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Immunology Behind the Granuloma et Causa Filler

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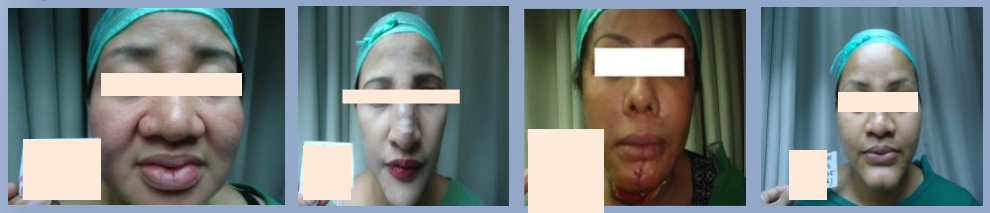
INTRODUCTION

All injected filler may cause foreign body granulomas in some patient. Filler which has irregular surface and can not be phagocytized may eventually form of foreign body granulomas due to of frustrated macrophages. Microspheres size of the filler below 15 microns are generally phagocytized and may transport to local lymph node. Larger microspheres are encapsulated with fibrous tissue and escape from phagocytosis, may cause granuloma



METHODS

We measures the immunology of the patient with granuloma ec silicone injection. We measured inflammation cytokines such as TNF-α, IFN-γ, and anti inflammation cytokines or immune tolerance marker such as IL-10, Indoleamine 2,3-dioxygenase (IDO), Treg ($CD4^+CD25^+$). We took the blood from the patient and do the biopsy at chin granuloma as tissue from the injection site, also submental skin as skin surrounded the injection site.

CONCLUSIONS

This study showed, the cytokines which produced by macrophages and IDO as immune tolerance play the rule of granuloma formation due to silicone injection.

REFERENCES

1. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133:775-87.
Mottet C, Golsbayan D. CD4+CD25+Foxp3+ regulatory T cells: from basic research to potential therapeutic use. Swiss Med Wkly. 2007;137:625-34.
2. Mellor AL, Munn DH. IDO expression by dendritic cells: Tolerance and tryptophan catabolism. Nat Rev Immunol. 2004;4(10):762-74.
3. Guillonneau C, Hill M, Hubert FX, Chiffoleau E, Herve C, Li XL et al. CD40Ig treatment results in allograft acceptance mediated by $CD8^+CD45RC^{low}$ T cells, IFN-g and indoleamine 2,3-dioxygenase. J Clin Invest. 2007;117(4):1096-106.
4. Abbas AK, Lichtman AH, Pillai S. Properties and overview of immune system. Dalam: Cellular and molecular immunology. Philadelphia: Saunders Elsevier Inc; 2015.h.1-12.
5. Abbas AK, Lichtman AH, Pillai S. Immunological tolerance and autoimmunity. Dalam: Cellular and molecular immunology. Philadelphia: Saunders Elsevier Inc; 2015.h.315-38.
6. Curti A, Trabanelli S, Salvestrini V, Baccarani M, Lemoli RM. The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: focus on hematology. Blood. 2009;113(11):2394-401.
7. Xiang C, Zhu BT. Indoleamine 2.3-dioxygenase tissue distribution and cellular localization in mice: Implication for its biological function. J histochem cytoche. 2010;58(1);17-28.

RESULTS (CLICK TO EDIT)

Cytokines level of TNF-α, IFN-γ, IL-10, IDO in supernatant blood cuture PHA, RPMI silicone between normal patient group and granuloma patient group

