Immunology Behind The Filler Which Causa Granuloma

by Ago Harlim

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Ago Harlim

Universitas Kristen indonesia

Introduction: All injected filler may cause foreign body granulomas in some patient. Fillers as an antigen and our immune system play a rule of granuloma formation. Larger microspheres fillers with irregular surface can not be phagocytized will encapsulated with fibrous tissue

Method: we measures the immunology of the patient with silicone injection. We measured inflammation cytokines such as TNF α, IFN γ, and Anti inflammation or immune tolerance marker such as IL-10, IDO, CD4CD25. We took the blood from the patient and chin granuloma as tissue from the injection site, also submental skin as skin surround the injection site.

Result: In this study showed the cytokines which produced by macrophages and indoleamine 2,3-dioxygenase (IDO) as immune tolerance play the rule of granuloma formation.

Conclusion : Immune tolerance system played the rule of granuloma formation due to silicone injection.

Key word: *macrophages, indoleamine 2,3-dioxygenase (IDO)*

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Ago Harlim

Universitas Kristen Indonesaia

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BACKGROUND

Silicone injection for cosmetics and surgery is still widely practiced in Indonesia. The result of Indonesian Association of Plastic Surgeons survey done during 2004 -2007, found 249 cases of silicone complications.[1] Epidemiological data in other countries were not clear because silicone injection had been banned. In 1990, more than 100,000 patients in United States had received silicone injection in their face.[3] In Indonesia, there had been no research on silicone injection and its complications, although the cases were abundant. The use of silicone injection for cosmetic treatment had been banned by Federal Food, Drug & Cosmetic America (FDA) since 1992.[3] Liquid silicone which was injected into the skin can migrate and cause morphological changes and uncontrolled inflammatory response. Liquid silicone in the tissue is persistent, so it will lead to chronic inflammation and granulomas formation. In severe cases, it could be followed by infection, necrosis, and abscess.[2-4] Silicone granuloma was difficult to evacuate and is still able to form new granuloma after the evacuation. Immune response of granuloma is mediated by T cells, Th1 secreted proinflammatory cytokines and Th2 secreted anti inflammatory cytokines.[18] The pathogenesis of granuloma formation due to silicone injection might be explained by the new theory of immune tolerance whichis played by Treg cells ($CD4^+CD25^+$) and Indoleamine-2,3-dioxygenase enzyme.[21-24] Until now, the pathogenesis of silicone granuloma has been studied, but the results are still controversial. The aim of this study is to analyze the pathogenesis of silicone granuloma in connection with immune inflammatory responses and tolerance.

METHOD OF STUDY

This research is descriptive analytic study, which included: (1). Cross-sectional study, to compare immune response in three groups, namely the chin granuloma tissue, submental skin and skin tissue from healthy individuals (control), and to assess the clinical correlation,

histopathological, and immune responses. Samples were 31 cases of silicone granulomas tissue and submental skin, and 37 normal skin tissues. All tissues were examined histopathologically (HE staining) to see the degree of foreign body reaction (FBR) and immunohistochemistry to assess the expression of TNF-α, IFN-γ, IL-10, IDO, and Treg cells $CD4^+CD25^+$; (2) Laboratory experimental performed to assess blood cytokine levels with: (a) Culturing whole blood cells from granuloma patient and normal individuals, using RPMI medium, RPMI stimulated by PHA, and stimulated by 3% of silicone industry. (b) Examined cytokine levels from cell culture supernataned on day 3, included TNF-α, IFN-γ, and IL-10. Allthe cytokines were analyzed with Luminex and IDO with ELISA. The research was conducted in specialist skin clinics, Faculty of Mathematics and Sciences University of Indonesia, Faculty of Medicine University of Indonesia, Faculty of Medicine Airlangga University, and Eijkman Institute, started in November, 2012 and continued until September, 2014

RESULT AND DISCUSSION

Generally, patients with silicone injections on their chin were injected in the salon. They came for treatment approximately 12.5 years after injection, shape of chin changed in the 4^{th} years, and the color of the skin changed in the 5^{th} years. Nose and chin were the main area of silicone injections, 54.8% of patients with silicone injection complications do not know that the injected substance was liquid silicone. Interestingly, silicone was also present in normal skin with statistical mean 44,07μg/g, while the silicone level in the submental skin (944 μg/g) was significantly higher than the silicone level in the granuloma (688 μg/g).

There was no statistical difference in the levels of TNF-α, IFN-γ, IL-10 and IDO from blood cultures stimulated by 3% of liquid silicone compared with negative control. Differences were seen significantly between negative control and the positive control (PHA), ($p<0.001$).

