USE OF GRAM NEGATIVE SPECIES TO TREAT ATOPIC DERMATITIS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Application No. 16/042,939, filed July 23, 2018, which is hereby incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0002] This disclosure relates to the field of dermatology, specifically to the use of the topical application of viable Gram negative bacterial to treat atopic dermatitis.

BACKGROUND

[0003] The term "eczema," often used to describe atopic dermatitis, was coined in ancient Greece, and roughly translates as "to boil out." Modern science, however, recognizes the contribution of both host and environmental factors to this disease. Hallmarks of the disease include reduced barrier function, reduced innate immune activation, and susceptibility to infections with *Staphylococcus aureus*. Predisposing host factors are suggested by monogenic mutations in STAT3, filaggrin, and other genes associated with AD-like phenotypes (Lyons *et al.*, *Immunology and allergy clinics of North America* **35**, 161-183 (2015); published online Epub Feb (10.1016/j.iac.2014.09.008)). Host genetic influences can be therapeutically modulated through topical steroids or calcineurin inhibitors (Boguniewicz and Leung*, J Allergy Clin Immunol* **132**, 511-512 e515 (2013); published online (Epub) Aug (10.1016/j.jaci.2013.06.030)). *S. aureus* contributes to AD pathogenesis, and can be mitigated by antibiotics (Boguniewicz and Leung, *supra;* Kobayashi *et al.*, *Immunity* **42**, 756-766). Recent work has revealed that the skin microbiome is significantly different between healthy controls and patients with AD and that symptoms are associated with a loss of commensal diversity (Kong *et al.*, *Genome research* **22**, 850-859 (2012); published online (Epub) May (10.1101/gr.131029.111)). A need remains for methods for therapeutically targeting this dysbiosis and treating atopic dermatitis.

SUMMARY OF THE DISCLOSURE

[0004] It is disclosed herein that culturable Gram negative bacteria (CGN) from the skin of healthy subjects were associated with activation of innate immunity, enhanced barrier function, and control of S. aureus. These Gram negative bacteria are of use for treating atopic dermatitis in subjects with this condition.

[0004a] In one aspect, provided herein is a pharmaceutical composition, comprising: at least one strain of *Roseomonas mucosa*, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3; and a

pharmaceutically acceptable carrier.

[0004b] In one aspect, provided herein is a pharmaceutical composition, comprising: at least one strain of *Roseomonas mucosa*, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12; and a pharmaceutically acceptable carrier.

[0005] In some embodiments, pharmaceutical compositions are disclosed that include a therapeutically effective amount of a purified viable Gram negative bacteria and a pharmaceutically acceptable carrier, wherein a) a lysate and/or component of the Gram negative bacteria inhibits growth of *S. aureus* in an *in vitro* assay; b) the Gram negative bacteria stimulates human keratinocytes; c) the Gram negative bacteria induces cytokine expression from human cells; and d) the Gram negative bacteria is non-pathogenic when administered to the skin of the subject. The pharmaceutical compositions are formulated for topical administration.

[0006] In some embodiments, a pharmaceutical composition is provided herein comprising a therapeutically effective amount of a purified viable Gram negative bacteria and a pharmaceutically acceptable carrier, wherein the purified viable Gram negative bacteria comprises at least one strain of *Roseomonas mucosa*, and wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of (i) SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, or (ii) SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 2. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 3. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-A, RM-B, or RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-A. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-B. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-A, RM-B, or RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-A. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-B. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* consists of isolates RM-A, RM-B, and RM-C. In further embodiments, the

at least one strain of *Roseomonas mucosa* is present in a total amount of 10^4 to 10^{12} colony forming units. In further embodiments, the pharmaceutical composition is in a topical dosage form. In further embodiments, the topical dosage form is a cream, gel, foam, ointment, or liquid. In some embodiments, a bandage is provided comprising the pharmaceutical composition of any one of the foregoing embodiments. In some embodiments, a spray bottle is provided comprising the pharmaceutical composition of any one of the foregoing embodiments. In some embodiments, a kit for treating atopic dermatitis is provided, comprising: a container comprising the pharmaceutical composition of the foregoing embodiments; a container comprising a pharmaceutically acceptable carrier; and instructions for applying the pharmaceutical composition with the pharmaceutically acceptable carrier topically to the skin.

