

1 **Lipoteichoic Acid and molecular weight of hyaluronic acid could explain the**
2 **late inflammatory response trigger by Hyaluronic acid fillers.**

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17 **ABSTRACT**

18 Introduction: Hyaluronic acid is a safe dermal filler, but sometimes late granuloma are generated.
19 This adverse effect is an inflammatory process, and its causes is not clear. Late granuloma
20 generation could be due to reaction to residual components of the bacterial wall present into
21 hyaluronic acid, such as lipoteichoic acid (LTA). Other possibility is hyaluronic acid degraded
22 could be trigger this inflammatory reaction.

23 Objective: Study possible molecular mechanism that could be implicated into the late granuloma
24 formation. We wonder whereas inflammatory response activation is triggered by lower molecular
25 weight hyaluronic acid or gram-positive bacterial components as LTA.

26 Methods: We analyzed one adverse case generated by hyaluronic acid injections. Our study
27 with one nodule through chemical and immunofluorescence histologic technics.

28 Results: In this case observe a late granuloma without infectious process. Histological analysis
29 shown few large Langerhans cells around fillers and multiple immunological cells infiltrated.
30 Immunofluorescent study shown immunological cells (CD45 positives cells) with high TLR2
31 expression (hyaluronic acid and LTA receptor).

32 Limitations: The difficulty of obtaining biopsy samples of nodules implies that the number of
33 cases analyzed is very low.

34 Conclusion: New model is proposed in which weight of hyaluronic acid and LTA could be able
35 to trigger inflammation. This process could be mediated by TLR2 expressed in infiltrated immune
36 cells.

37 INTRODUCTION

38 Hyaluronic acid (HA) is a common dermal filler used in multiple aesthetic procedures. Although,
39 HA is a safe filler, sometime produce adverse effects as acute inflammation or foreign body
40 reaction. Acute inflammation is a normal process after injection, and its resolve inside of 2 or 4
41 weeks. Foreign body reaction is a complex inflammation event and could form granuloma (2).

42 However, the formation of theses granulomas is not clear, and it is necessary understand the
43 molecular properties of HA and how interacts with immunological system to prevent granulomas
44 or other inflammation effects.

45 Commercial hyaluronic acid is manufactured with strains of Gram-positive bacteria. Theses
46 bacteria produce HA for its cellular wall. This manufactured method could remain in the final
47 product some immunogenic particles and promote an immunological response (3).

48 Gram-positive bacteria have in its wall immunogenic molecules as Teichoic acid (TA) or
49 Lipoteichoic acid (LTA). These molecules promote inflammatory response, in which response is
50 implicated Toll Like Receptor (TLR) (4-5).

51 TLR are a family of receptors implicated in innate immunological response. For example, TLR2
52 is upregulated in myeloid lineage cells by gram positive bacteria. This receptor binds LTA and
53 hyaluronic acid (6-7).

54 MATERIAL AND METHODS

55 We had studied the case of 43-year-old female, which present spherical nodules in her neck
56 (Fig.1A). This patient was treated with 1 ml of HA distributed in three sessions; HA was injected
57 intradermal into neck region. Just after injections, the patient did not show inflammation. Six
58 months after the last intradermal injections season, the patient shown several nodules into the
59 neck. She did not feel pain or high temperature in this area.

60 Nodules were dissected with a 4mm punch and analyzed histologically through
61 hematoxylin/eosin and immunofluorescent staining. Immunostaining was performed with rabbit
62 anti-toll like receptor 2 (1:500; cell signaling), rat anti-CD45 (1:500, Serotec) Secondary
63 antibodies were conjugated to Alexa-594 and Alexa-488 (1:500; Invitrogen). Sections were
64 counterstained with DAPI (1 µg/ml, Vector Laboratories). Negative controls were only stained
65 with secondary antibodies.

66 **RESULTS**

67 Cutaneous punch shown a deep granulomatous inflammatory reaction. this is formed by
68 granulomatous aggregates of epithelioid histiocytes with abundant multinucleated giant cells.
69 They are situated surrounding deposits of HA. Among the granuloma, there is a discrete
70 lymphocyte component. Polymorphonuclear neutrophils have not been identified in significant
71 numbers neither suppurative events that could be suggest an infectious process (fig.1B).

72 Infiltrated lymphocytes were analyzed by CD45 staining; this molecule is localized in the surface
73 throughout lymphoid linage. The biopsy showed a multiple lymphocyte infiltrated into the affected
74 are, this confirms the observed results with hematoxylin and eosin staining (fig.2). We show as
75 TLR2 receptor is presented in CD45 positive cells (fig.2), but not in the others cell.

76 **DISCUSSION**

77 Late granuloma is a common adverse effect in aesthetics procedures with HA (2). This
78 inflammatory response appears from one month until one year after HA infiltration and is
79 characterized by nodules into infiltrated area. The most studies describe this process as foreign
80 body reaction, in which lymphocytes and macrophages react against filler.