Cytokines	Culture	Cytokines level in normal group n=37	Cytokines level in granuloma group n=31	P value
TNF-α pg/mL	PHA	757,5 (24,6-13274,3)	2689,6 (29,5-7573,6)	p= 0,056[m]
	RPMI	91,6 (21,9-1253,8)	185,1 (23,5-1469,4)	**p= 0,021[m*]**
	Silicone	74 (22,8-3710)	127,1(18,1-2910,45)	p= 0,100[m]
IFN-γ pg/mL	PHA	372,4 (27,1-52503,7)	6868,3 (12,5-81372,5)	p= 0,075[m]
	RPMI	84,5 (6,6-864,9)	175,4 (9-5329,45)	p= 0,304[m]
	Silicone	37,83 (5,7-12736,8)	169,40 (21,3-1021,5)	p= 0,310[m]
IL-10 pg/mL	PHA	191,6 (10.2-927)	192,9 (13,6-890,4)	p= 0,392[m]
	RPMI	23,1 (8,6-112,6)	17,6 (6,5-418,5)	p= 0,207[m]
	Silicone	22,4 (8,6-417,4)	17,13 (6,1-339,95)	p= 0,281[m]
IDO ng/mL	PHA	1112,7 (92,1-7146,7)	979,4 (106,7-7366,7)	p= 0,449[m]
	RPMI	784,2 (92,1-6280)	458,2 (97-6440)	**p= 0,049[m*]**
	Silicone	773,1 (66,7-5106,7)	471,5 (100,6-5600)	**p= 0,050[m*]**
Ratio TNF-α/ IL-10	PHA	7,6 (0,3-32)	10,7 (1-60,9)	p= 0,055[m]
	RPMI	3,8 (0,3-27,9)	10,6 (0,2-63)	**p= 0,002[m*]**
	Silicone	4,1 (0,7-25,4)	6,7 (0,3-51,1)	**p= 0,011[m*]**
Ratio TNF-α/ IDO	PHA	0,7 (0-5,9)	3,2 (0,0-14,5)	**p= 0,008[m*]**
	RPMI	0,1 (0-5,2)	0,3 (0-12,0)	**p= 0,008[m*]**
	Silicone	0,11 (0-7,0)	0,17 (0-12,2)	**p= 0,026[m*]**

Notes :
PHA : *phytohemaglutinin*, RPMI : culture using *Roswell park memorial institute medium* / normal serum, Silicone : industrial silicone 3%
[M] Mann Whitney test, ng/mL : nanogram/milliliter, pg :picogram/milliliter.
All the data above is median numbered (minimum-maksimum)
*meaningful in P <0,05 ; **very meaningful in P<0,005

In this study, we took the blood and measure the inflammatory cytokines (TNF-α, IFN-γ), and anti inflammation cytokines (IL-10) also immune tolerance enzyme (IDO). From this study, we can see the granuloma group have higher inflammation immune response, but lower immune tolerance. So the granuloma can develop if someone have high inflammation immune response but don't have a good immune tolerance.

Imunohistochemistry examination between granuloma tissue/ chin and surrounded skin/submental skin

		GRANULOMA (n=31)				
Variabel		TNF-α	IFN-γ	IL-10	IDO	Treg
SURROUNDED SKIN	TNF-α	p= 0,596[s] r= 0,099	p= 0,292[s] r= 0,196	p= 0,707[s] r = -0,070	p= 0,741[s] r= -0,062	p= 0,616[s] r= 0,094
	IFN-γ	p= 0,955[s] r= 0,01	p= 0,691[s] r= 0,074	p= 0,611[s] r= 0,095	p= 0,333[s] r= -0,180	p= 0,900[s] r= 0,024
	IL-10	p= 0,541[s] r= 0,114	p= 0,816[s] r= 0,044	**p= 0,021[s*] r= 0,412 R^2= 0,170**	p= 0,506[s] r= 0,124	p= 0,777[s] r= -0,053
	IDO	**p= 0,009[s*] r= 0,460 R^2= 0,211**	**p= 0,003[s**] r= 0,512 R^{2*} 0,262**	**p= 0,012[s*] r= 0,445 R^2= 0,199**	**p= 0,026[s*] r= 0,399 R^2= 0,160**	p= 0,598[s] r= 0,098
	Treg	p= 0,838[s] r= - 0,038	p= 0,367[s] r= 0,168	p=0,293[s] r= 0,195	**p= 0,034[s] r=0,381 R^2= 0,145**	p=0,598[s] r= 0,098

Note :

[s] Spearman test, *meaningful in P <0,05, **meaningful in P<0,005 correlation power(r) 0,00-0,199 very weak, 0,20-0,399 weak, 0,40-0,599 medium, 0,60-0,799 strong, 0,80-1,00 very strong. R^2 : Determination coeficient

We also did the biopsy in granuloma site and the skin surround the granuloma. And then we stained the tissue with immuno histochemistry. In this table, you can see the anti inflammation cytokines (IL-10) and immune tolerance (IDO and T-reg) play a role in skin surround the granuloma. Inflammation cytokines appear only in the granuloma site but the anti-inflammation or immune tolerance moved from granuloma site to surrounded skin. So it means, our body do something for protection, by trying to block the inflammation by anti inflammation cytokines or immune tolerance to make the inflammation not spread anywhere. This means, immune tolerance play a role in foreign body reaction due to the fillers in the patient with the granuloma.

