hair growing cycle [5]. One main characteristic of

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

absent hair is thought to be the physical feature of habitats for distinctive microbiome on skin surface. Our group recently demonstrated that microbiome induces MC maturation in the skin, since it induces stem cell factor (SCF) production in keratinocytes, essential for MC survival [6, 7]; therefore, we hypothesized that: changes in the skin microbiome due to hair loss, could be an important factor in increasing the number of MCs present in the skin of hairless mice.

We first generated C57 HR (C57BL/6J hairless) mice that present with MC hyperplasia and hairless skin compared to the hairy skin of C57 wild type (WT) mice. We evaluated the number of MCs in the skin and found a significant increase in MCs, by counting the number of MCs from 5 tissue sections of 4 mice per group. The average number of MCs in C57 WT group was 24±4/mm^2, while in C57 HR mice 83±16/mm^2 (Figure 1A–1C). Interestingly, C57 HR newborn mice develop normal first fur, but pups start losing hair from the 14th day after birth. Therefore, to determine whether the discontinued hair cycle affects MCs, we verified the MC number in hairy skin from 1-week old C57 HR (1WO C57 HR) by avidin staining. As expected, we found the average number of MCs in 1WO C57 HR group was 51±3/mm^2 and that less than the average 85±7/mm^2 MCs in adult skin of C57 HR mice (Figure 1D–1F and Figure E3). When we compared the 1WO C57 WT with the 8WO, they had slightly less MCs. However, 1-week old C57 WT already had a significantly lower number of MCs when compared to the 1WO C57 HR. This difference could be considered the genetic component of HR mice for their number of MCs.

To assess the function of the MCs in the C57 HR mice, we examined mastcell-specific enzyme activities such as chymase and tryptase. Immunofluorescent staining showed that there was an elevated expression of chymase in C57 HR mice that correlated with the accumulation of excessive MCs in their dermis (Figure 1G–1J). A remarkably high *tryptase beta-2 (Tpsb2*) expression was found in the skin of C57 HR mice, but not C57 WT mice (Figure 1K). Likewise, a strikingly high expression of trypsin-like activity was found when we performed fluorometric analysis (Figure 1L). In addition, we evaluated MC function by measuring the diameter increase of the skin edema by compound 48/80. We found the C57 HR mice had a much bigger edema area than C57 WT mice, which indicated increased release of MC mediators (Figure 1M). We also measured MC reactivity using passive cutaneous anaphylaxis. A larger edema area was observed on C57 HR mice, suggesting MCs were activated by IgE (Figure 1N). These results demonstrate that MCs in C57 HR mice skin are functioning normally.

Given that our lab previously demonstrated that MC maturation and migration can be promoted by skin commensal gram-positive bacteria, through the TLR2-SCF pathway in keratinocytes [6, 7], we investigated the composition of the skin microbiome in C57 HR and C57 WT mice. The skin microbiome composition was assessed by16S rRNA sequencing performed on fourteen samples including eight C57 WT, four C57 HR and two 1-week old C57 HR. Principal coordinate analysis (PCoA) and Bray-Curtis algorithm analysis showed significant segregation based on the presence of hair, that is, haired mice versus hairless mice (Figure E1A). By comparing the representative sequence of OTU (operational taxonomic unit) with the microbial reference database, we found that hairless mouse skin contained a lower amount of bacteria, but that the bacteria composition exhibited a

significantly higher ratio of gram-positive bacteria, mainly *Staphylococcus* genus, than in C57 WT mice and haired 1-week old (1WO) C57 HR pups (Figure E1B–C).

To further investigate the correlation between gram-positive skin microbiome and MC skin migration, we examined the level of LTA (lipoteichoic acid, a major constituent of the cell wall of gram-positive bacteria) and SCF. Consistent with the microbiome sequencing data, hairless mice have more LTA in the epidermis (Figure E1D–E), and a higher expression of SCF in the epidermis than C57 WT mice (Figure E1F–G). We also confirmed the increased SCF expression in hairless mice skin by ELISA and RT-qPCR (Figure E1H–I). Since, it was previously reported that, in healthy human skin, perifollicular MCs have a strikingly immunoinhibitory phenotype [2]. Therefore, one important question to ask was whether and how alterations in MC-microbiome interactions impact on the previously reported switch of skin MCs from an immunoinhibitory to a pro-inflammatory phenotype. From our in vitro study on mouse MCs in contact with *S. Epidermidis* (Figure E2), we could observe that *S Epidermidis* does not affect IL-10 or TNF-α level. However, preconditioning MCs with mouse dermal fibroblasts increases TNF-α more than IL-10 release. Our future studies will focus on effects of *S. Epidermidis* on human MCs.