[0007] Methods of treating a topical dermatitis using these pharmaceutical compositions are also disclosed. In some embodiments, a method for treatment of atopic dermatitis is provided, comprising: topically administering to a subject in need thereof a purified viable Gram negative bacteria and a pharmaceutically acceptable carrier, wherein the purified viable Gram negative bacteria comprises at least one strain of *Roseomonas mucosa*, and wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of (i) SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 or (ii) SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, and wherein the at least one strain of *Roseomonas mucosa* is present in an amount sufficient for treatment of atopic dermatitis in the subject. In further embodiments, the at least one strain of *Roseomonas mucosa* is topically administered by spraying. In further embodiments, the at least one strain of *Roseomonas mucosa* is topically administered to the subject at least two times per a week. In further embodiments, the at least one strain of *Roseomonas mucosa* is topically administered to the subject every other day over a week. In further embodiments, the at least one strain of *Roseomonas mucosa* is topically administered to the subject daily over a week. In further embodiments, the subject is an adult. In further embodiments, the subject is an infant. In further embodiments, the subject is a child. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 2. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 3. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-A, RM-B, or RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa*

comprises isolate RM-A. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-B. In further embodiments, the at least one strain of *Roseomonas mucosa* comprises isolate RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-A, RM-B, or RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-A. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-B. In further embodiments, the at least one strain of *Roseomonas mucosa* is isolate RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* consists of isolates RM-A, RM-B, and RM-C. In further embodiments, the at least one strain of *Roseomonas mucosa* is present in a total amount of 10^4 to 10^{12} colony forming units. In further embodiments, the pharmaceutical composition is in a topical dosage form. In further embodiments, the topical dosage form is a cream, gel, foam, ointment, or liquid.

[0007a] In one aspect, provided herein is a use of at least one strain of *Roseomonas mucosa* in the manufacture of a medicament for the treatment of atopic dermatitis, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to a subject in need thereof, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, and wherein the at least one strain of *Roseomonas mucosa* is present in an amount sufficient for treatment of atopic dermatitis in the subject.

[0007b] In one aspect, provided herein is a use of at least one strain of *Roseomonas mucosa* in the manufacture of a medicament for the treatment of atopic dermatitis, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to a subject in need thereof, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, and wherein the at least one strain of *Roseomonas mucosa* is present in an amount sufficient for treatment of atopic dermatitis in the subject.

[0007c] In one aspect, provided herein is a bandage comprising the pharmaceutical composition as described herein.

[0007d] In one aspect, provided herein is a spray bottle comprising the pharmaceutical composition as described herein.

[0007e] In one aspect, provided herein is a kit for treating atopic dermatitis, comprising: (a) a container comprising the pharmaceutical composition as described herein; (b) a container comprising a pharmaceutically acceptable carrier; and (c) instructions for applying the pharmaceutical composition with the pharmaceutically acceptable carrier topically to the skin.

[0007f] In one aspect, provided herein is at least one strain of *Roseomonas mucosa* for use in therapy, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, or wherein the at least one strain

of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12.

[0008] The foregoing and other features and advantages of the invention will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

[0009] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0010] FIGs. 1A-1D: CGN isolates differ in presence and *S. aureus* inhibition between healthy volunteers and patients with AD. (A) Percentage of individuals with CGN isolate yield from HV (n=26) and AD (n=17) subjects. Individuals with multiple CGN isolates were counted per isolate for >100% total in HV; see Table 1 for details. (B) Eight strains of *S. aureus* isolated from HV and AD patients were grown in the presence of either CGN supernatant or control media. Each data point represents the effect on *S. aureus* growth of supernatant from one CGN isolate compared to media control (HV isolates = 9, AD isolates = 7); shapes represent sourcing of *S. aureus* as either the participant's autologous *S. aureus*, or one *S. aureus* isolate from HV or AD patients. Data points with a * symbol represent one HV- and one AD-derived CGN isolate selected for subsequent human challenge and mouse model experiments. Data is alternatively presented by CGN species, see FIG. 4. (C) Healthy mouse ears were co-inoculated with *S. aureus* (strain SAAS9) and *R. mucosa* (*Rm*) from HV or AD patients, or *P. aeruginosa* from a HV for 10 days. Day 12 ears were homogenized and plated by serial dilution for CFU. Percent change in growth versus diluent (no CGN added) control is shown. (D) CGN CFU yield taken from same mice as panel C. Data shown are a combination of three or more independent experiments (B) or representative of two independent experiments (C-D) and displayed as mean + sem. SA = *S. aureus*, HV = Healthy Volunteer, AD = Atopic Dermatitis, CGN = Culturable Gram negative, Rm = *Roseomonas mucosa*, Pa = *Pseudomonas aeruginosa*. Significance determined by Student's t test (B) or ANOVA with Bonferroni's correction (C-D).