IMMUNOLOGY BEHIND THE GRANULOMA ec FILLER

Ago Harlim

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1. BACKGROUND

Silicone injection for cosmetics and surgery is still widely practiced in Indonesia. The result of Indonesian Association of Plastic Surgeons survey start from 2004 to 2007, found 249 cases of silicone complications.[1] Epidemiological data in other countries was 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, if severe, it could be followed by infection, necrosis, and abscess.[2-4] Silicone granuloma is 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 pro inflammatory cytokines and Th2 secreted anti inflammatory cytokines.[18] The new theory of immune tolerance played by Tregs ($CD4^+CD25^+$) and Indoleamine-2,3-dioxygenase enzyme might be explained obviously the pathogenesis of granuloma formation due to silicone injection.[21-24] Until now, the pathogenesis of silicone granuloma has been studied, but the result are still controversial. The aimed of this study is to analyse the phatogenesis of silicone granuloma in respect to immune inflammatory response and tolerance.

2. METHOD OF STUDY

Descriptive analytic method was conducted in this 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 tissue. 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 supernatant on day 3, included TNF-α, IFN-γ, and IL-10. All analyzed with Luminex and IDO with ELISA. The research was conducted in specialist clinics, Faculty of Mathematics and Sciences University of Indonesia, Faculty of Medicine University of Indonesia, Faculty of Medicine Airlangga University, and Eijkman Institute, start from November 2012 until September 2014.

3. RESULT AND DISCUSSION

Generally, patients with silicone injections in their chin were injected in the salon. They came for treatment approximately 12.5 years after injection, shape of chin changed in 4^{th} years, the color of the skin changed in 5^{th} years. Nose and chin were main area of silicone injections, 54.8% of patients with silicone injection complications were not know that the injected substance was liquid silicone. 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 significantly 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 seen significantly between negative control and the positive control (PHA), ($p<0.001$).

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

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

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

Based on a significant correlation between the expression of cytokines TNF-α, IFN-ɣ, IL-10 and IDO on inflammatory cell surface in chin granuloma, submental skin of granuloma and normal skin with cytokines levels in blood, thus the **minor hypothesis 1**.

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

Based on the correlation between the expression of cytokines TNF-α, IFN-ɣ, IL-10 and immune tolerance (Treg cells ($CD4^+CD25^+$), IDO in chin granuloma and submental skin of granuloma, **minor hypothesis point 2 was accepted**.

1. There was a significant correlation between histopathologic features of granulomas with submental skin of granuloma ($p=0.004$, $r=0.507$), due to silicone spreading, thus the foreign body reaction also occured in the submental skin of granuloma (Table 2).
2. Anti inflammatory cytokines in submental skin of granuloma were significantly correlated with cytokines level in granulomas tissue. Level of IL-10 in submental skin of granuloma correlated significantly with IL-10 in granuloma tissue ($p=0.021$, $r=0.412$), IDO in submental skin of granuloma 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 $p=0.026$ $r=0.399$). Population of Treg cells in submental skin of granuloma was significantly correlated with the expression of IDO on inflammatory cell surface in granuloma ($p=0.034$, $r=0.381$) (Table 3). Based on these results, the submental skin of granuloma occurs immune tolerance to prevent damage due to inflammation by silicone.

Histopathological features of chin granulomas and submental skin of granuloma related with period of injection, silicone levels and the degree of clinical severity

Based on correlation of histopathological features of chin granulomas and submental skin with period of injection, silicone levels and clinical severity, so the **hypothesis minor 3 points 2, 3, 4 are accepted.**

