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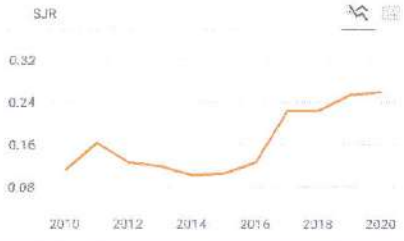

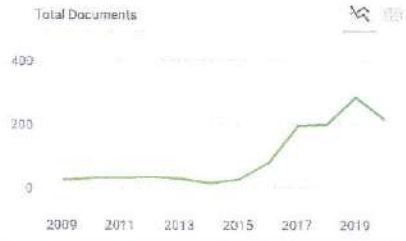

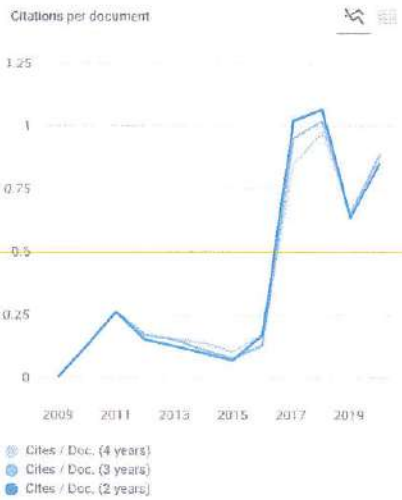

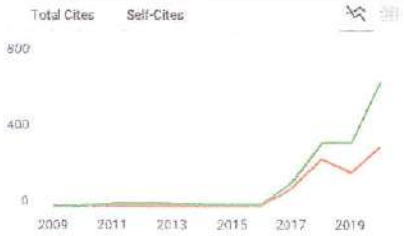

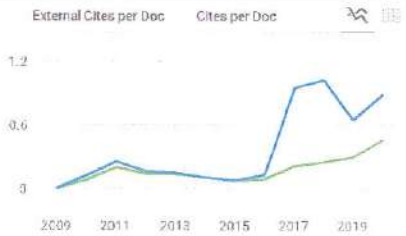

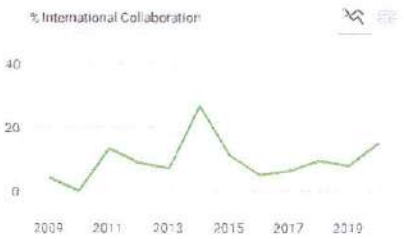

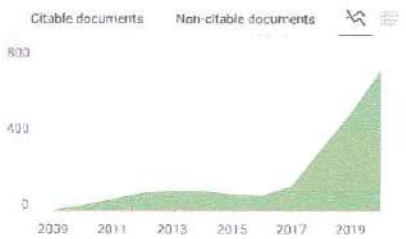

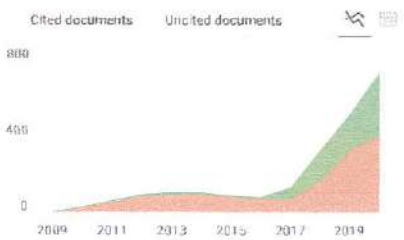

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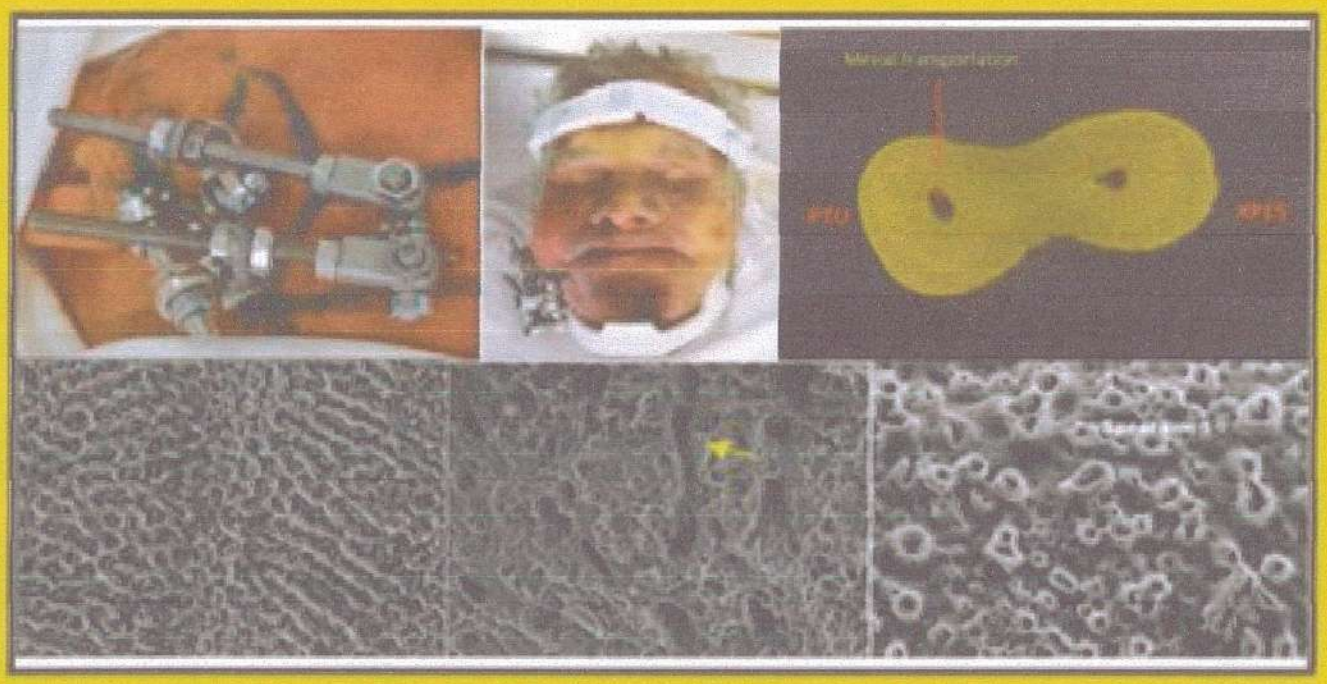