To confirm the importance of the gram-positive bacteria-LTA-TLR2-SCF signaling pathway on MC skin recruitment, we generated *Tlr2*$^{-/-}$ hairless mice (TLR2$^{-/-}$ HR). We found a significant decrease in the number of MCs in the skin of TLR2$^{-/-}$ HR mice. These results prove that MCs can be recruited by skin commensal gram-positive bacteria through the TLR2-SCF pathway. The number of MCs in the skin of TLR2$^{-/-}$ HR mice was still above the number in C57 WT mice. Interestingly the number of the MCs in adult TLR2$^{-/-}$ HR mice is equivalent to the number of MCs in the skin of 1WO HR mice, confirming that the slightly higher number of MCs present at birth in the HR mice cannot further increase in the absence of TLR2 and is due to their genetic (Figure E1J–L), while the big increase after birth is due to the change in microbiome, especially gram positive. TLR2$^{-/-}$ HR mice skin express less SCF production (Figure E1K and Figure E4) further suggesting the impact of this MC-microbiome interaction through keratinocytes.

Since the perifollicular mesenchyme in both murine and human skin itself harbors resident MC progenitors from which mature MCs can rapidly differentiate in loco and under ex vivo conditions, i.e. in the absence of bone marrow and blood perfusion [8], we evaluated Ki-67 expression in skin MCs of C57WT and C57HR mice. We found that C57HR express more Ki-67 in their skin and show higher proliferation activity of skin cells in general and of mature MCs specifically (Figure 1O–1Q).

We demonstrated that MC activity and numbers greatly increase in hairless mice skin, along with a different skin bacteria population. In addition, we verified that the number of MCs increases during the transition to hair loss. We built on our previously published data and showed that a higher ratio of gram-positive bacteria in the skin of hairless mice stimulates keratinocytes to produce more SCF, which leads to an increase of MCs in the skin. We further demonstrated that MC recruitment can be achieved by controlling TLR2 signaling.

Our present study focuses specifically on the skin surface microbiome and on its larger impact on skin mast cells. However, for a future comprehensive understanding of skin MCs development and function in hairless environment, it would be beneficial to center our investigations on the epithelial utriculi of hairless mouse skin and compare them with WT mouse follicle microbiome. We can speculate that, as skin surface microbiome changes when hairs are absent, epithelial utriculi of HR mice will change as well, and they may harbor their own specific microbial communities.

How MCs are recruited at the skin site is still a matter of debate[1]. Although we cannot address how MCs are recruited during embryogenesis in the present study, we proved that C57 HR adult mice maintain their MC population by local mitosis of mature MCs as a response to the microbiome induced SCF release. Perhaps, further research will focus on understanding if MCs that are present at birth in the C57 HR mice are still of yolk sac derivation and less subject to microbiome stimulation but only controlled by genetic variation, like the TLR2-HR double mutant experiment suggests. In conclusion, this study confirms that the microbiome composition influences MC recruitment and SCF expression in the skin. Moreover, that the microbiome, and its induced cytokines are strongly influenced by the presence of hair.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Founding Sources: Supported by the National Institutes of Health (NIH) / National Institute of Allergy and Infectious Diseases through NIH grant 5R01AI106874 to Anna Di Nardo

Disclosure of potential conflict of interest: Chia-Chi Wu, Zhenping Wang and Anna DiNardo received grant funding from the National Institutes of Health (NIH).