1. Clinical severity was not associated with and silicone levels in patient with chin granuloma caused by silicone. The degree of clinical severity was not determined by period of silicone injection or silicone level, but by individual immune response.
2. Histopathologic features with three phases of granuloma significantly associated with clinical severity ($p=0{,}020_{ch}$*). When clinical features became more severe, histopathological features tends to be fibrosis ($r=0.456$, $p=0{,}010_{s}$*, $R_2=0.207$) (Figure 4)
3. Histopathological features with eight stages of granuloma significantly associated with period of silicone injection in granuloma tissue ($p = 0.020$), and submental skin of granuloma ($p=0.046$) (Figure 5). Peak of inflammation was reached around 10-19 years after silicone injection and decreased after 19 years due to individual immune tolerance.
4. Histopathological features with eight stages of granuloma significantly associated with higher levels of silicone in submental skin of granuloma ($p=0.047$), but not in the granuloma tissue. it can be seen in figure 4.10, that the inflammation increased concomitantly with silicone level in submental skin of granuloma and shifted toward fibrosis gradually when silicone started to be decreasing.[20,23,27] Silicone level in submental skin of granuloma was more stable than in granuloma tissue.

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

Based on the correlation between Treg ($CD4^{+}CD25^{+}$) population as immune tolerance properties, expression of IDO on inflammatory cell surface in granuloma tissue and submental skin with period of silicone injection and silicon level, the **minor hypothesis 4 was accepted**, described in table 4 and 5.

Expression of IDO on inflammatory cell surface did not correlate with period of silicone injection and silicone level. Population of Treg cells did not correlate with period of silicone injection, but Treg population in granuloma tissue correlated significantly with silicone level ($p=0.033$, $r=0.383$), (Table 4 and 5). Each individual have a difference immune tolerance,

depend on antigen level. Silicone need plasma proteins on its surface to trigger immune responses. Phases of protein adsorption on silicone surface are dynamic process and difficult to be predicted.[31] Patients with chin silicone injection have delayed-type hypersensitivity (DTH) reaction which would recruit lymphocytes. Silicone captured by lymphocytes via its receptor, then lymphocytes secreted both proinflammatory and antiinflammatory cytokines, and then, in this process, Treg played a role to mantain homeostasis, thus the silicone level correlated with Treg population in granuloma tissue. [27]

Based on the correlation between the expression of IDO on inflammatory cell surfacein granuloma tissue, submental skin of granuloma and blood with clinical and histopathologic severity, then the **minor hypothesis 5 was accepted** and can be seen in table 6.

Histopathologic features of granulomas were not associated with the expression of IDO on inflammatory cell surfacein granulomas, and whole blood culture with all stimulants, but histopathologic with eight phases in submental skin of granuloma was associated significantly with the expression of IDO on inflammatory cell surface in the submental skin of granuloma ($p=0.038$, Table 6). Expression of IDO on inflammatory cell surface in the submental skin of granuloma correlated with almost all cytokines in granuloma tissue. It is not surprising if IDO also correlated significantly with histopathological features. IDO seems to play an important role in the submental skin of granuloma so that IDO can be used as predictive tool for immune tolerance to silicone injection.

Treg did not associate with histopathologic and clinical severity, but Treg population in submental skin of granuloma significantly associated with clinical severity ($p=0.011$, Table 7). **Minor hypothesis 6 is accepted**, so, Treg population in the submental skin of granuloma can be used as predictive tool for observing the immune response and clinical features.

Based on the correlation between IDO and TNF-α and IFN-ɣ, in chin granuloma, submental skin of granuloma, as well as the blood level of cytokines, then the minor hypothesis at point seven is accepted and can be seen in Table 8.

1. Level of pro inflammatory cytokines, TNF-α and IFN-γ, in whole blood culture is not associated with the expression of IDO on inflammatory cell surface in both tissues but the expression of proinflammatory cytokines, TNF-α and IFN-γ, in granuloma significantly correlated with the expression of IDO in both tissues (TNF-α, $r=0.592$, $p<0.001$; IFN-γ, $r=0.603$, $p<0.001$, table 8). IDO has a primary role in submental area for controlling

inflammation from silicone-consuming macrophages with IDO secretion and helps to mantain immune tolerance, so tissue damage caused by inflammation could be prevented.[32-34] IDO activity can be used as predictive tool for observing immune response in granuloma.