2020 - Vol. 13 – No. 2

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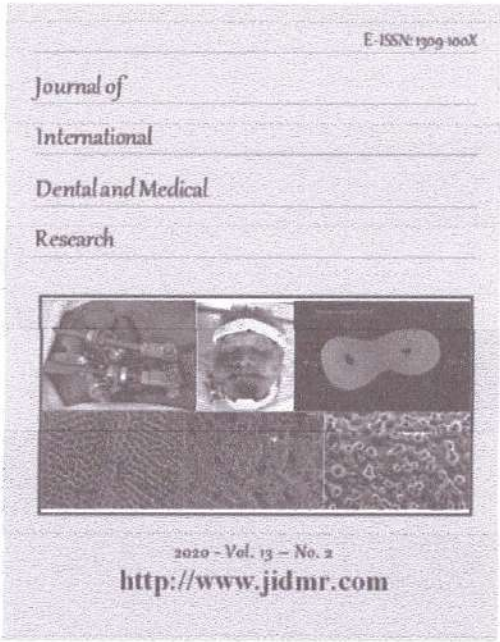

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Table of Contents 2020 Vol.13–No.2

[http://www.jidmr.com/journal/wp-content /uploads/2020/06 /1.2_13_20_Table_of_Contents..pdf]

DENTISTRY

EXPERIMENTAL ARTICLE

1. Mandible Exoskeleton – First Results of Development and Implementation

Alexandr A. Vorobyev, Denis Yu. Dyachenko, Yuliya A. Makedonova, Dmitriy V. Mikhalchenko, Evgeniy V. Fomichev, Karen A. Sargsyan

Pages 400-406 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/1-D20_1061_Yuliya_A_Makedonova_Russia.pdf]

EXPERIMENTAL ARTICLE

2. Evaluation of the Shaping Ability of XP Endo Shaper: A Micro-Computed Tomography Study

Sarah Mubarak Alkahtany, Sara Suliman Alrumaih, Mona Abdullah Alhassan, Basmah Ahmad Alnashmi, Ebtissam M. Al-Madi

Pages 407-411 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/2-D19_1017_Sarah_M_Alkahtany_Saudi_Arabia.pdf]

EXPERIMENTAL ARTICLE

3. Viability Test of Fish Scales Collagen from Oshphronemus Gouramy on Osteoblast Cell Culture

Agung Krismariono, Novia Wiyono, Chiquita Prahasanti

Pages 412-416 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/3-D20_1028_Agung-Krismariono_Indonesia.pdf]

EXPERIMENTAL ARTICLE

4. Isolation and Antimicrobial Activity of Lactic Acid Bacteria against Streptococcus Mutans

Nor Zaihana Abdul Rahman, Rohazila Mohamad Hanafiah, Siti Aisyah Abd Ghafar, Norafiqa Abdullah, Nur Nabilah Azman

Pages 417-421 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/4-D19_979_NOR_ZAIHANA_BINTI_ABDUL_RAHMAN_Malaysia.pdf]

EXPERIMENTAL ARTICLE

5. Clinical Control of Denture Base Acrylics Polymerization for the Quality Assurance: Pilot Study of Spectroscopic Approach