There was significant difference in the expression of TNF-α, IFN-γ, IL-10 and IDO on inflammatory cells surface in normal skin compared to granuloma or submental skin ($p<0.001$). However, there was no statistical difference between granuloma and submental skin. Moreover, there was no statistical difference in Treg cells population between granuloma and normal skin, but there was significant difference between normal skin and submental skin ($p<0.001$).

Histopathological feature (with HE staining) showed that giant cells and fibrosis area were not found in normal skin tissue. Histopathological feature of granuloma showed that granuloma tissue was more inflammative than submental skin.

Correlation between immune response in chin granuloma due to silicone injections, submental skin and blood.

There was a significant correlation between TNF-α in supernatant of blood culture with the expression of cytokines TNF-α on inflammatory cell surface in chin granuloma. (p= 0.017, r= 0,426).

Correlation between immune response in chin granuloma due to silicone injections with submental skin.

There was a significant correlation between histopathological feature (8 categories) of granulomas with submental skin (p=0.004, r=0.507), due to silicone spreading, thus the foreign body reaction also occurred in the submental skin.

1. In this study the folowing data were found:

a. Anti inflammatory cytokines in submental skin were significantly correlated with cytokines expression in granulomas tissue. The expression of IL-10 in submental skin was significantly correlated with IL-10 in granuloma tissue (p=0.021, r=0.412), while IDO in submental skin was significantly correlated with almost all cytokines (TNF-α p=0.009, r=0.460; IFN-γ p=0.003 r=0.512; IL-10 p=0.012; r=0.445; IDO p=0.026 r=0.399).
b. Population of Treg cells in submental skin was significantly correlated with the expression of IDO on inflammatory cells surfacein granuloma (p=0.034, r=0.381).

The submental skin occurred immune tolerance to prevent damage due to inflammation by silicone.

Degree of clinical severity, histopathological feature of chin granulomas and submental skin related with period of granuloma development, and silicone levels

Clinical severity was not associated with period of granuloma development and silicone levels in patient with chin silicone granuloma. The degree of clinical severity was not determined by period of silicone injection or silicone level, but by individual immune response.

1. Histopathological feature with three phases of foreign body reaction (FBR)was significantly associated with clinical severity ($p=0.020^{ch*}$). When clinical feature became more severe, histopathological features tend to be more fibrosis ($r=0.456$, $p=0,010^{s*}$, $R^2=0.207$)
2. Histopathological feature with eight categories (stages) of granuloma was significantly associated with period of granuloma development in granuloma tissue ($p = 0.020$)and submental skin ($p=0.046$) (Figure 4.7 and 4.9). Peak level of inflammation was reached at around 10-19 years after silicone injection and decreased after 19 years due to individual immune tolerance.
3. Histopathological feature with eight categories of granuloma was significantly associated with higher levels of silicone in submental skin ($p=0.047$), but not in the granuloma tissue, it can be seen in figure 4.10.

The inflammation increased concomitantly with silicone level in submental skin and shifted toward fibrosis gradually when silicone started to be decreasing.[20,23,57] Silicone level in submental skin was more stable than in granuloma tissue.

The role of proinflammatory and anti inflammatory cytokines to the occurrence of immune tolerance in patients due to silicone injections into their chin, which was assessed in granuloma tissue, skin and blood

Expression of IDO on inflammatory cells surface and statistically not correlated with the period of granuloma formation and silicone level. Population of Treg cells was not correlated with period of granuloma formation, but Treg population in granuloma tissue was significantly correlated with silicone level ($p=0.033$, $r=0.383$).

Each individual has different immune tolerance, depending on antigen level. Silicone needs plasma proteins on its surface to trigger immune response. Phases of protein adsorption on silicone surface are dynamic process and difficult to be predicted.[92] Patients with chin silicone injection had delayed-type hypersensitivity (DTH) reaction which would recruit lymphocytes. Silicone is captured by lymphocytes via its receptor, then lymphocytes secrete both proinflammatory and anti inflammatory cytokines, and then, in this process, Treg cells play a role to mantain homeostasis, thus the silicone level was correlated with Treg population in granuloma tissue. [27]

This study showed that histopathological feature of granulomas (FBR in 3 phase) was not statistically associated with the expression of IDO on inflammatory cells surface in granulomas, and whole blood culture with all stimulants, but histopathological with eight categories in submental skin was significantly associated with the expression of IDO on inflammatory cells surface in the submental skin (p=0.038).