81 The inflammatory process could be generated by some bacterial components presents in HA.
82 LTA is present in outer membrane of gram-positive bacteria, which are used by industry to make

83 different fillers with hyaluronic acids (3,4). LTA triggers inflammatory response via TLR2
84 receptors (5,8). We have observed high levels of TLR2 in CD45 positive cells near of HA in our
85 case. The possibilities that LTA presents in bacterial wall while manufacturing HA filler could be
86 implicated in the late inflammation.

87 On the other hand, this scene could be more complex, because the injection of hyaluronic acid
88 is not enough to promote an inflammatory response. For this reason, it is possible that HA
89 modulate the immunological response. While, low molecular weight (LMWHA) triggers
90 inflammatory processes, high molecular weight (HMWHA) inhibits them.

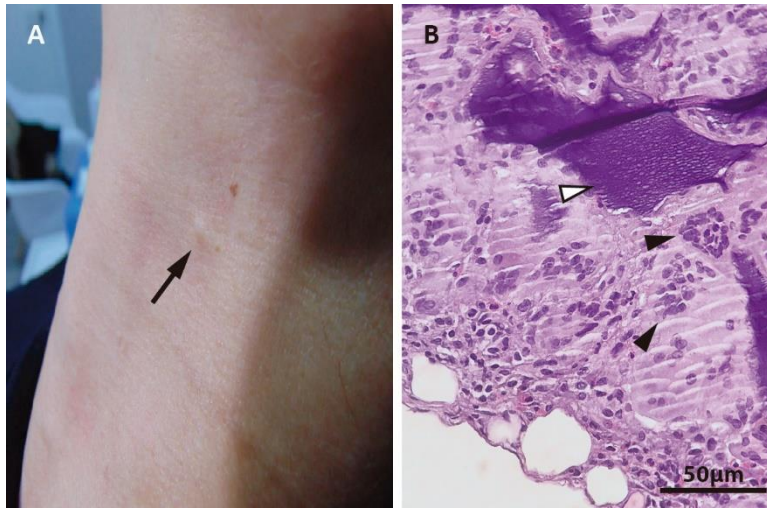
91 **CONCLUSION**

92 We propose a molecular mechanism in which HMWHA avoid early inflammatory process;
93 HMWHA is degraded by human hyaluronidases and LMWHA is generated; this HA with low
94 molecular weight together LTA or other bacterial antigen trigger inflammatory response that end
95 with late granulomas formation (6-10). TLR2 could be the receptor implicated in this mechanism
96 to trigger inflammatory response (Fig. 3).

97 In the future is necessary to study LTA or other bacterial components of the wall into HA fillers,
98 and what is the different mechanisms implicated in HA degradation. More biopsies and
99 molecular analyzes are necessary, but it is difficult to obtain theses material, because extract
100 this tissue implicate to do a little scar.

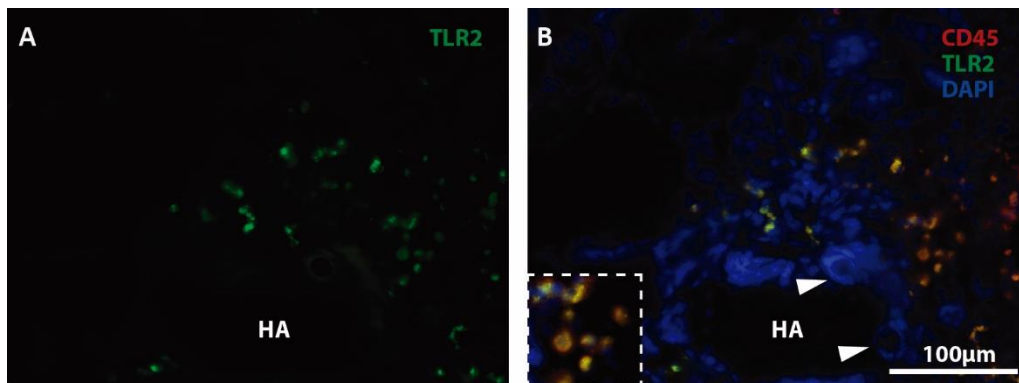
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126 **FIGURES**