Yuriy Lokota, Ivan Paliichuk, Volodymyr Paliichuk, Myroslav Goncharuk-Khomyn

59. Recent Updates of the Oral Benefits of Mangosteen Plant Extracts: Review

Mohammed Aljunaid, Ninuk Hariyani, Retno Indrawati Roestamadji, Rini Devijanti Ridwan, Tuti Kusumaningsih, Huda Rashad Qaid

Pages 752-757 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/59-D20_1068_Mohammed_Ahmed-Al_junaid_Indonesia.pdf]

MEDICINE

EXPERIMENTAL ARTICLE

60. The Effect of the Neurotransmitter Dopamine, Lead Acetate, L-NAME, and Verapamil on the Metabolic Pathway in the Longitudinal Smooth Muscle

Rrahman Ferizi, Nora Shabani, Ragip Shabani, Naim Haliti

Pages 758-768 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/60-M19_975_Rrahman_Ferizi_Kosovo.pdf]

EXPERIMENTAL ARTICLE

61. In Vitro Assay of Cornea Artificial Properties

Prihartini Widiyanti, Reni Prastyani

Pages 769-773 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/61-M19_942_Prihartini_Widiyanti_Indonesia.pdf]

EXPERIMENTAL ARTICLE

62. Smoke Effects of Disturbances Folliculogenesis (Mda, Gnrh, Hsp70, Apoptosis, and Follicles) in Ovarian on Mice Balb / C

Eny Susanti, I Ketut Sudiana, Hendy Hendarto

Pages 774-777 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/62-M19_1018_Eny_Susanti_Indonesia.pdf]

EXPERIMENTAL ARTICLE

63. Lung Dendritic Cells Express Higher Stress Proteins on Higher Allergen Dose Exposure and Contribute to Allergen Tolerance Induction

Gatot Soegiarto, Agustina Tri Endharti, Wibi Riawan, Anang Endaryanto, Subijanto Marto Sudarmo

Pages 778-784 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/63-M20_1056_Gatot_Soegiarto_Indonesia.pdf]

EXPERIMENTAL ARTICLE

64. The Increasing Inflammatory Cells, Degeneration and Duodenal Necrosis on Infant Mice from Carbofuran Exposed Mothers

1. Z. Yanti, H. A. Hermadi, P. S. Rejeki, Y. Dhamayanti, Widjiati, E. M. Luqman

Pages 785-790 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/64-M20_1074_Epy_Muhammad_Luqman_Indonesia.pdf]

CLINICAL ARTICLE

65. The Effect of Serum and Follicular Fluid Vitamin D on Intracytoplasmic Sperm Injection Outcome

Israa Majeed, Mohammad Oda Selman, Ban J. Qasim, Ghasak Ghazi faisal

Pages 791-795 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/65-M19_1008_Ghassak_Ghazi_Faisal_Malaysia.pdf]

CLINICAL ARTICLE

66. Predictors of Time Delay in Commencing Primary Coronary Intervention in STEMI Kosova Case-Pilot Study

Hajdin Çitaku, Ramë Miftari, Fatmir Ferati, Xhevdet Krasniqi

Pages 796-800 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/66-M19_1003_Hajdin_Çitaku_Kosova.pdf]

CLINICAL ARTICLE

67. Profile of Predictive Factors of Response to Therapy in Patients with Diffuse Large B-cell Lymphoma in dr Soetomo General Teaching Hospital Surabaya

Mochammad Dilliawan, Siprianus Ugroseno Yudho Bintoro, Putu Niken Ayu Amrita

Pages 801-807 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/67-M20_1102_Hendrik_Setia_Budi_Siprianus_U_Y_B_Indonesia.pdf]

CLINICAL ARTICLE

68. Evaluating the Treatment of Patients with Appendicitis, Perspectives on Challenges Professional Work

Kadir Hysein, Valon Morina, Zeqir Hashani, Qenan Maxhuni, Rrahman Ferizi

Pages 808-815 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/68-M20_1063_Rrahman_FERIZI_Kosova.pdf]

CLINICAL ARTICLE

69. Comparison Study between Angio CT and USG Doppler for Early Detection of Arterial Stenosis of Lower Extremities in University Clinical Center of Kosovo

Lavdim H. Ymeri, Vjosa A.Zejnullahu, Serbeze Kabashi Muqaj, Muharrem Sadiku, Valon A.Zejnullahu

Pages 816-823 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/69-M20_1070_Valon_Zejnullahu_Kosovo.pdf]

REVİEW

70. Curcumin as Adjuvant Therapy in COVID-19: Friend or Foe?

Jeanne Adiwinata Pawitan

Pages 824-829 Full Text PDF [http://www.jidmr.com/journal/wp-content /uploads/2020/06/70-M20_1144_Jeanne_Adiwinata_Pawitan_Indonesia.pdf]

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Lung Dendritic Cells Express Higher Stress Proteins on Higher Allergen Dose Exposure and Contribute to Allergen Tolerance Induction

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Abstract

Different doses of allergen lead to different T lymphocyte responses which partly explain the phenomenon of low or high dose tolerance. Dendritic cells (DC) are responsible for driving the T lymphocyte responses to a variety of exogenous stimuli, but the mechanisms are not completely understood.