2. Treg population did not correlate with TNF-α and IFN-γ in granuloma tissue and submental skin of granuloma, also in blood plasma, as well as silicone-stimulated blood, but Treg population in granuloma inversely correlated with TNF-α and IFN-γ in PHA-stimulated blood (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 happen because Treg works as an anti inflammatory, whereas TNF-α and IFN-γ are pro inflammatory cytokines.[17,27]

Based on the correlation between Treg population ($CD4^+CD25^+$) and IDO with ratio TNF-α/IL-10 and IFN-γ/IL-10 in whole blood culture and both tissue, then minor hypothesis at point eight is accepted and described on the table 9.

1. IDO in granuloma and submental skin of granuloma did not correlate to the ratio of TNF-α/IL-10 and IFN-γ/IL-10 in blood plasma and PHA-stimulated blood, as well as granuloma tissue but the IDO in granuloma significantly correlated with the ratio of TNF-α/IL-10 in silicone-stimulated blood and submental skin of granuloma (blood, r=0.418, p=0.019; submental skin of granuloma, r= -0.363, p=0.045). Based on data, IDO activity correlated with Treg function, thus the expression of IDO on inflammatory cell surface in granuloma can predict immune responses.
2. Treg population in granuloma and submental skin did not correlate to the ratio of TNF-α/IL-10 and IFN-γ/IL-10 in PHA-stimulated blood and silicone-stimulated silicone, as well as in granuloma and submental skin, but Treg population in granulomas inversely correlated with the ratio of TNF-α/IL-10 in blood plasma (r= - 0.460 p=0.009). This data prove that Treg function work through IL-10.

The level of cytokines ratio in blood for granuloma prediction

By assessing and comparing the inflammation that was occured with ability of the body to inhibit inflammation with anti inflammatory cytokines or tolerance mechanism, as played by IL-10 and IDO, the ratio of TNF-α/IL-10 or TNF-α/IDO would be more accurate to be used as predictive tool. Relationship between ratio of TNF-α/IL-10 and TNF-α/IDO with period of

silicone injection can be seen in table 10. The ratio of TNF-α/IL-10 in PHA-stimulated blood and blood plasma and TNF-α/IDO in silicone-stimulated blood and blood plasma can be used as predictive tool. Inverse correlation means that the lower the ratio, the longer onset period of granuloma.

According to the table 10, the ratio of TNF-α/IL-10 and TNF-α/IDO in blood plasma can be used as predictors of the onset period of granuloma (p=0.038; p=0.028). Table 1 showed the significant difference between the normal and granuloma patients. Ratio of TNF-α/IL-10 in blood plasma of normal patients differ significantly with granuloma patients (p=0.002). Ratio of TNF-α/IDO in blood plasma of normal patients differ significantly with granuloma patients (p=0.008).

The level of cytokines in tissue for granuloma prediction

Tissue examination is required if there are any indecision in the existing examination, either physical or blood cytokine examination.

Based on the results, in table 11, there was seen some cytokines correlated with clinical features and period of silicone injection. TNF-α expression on inflammatory cell surface in granuloma tissue significantly correlated with clinical features and period of silicone injection, but clinically most patients do not want to do the biopsy, i.e. at chin, so the submental skin of granuloma areas should be selected to represent the immune response that was occured.

Based on table 11, Treg and IL-10 in submental skin of granuloma can be used as predictors of immune response that would happen. In accordance with the results, table 7 showed Treg had a significant correlation with clinical severity of submental skin of granuloma, whereas IL-10 in submental skin of granuloma significantly correlated with IL-10 in the granuloma tissue.

The numerical data need to be transformed to categorical data to measure prediction by mean of multivariate logistic regression. Treg population in submental skin of granuloma were splitted into two category with a cut-off limit 0.5 based on ROC analysis between Treg with the clinical features.

Result of multivariate logistic regression analysis between clinical severity with IL-10 were obtained (IL-10 p=0.028 and Treg p=0.057). It was concluded that IL-10 in submental skin of granuloma can be used as the best predictor. Based on ROC analysis between IL-10 in

submental skin of granuloma and clinical features, expression limit of IL-10 in submental skin of granuloma is 138.

The higher degree of fibrosis showed immunologically improvement, but also became a problem for patients. IL-10 is an anti inflammatory cytokine and one of the cytokines produced by Treg, in addition to TGF-β.[20,27,28] TGF-β played a role in fibrosis. If IL-10 level more than 138 in submental skin of granuloma, granuloma did not need to be evacuated, actually. Evacuation procedure can be done on cosmetic indications, and must be followed-up through a standard blood test of normal patient, if IL-10 level less than 138, the patient should be recommended to treat with anti TNF-α or immunomodulatory, in order to avoid granuloma formation due to remaining-silicone in tissue.