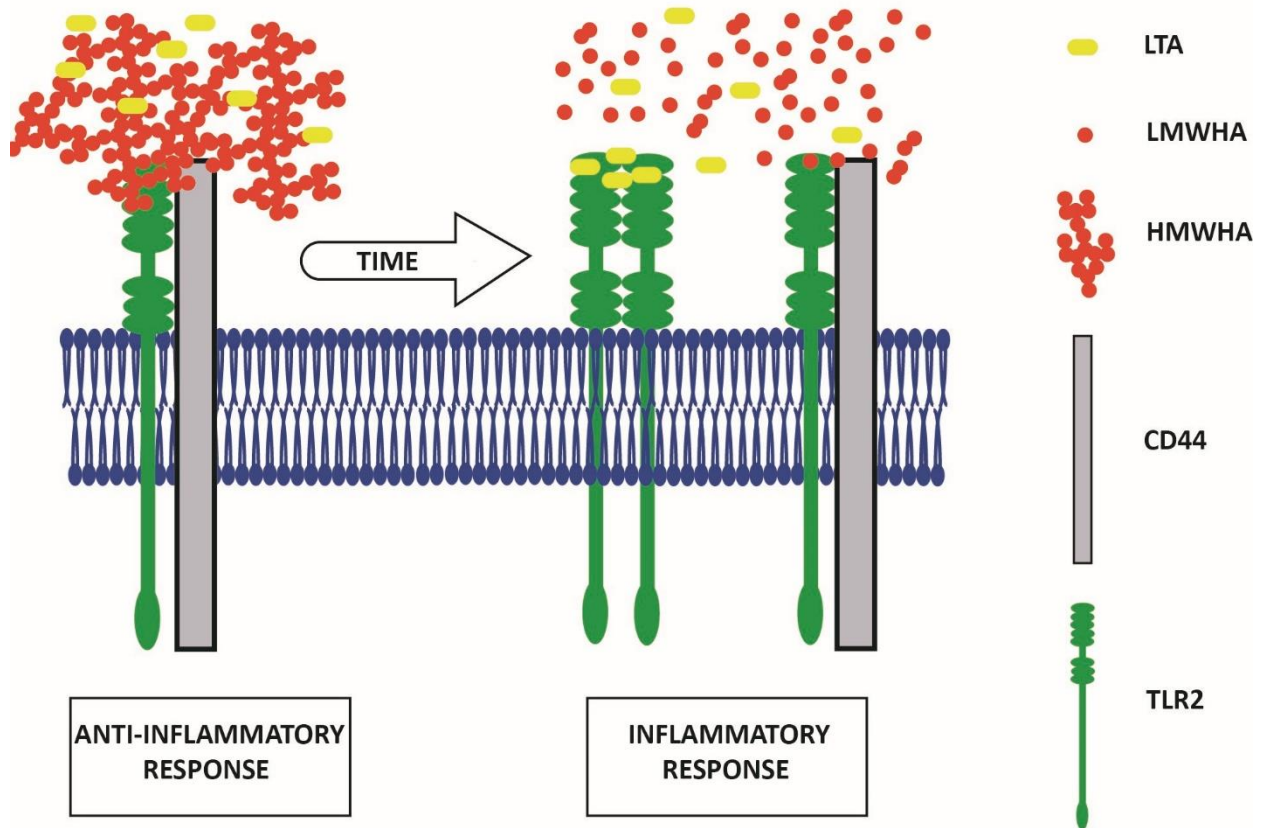
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128 **Figure 1. Late granuloma generated by hyaluronic acid injection.** A, visible nodules on the
 129 woman's neck (arrow). B, Biopsy stain with Hematoxylin-Eosin show hyaluronic acid filler (white
 130 head of arrow) into tissue surrounded by granulomatous aggregates of epithelioid histiocytes
 131 with abundant multinucleated giant cells (black head of arrow)



132

133 **Figure 2. Late granuloma generated by hyaluronic acid shows immune system cells with**
 134 **high levels of TLR2 protein.** A, Immunofluorescence staining shows high levels of TLR2
 135 (green) around HA filler. B, All TLR2 positive cells (green) are immune system cells mark with
 136 CD45 positives (red). Nuclear counterstaining with Dapi in blue. HA, Hyaluronic Acid. White head
 137 of arrow indicate multinucleated giant cells.



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139 Figure 3. **Possible molecular model explains late granuloma generated by hyaluronic acid**
 140 **injection.** When Hyaluronic acid is injected, high weigh molecular hyaluronic acid (HWMHA)
 141 prevents in first step to inflammatory response. Hyaluronidases degrade HWMHA in low weight
 142 molecular hyaluronic acid (LWMHA) and promote inflammatory response via TLR2 and
 143 lipoteichoic acid or TLR2:CD44 and LWMHA.

144 **Ethical Statement for Solid State Ionics**

145 Hereby, I Francisco Nieto-Lopez consciously assure that for the manuscript Lipoteichoic Acid
146 and molecular weight of hyaluronic acid could explain the late inflammatory response trigger by
147 Hyaluronic acid fillers. the following is fulfilled:

- 148 1) This material is the authors' own original work, which has not been previously published
149 elsewhere.
- 150 2) The paper is not currently being considered for publication elsewhere.
- 151 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 152 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 153 5) The results are appropriately placed in the context of prior and existing research.
- 154 6) All sources used are properly disclosed (correct citation). Literally copying of text must be
155 indicated as such by using quotation marks and giving proper reference.
- 156 7) All authors have been personally and actively involved in substantial work leading to the paper
157 and will take public responsibility for its content.

158 The violation of the Ethical Statement rules may result in severe consequences.

159 I agree with the above statements and declare that this submission follows the policies of Solid-
160 State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

161

162 Date: 11th July 2022

163 Corresponding author's signature:

A handwritten signature in blue ink, appearing to be 'F. Nieto-Lopez', written over a light blue circular stamp.