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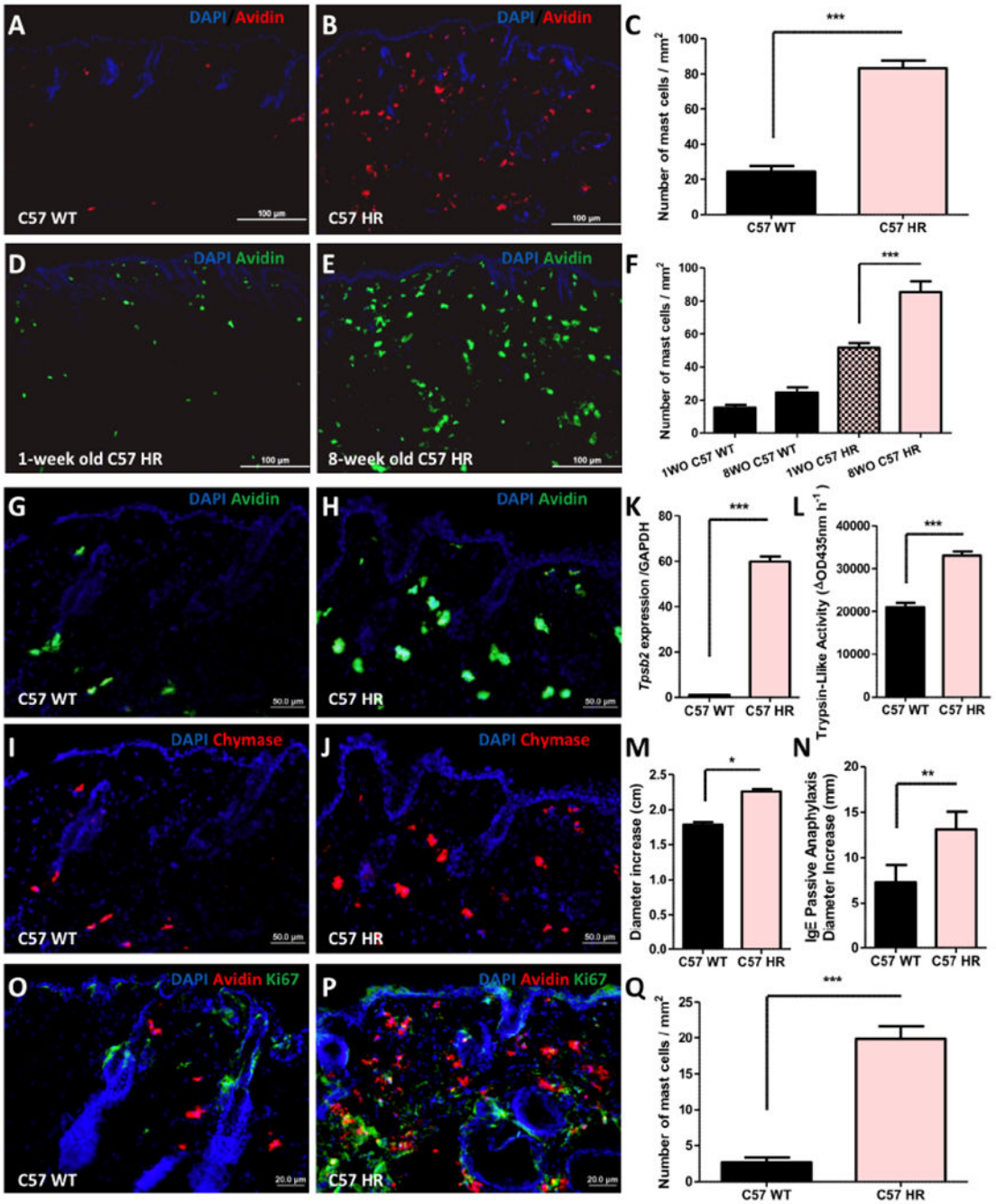

Figure 1.

An increased number of MCs are present in the skin of C57 HR mice. **(A-B)** MCs were identified in C57 WT (A) and C57 HR (B) mouse skin by staining with rhodamine-avidin (red). **(C)** Numbers of MCs were counted in the skin of C57 WT, C57 HR, and haired newborn C57 HR. **(D-E)** MCs stained with FITC-avidin (green) in 1-week old C57 HR (D) and 8-week old C57 HR (E) mouse skin. **(F)** Numbers of MCs were counted in the skin of 1-week and 8-week old C57 HR (see supplemental material for tissue images) **(G-J)** MC specific avidin staining (green) was co-localized with chymase staining (red) in C57 WT (G,

I) and C57 HR (H, J) mice skin. **(K)** *Tpsb2* (Tryptase beta-2) mRNA expression in mouse skin measured by qPCR. **(L)** Trypsin-like activity in mouse skin analyzed by fluorometry. **(M)** Diameter of swollen skin area between 30 and 60 minutes after compound 48/80 injection was measured. **(N)** Passive cutaneous anaphylaxis determined by measuring the diameter of swollen skin area between 0 and 30 minutes. **(O-Q)** MC specific avidin staining (red) was co-localized with Ki-67 staining (green) in C57 WT (O) and C57 HR (P) mice skin. (Q) Numbers of Ki-67 co-localizing MCs were counted in the skin of C57 WT and C57 HR. *P<0.05, **P<0.01, and ***P<0.001.