Based on these data, cut off point for prediction of granuloma was establish. The median of ratios of granuloma patients can be used as cut-off point for predicting the onset of granulomas. Cut off point ratio of TNF-α/IL-10=3.8 and TNF-α/IDO=0.1 as a lowest level. If the ratio below the cut off, so we need follow up every 6 month, if above the cut off, therpy anti TNF-α is needed.

4. CONCLUSIONS

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 chin changed in 4th years, the color of the skin changed in 5th years.
2. Level of pro inflammatory 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 tend to be lower than normal patients. Histopathological features of granuloma caused by silicone are more inflammation, while nomal skin were more fibrosis.
3. There is a significant correlation between proinflammatory cytokines TNF-α, in blood with TNF-α expression on inflammatory cell surface in granuloma tissue. Level of TNF-α in blood can be used as predictor to assess the immune response due to silicone injection.
4. IL-10 in submental skin of granulomas significantly correlated with cytokines in granulomas. IL-10 played a role in submental skin of granuloma and can be used as the best predictor to assess the immune response in submental skin due to silicone injection.

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

REFERENCE

1. Prasetyono TOH. Data survey kasus akibat suntikan silikon di Indonesia. PERAPI. 2007.
2. Maria imelda. Unusual beauty. Penyalahgunaan silikon ternyata masih banyak. Tersedia di: http://beautyonwatch.wordpress.com/2009/03/20/penyalahgunaan-silikon-ternyata-masih-marak/. Diunduh 11 Agustus 2011.
3. Peters W, Fomarsier V. Complication from injectable material used for breast augmentation. The canadian journal of plastic surgery. Autum 2009; 17(3). Tersedia di: http://www.ncbi.mlm.nih.gov/june/article/PMC740603. Diunduh 16 Oktober 2010.
4. James C. Mc Kinley Jr. Woman charged indeath caused by silicone injection. New York times. Maret, 2014. Tersedia di: http://nyti.ms/1foy4dr. Diunduh 18 Agustus 2014.
5. Aladiw. Radang payudara malinda karena suntik silikon [Internet].2011 [updated 2011 Jun 11;cited 2011 Jun 28]. Tersedia di:
   - http://aladiw-us/radang-payudara-malinda-karena-suntikan-silikon/'
6. Alcon Laboratories, Inc. Liquid silikon injection. Tersedia di: http://www.yestheyrefake.net/liquid_silicone_risks.htm. Diunduh 11 Agustus 2010.
7. Nitzan D, Yahalom R, Taicher S. Silicone granuloma of lip. Harefuah. 2004;143(5):335-8, 391.
8. Takenaka M, Tanaka M, Isobe M, Yamagichi R, Kojiro M, Sirouzu K. Angiosarcoma of the breast with silicone granuloma: A case report. Kurume Med J. 2009;56:33-7.
9. Syalendra, M. Aura Cantik Berkharisma Cahaya Mutiara [Internet]. 2008 [cited 2011 Aug 8]. Tersedia di:
   - http://www.mariasyailendra.com/produk.html.
10. Chen YC, Chen ML, Chui YM. A case mimicking angioedema: chin silicone granulomatous reaction spreading all over the face after receiving liquid silicone injection forty years previous. Chin Med J. 2011;124(11):1747-50.
11. James S J, Pogribna M, Miller BJ, Bolon B, Muskhelishvill L. Characterization of cellular response to silicone implants in rat: implications for foreign body carcinogenesis. Biomaterial. 1997;18(9):667-75.
12. Kumar V, Abbas AK, Fausto N, Aster JC. Acute and chronic inflamation. Dalam: Kumar V, Abbas AK, Fausto N, Aster JC, ed. Robbins and Cotran. Pathologic Basic of Disease. Edisi ke-8. Philadelphia: Saunders Elsevier Inc; 2004.h.45-77.
13. Baratawijaya KG, Rengganis I. Imunologi dasar. Edisi ke-10. Jakarta: Balai penerbit FK UI; 2010.h.257-86.
14. Cakmak O, Turkoz HK, Polat S, Serin GM, Hizal E, Tanyeri H. Histopathologic response to highly purified liquid silicone injected intradermally in rat' skin. Aesth Plast Surg. 2011;35:538-44.
15. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. Aesth Plast Surg. 2003;27:354-66. Doi:10.1007/s00266-003-3022-1.