Expression of IDO on inflammatory cell surface in the submental skin correlated with almost all cytokines in granuloma tissue. It was not surprising if IDO also significantly correlated with histopathological feature. IDO enzyme seems to play an important role in the submental skin, so that IDO can be used as a predictive factor for developing of immune tolerance to silicone injection.

Treg was not statistically associated with histopathological and clinical severity, but Treg population in submental skin was significantly associated with clinical severity (p=0.011). Treg population in the submental skin can be used as a predictive factor for assessing the immune response and clinical features.

This study found that level of proinflammatory cytokines, TNF-α and IFN-γ, in whole blood culture was not statistically associated with the expression of IDO on inflammatory cells surface in both tissues; but the expression of TNF-α and IFN-γ cytokines in granuloma was significantly correlated with the expression of IDO in both granuloma and submental skin (TNF-α, r=0.592, p<0.001; IFN-γ, r=0.603, p<0.001). IDO has a primary role in submental area for controlling inflammation and helps to mantain immune tolerance, so tissue damage caused by inflammation could be prevented.[21-23] IDO activity can be used as a predictive factor for developing immune response in granuloma.

The result of this study described that Treg population was not statistically correlated with TNF-α and IFN-γ in granuloma tissue and submental skin, but also in supernatant of blood culture, as well as silicone-stimulated blood culture; but Treg population in granuloma was inversely correlated with TNF-α and IFN-γ in PHA-stimulated blood culture (TNF-α, r= -0.450, p=0.011; IFN-γ, r= -0.367, p=0.042).

Lymphocytes will be stimulated by PHA. Treg cells are subset of lymphocytes that will mantain immune tolerance in granuloma caused by silicone injection. Inverse correlation happens because of Treg works as an anti inflammatory, whereas TNF-α and IFN-γ are pro inflammatory cytokines.[13,27]

The ratio of TNF-α/IL-10 and IFN-g/IL-10 are factors that show the function of T reg. There were correlation between Treg population ($CD4^{+}CD25^{+}$) and IDO with ratio TNF-α/IL-10 and IFN-g/IL-10 in whole blood culture and both tissue. IDO in granuloma and submental skin was not statistically correlated to the ratio of TNF-α/IL-10 and IFN-γ/IL-10 in supernatant of blood culture and PHA-stimulated blood culture; but IDO expression in granuloma was significantly correlated with the ratio of TNF-α/IL-10 in silicone-stimulated blood culture and submental skin (blood, r=0.418, p=0.019; submental skin, r= -0.363, p=0.045). According to these findings, IDO activity correlated with Treg function, thus the expression of IDO on inflammatory cells surface in granuloma can predict immune responses. Treg population in granuloma and submental skin were not statistically correlated to the ratio of TNF-α/IL-10 and IFN-γ/IL-10 in PHA-stimulated blood culture and silicone-stimulated silicone, as well as in granuloma and submental skin. Treg population in granulomas inversely correlated with the ratio of TNF-α/IL-10 in supernatant of blood culture (r= - 0.460 p=0.009). This data prove that Treg function work through IL-10.

Conclusion

1. Generally, patients with chin granuloma due to silicone injection were injected in the salon. They came for treatment approximately 12.5 years after injection, the shape of the chin changed in 4^{th} years, the color of the skin changed in 5^{th} years.
2. Level of proinflammatory cytokines tend to be higher in patients with granuloma due to silicone injection compared to the normal patients, while anti inflammatory cytokines levels of blood cultured supernatant tend to be lower than normal patients. Histopathological feature of silicone granuloma was more dominated by inflammation, while in submental skin more fibrosis was found.
3. There was a significant correlation between proinflammatory cytokines TNF-α in supernatant of blood culturewith TNF-α expression on inflammatory cellssurface in granuloma tissue. Level of TNF-α in supernatant of blood culturecan be used as predictor to assess the immune response due to silicone injection. The ratio of TNF-α/IL-10 and TNF-α/IDO in supernatant ofblood culturecan be used as predictors for granuloma formation.
4. IL-10 in submental skins was significantly correlated with cytokines in granulomas. IL-10 played a role in submental skin and can be used as the best predictor to assess the immune response in submental skin due to silicone injection.

5. Clinical severity was significantly correlated with histopathological feature of granuloma. Period of granuloma development related to histopathological feature in granulomas and submental skin. Histopathological feature in submental skin was associated with higher level of silicone.
6. TNF-α played a role in immune respons due to inflammation in granuloma, while IL-10, Treg cells and IDO enzyme played a role in immune tolerance due to silicone injection.

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