[0011] FIGs. 2A-2D: CGN from HV enhances innate immunity and barrier function. (A) Cytokine analysis for in vivo human blister challenge on healthy controls showing cytokine responses to a representative strain of *R. mucosa* from a healthy control versus one isolated from a patient with AD as compared to the saline control well (dotted line), N= 7. (B) Paired analysis of data presented in panel A showing the cytokine production in the blister well exposed to the

CLAIMS

We claim:

1. A pharmaceutical composition, comprising:
   at least one strain of *Roseomonas mucosa*, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3; and
   a pharmaceutically acceptable carrier.
2. The pharmaceutical composition of claim 1, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 1.
3. The pharmaceutical composition of claim 1, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 2.
4. The pharmaceutical composition of claim 1, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 3.
5. The pharmaceutical composition of claim 1, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.
6. The pharmaceutical composition of any one of claims 1 to 5, wherein the at least one strain of *Roseomonas mucosa* is present in a total amount of 10^4 to 10^{12} colony forming units.
7. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition is in a topical dosage form.
8. The pharmaceutical composition of claim 7, wherein the topical dosage form is a cream, gel, foam, ointment, lotion, or liquid.
9. A bandage comprising the pharmaceutical composition of any one of claims 1-8.
10. A spray bottle comprising the pharmaceutical composition of any one of claims 1-8.
11. Use of at least one strain of *Roseomonas mucosa* in the manufacture of a medicament for the treatment of atopic dermatitis, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to a subject in need thereof, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID

NO: 2, or SEQ ID NO: 3, and wherein the at least one strain of *Roseomonas mucosa* is present in an amount sufficient for treatment of atopic dermatitis in the subject.

12. The use of claim 11, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered by spraying.

13. The use of claim 11, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to the subject at least two times per a week.

14. The use of claim 11, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to the subject daily over a week.

15. The use of claim 11, wherein the subject is an adult, child, or infant.

16. The use of claim 11, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

17. The use of claim 11, wherein the at least one strain of *Roseomonas mucosa* is present in a total amount of 10^4 to 10^{12} colony forming units.

18. A kit for treating atopic dermatitis, comprising:
    a) a container comprising the pharmaceutical composition of any one of claims 1 to 8;
    b) a container comprising a pharmaceutically acceptable carrier; and
    c) instructions for applying the pharmaceutical composition with the pharmaceutically acceptable carrier topically to the skin.

19. A pharmaceutical composition, comprising:
    at least one strain of *Roseomonas mucosa*, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12; and
    a pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 19, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 10.

21. The pharmaceutical composition of claim 19, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 11.

22. The pharmaceutical composition of claim 19, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 12.

23. The pharmaceutical composition of claim 19, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

24. The pharmaceutical composition of any one of claims 19 to 23, wherein the at least one strain of *Roseomonas mucosa* is present in a total amount of 10^4 to 10^{12} colony forming units.

25. The pharmaceutical composition of any one of claims 19 to 24, wherein the pharmaceutical composition is in a topical dosage form.

26. The pharmaceutical composition of claim 25, wherein the topical dosage form is a cream, gel, foam, ointment, lotion, or liquid.

27. Use of at least one strain of *Roseomonas mucosa* in the manufacture of a medicament for the treatment of atopic dermatitis, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to a subject in need thereof, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, and wherein the at least one strain of *Roseomonas mucosa* is present in an amount sufficient for treatment of atopic dermatitis in the subject.

28. The use of claim 27, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered by spraying.

29. The use of claim 27, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to the subject at least two times per a week.

30. The use of claim 27, wherein the at least one strain of *Roseomonas mucosa* is to be topically administered to the subject daily over a week.

31. The use of claim 27, wherein the subject is an adult, child, or infant.

32. The use of claim 27, wherein the at least one strain of *Roseomonas mucosa* comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

33. The use of claim 27, wherein the at least one strain of *Roseomonas mucosa* is present in a total amount of 10^4 to 10^{12} colony forming units.

34. A bandage comprising the pharmaceutical composition of any one of claims 19 to 26.

35. A spray bottle comprising the pharmaceutical composition of any one of claims 19 to 26.

36. A kit for treating atopic dermatitis, comprising:

a) a container comprising the pharmaceutical composition of any one of claims 19 to 26;

b) a container comprising a pharmaceutically acceptable carrier; and

c) instructions for applying pharmaceutical composition with the pharmaceutically acceptable carrier topically to the skin.

37. At least one strain of *Roseomonas mucosa* for use in therapy, wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, or wherein the at least one strain of *Roseomonas mucosa* comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12.