16. Pasternack FR, Fox LP, Engler DE. Silicone granulomas treated with etanercept. Arch Dermatol. 2005;141(1):13–15.
17. Agustini C, Semenzato G. Biology and immunology of granuloma. Dalam: James DG, Zumla A ed. Granulomatous disorders. United Kindom: Cambrige press; 1999.h.3-16.
18. Bondurant S, Ernster V, Herdman R. Antinuclear antibodies and silicone breast implantts. Dalam: Safety of Silicone Breast Implants. Washington: The National Academy Press;1999.h.198-214.
19. Bondurant S, Ernster V, Herdman R. Immunology of silicone. Dalam: Safety of Silicone Breast Implants. Washington: The National Academy Press; 1999.h.179-97.
20. Baratawijaya KG, Rengganis I. Toleransi imun. Dalam:Imunologi dasar. Edisi ke-10. Jakarta: Balai penerbit FK UI; 2012.h.287-312.
21. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133:775-87.
22. Mottet C, Golsbayan D. CD4+CD25+Foxp3+ regulatory T cells: from basic research to potential therapeutic use. Swiss Med wkly. 2007;137:625-34.
23. MellorAL, Munn DH. IDO expression by dendritic cells: Tolerance and tryptophan catabolism. Nat Rev Immunol. 2004;4(10):762-74.
24. Guillonneau C, Hill M, Hubert FX, Chiffoleau E, Herve C, Li XL et al. CD40 Ig treatment results in allograft acceptance mediated by $CD8^{+}CD45RC_{low}$T cells, IFN-γ, and indoleamine 2,3-dioxygenase. Clin invest J. 2007; 117(4):1096-106.
25. Egen JG, Rothfuchs AG, Feng CG, Winter N, Sher A, Germain RN. Macrophage and T cell dynamics during development and disintegrasion of mycobacterial granulomas. Immunity. 2008; 28: 271-84.
26. Zaremba J, Losy J. The levels of TNF-alpha in cerebrospinal fluid and serum do not correlate with counts of the white blood cells in acute phase of ischaemic stroke. Folia Morphol. 2001; 60(2): 91-7.
27. Abbas AK, Lichtman AH, Pillai S. Immunological tolerance and autoimmunity. Dalam: Cellular and molecular immunology. Philadelphia: Saunders Elsevier Inc; 2015.h.315-38.
28. Rifa'i M. Perkembangan Sel T Regulator periferal dan mekanisme supresi *in vitro*. J Exp life Sci. 2010;1:1.
29. Kresno SB. Imunologi: Diagnosis dan Prosedur Laboratorium. Edisi ke-5. Jakarta: Balai Penerbit FKUI. 2010.h.50-97.
30. Wick G, Grundtman C, Mayerl C, Wimpissinger T, Feichtinger J, Zelger B, et al. The immunology of fibrosis. Annu. Rev. Immunol. 2013; 31: 107-35.
31. Zhang J M, Xiong J. Cytokines, inflammation and pain. Int Anesthesiol Clin. 2007;45(2):27-37.
32. Opitz CA et al. The indoleamine-2,3-dioxygenase (IDO) inhibitor 1-methyl -D-trypthophan upregulates IDO1 in human cancer cells. PLoS ONE.2011;6(5):e19823. DOI:10.1371/journal.pone.0019823.
33. Romani L, Fallarino F, Luca AD, Montagnoli C, D'Angelo C, Zelante T, et al. Defective tryptophan catabolism underlies inflamation in mouse chronic granulomatous disease. Nature. 2008 ;451:211-5.
34. Luft T, Maraskovsky E, Schnurr M. IDO production, adaptive immunity, and CTL killing. Blood. 2005;106:2228-9.
35. Lu Y, Giver CR, Sharma A, LI JM, Darlak KA, Owen LM, Roback JD, Galipeau J, Waller EK. IFN-γ and indoleamine 2,3-dioxygenase signalig between donor dendritic cells and T cells regulates graft versus host and graft versus leukemia activity. Blood. 2012;119(4):1075-85.