Elucidate the mechanisms of how DCs drive the differentiation of T lymphocytes in response to different doses of allergen.

Groups of male BALB/c mice (n=4-5) were sensitized intraperitoneally with sham or different doses (low: 10 μg, high: 1000 μg) of Der P1 (house dust mite allergen). They were exposed daily to aerosolized Der p1 allergen for 7 consecutive days. Different concentrations of Der p1 solution were nebulized: sham, low dose (15 μg/mL), or high dose (1500 μg/mL). Bronchoalveolar lavage fluid (BALF) was obtained from the lungs. Levels of IL-12 in BALF were measured. Lung tissue sections were then stained to detect the expression of Hsp70 by lung DCs.

Lung DCs exposed to a higher sensitizing doses of Der p1 allergen tend to express significantly higher levels of Hsp70 and secrete higher levels of IL-12 in BALF. There was a significant positive correlation between the levels of Hsp70 expression by lung DCs with IL-12 levels in BALF (r=0.581).

Higher exposure doses of allergen puts lung DCs under stressed condition thereby induced high expression of 'stress proteins' which may explain the mechanism of DCs drive the T lymphocyte response.

Experimental article (J Int Dent Med Res 2020; 13(2): 778-784)

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Introduction

Different exposure doses of allergen leads to different direction of T lymphocyte responses which partly explain the phenomenon of low or high dose tolerance [1–3]. Dendritic cells are cells that responsible for driving the appropriate T lymphocyte responses to a variety of exogenous stimuli [4,5], but the mechanisms by which they perform that task are not completely understood.

Other studies have shown that dendritic cells exposed to some stressful conditions from the environment (heat, ultraviolet radiation or heavy metals), pathological insults (infections or malignancies), or physiological stimuli (growth factors or cell differentiation) induce a marked increase in heat shock proteins (HSPs) synthesis, a phenomenon known as the stress response[6–8].

These HSPs are shown to induce the production and release of a variety of pro-inflammatory cytokines, including IL-12, nitric oxide and C-C chemokines by monocyte, macrophage and dendritic cells. They also induce the maturation of dendritic cells which enable these cells to activate T lymphocytes as demonstrated by the up-regulation of MHC class

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I and II molecules, co-stimulatory molecules such as B7-1 and B7-2[9–11]. Recently, it has been shown that HSPs play an important role in antigen direct and cross-presentation because they can bind antigenic peptides generated within the cells, form HSP-peptide complexes. When released into extracellular compartment the HSP-peptide complexes can be recognized and taken up by other dendritic cells via CD91-mediated endocytosis, resulting in representation of the antigenic peptide by dendritic cells to T lymphocytes with the peptide specific receptor [9,12,13].

Using cellular stress and homeostasis paradigm, it can be speculated that dendritic cells exposed to high dose of allergen are under stressful condition and thereby release or produce stress protein such as HSPs and certain cytokines. The present study aim to answer the following questions: can the mechanism by which dendritic cells drive the differentiation of T lymphocytes under different exposure doses of allergen be explained through the 'cellular stress concept', and are dendritic cells that exposed to high doses of allergen indeed under stress and thereby express or secrete high amount of 'stress proteins' which also act as 'danger signals' that used by dendritic cells to drive the differentiation of T lymphocytes?

Materials and methods

Mice

Male inbred BALB/c mice of about 8-10 weeks old were purchased from Japan SLC, Inc. (Shizuoka, Japan) via PN Biofarma (Bandung, Indonesia). Food and water were provided ad libitum and mice were kept in a 12 h-light, 12 h-dark cycle in a specific pathogen free condition according to Federation of European Laboratory Animal Science Associations (FELASA) recommendation [14]. The local Ethical Committee (ACUC Veterinary Faculty Universitas Airlangga) approved the in vivo manipulations used in this study with certificate No. 150-KE.

Sensitization and Allergen Challenge Protocol

Sensitization and challenge were performed according to the method by Tournoy et al [15] with some modifications. The lyophilized natural major house dust mite allergen (Der p1) was purchased from Greer Laboratories (Lenoir, N.C., USA). Groups of mice (n=4-5) were sensitized intraperitoneally with sham (phosphate-buffered saline/PBS), or low dose (10 μg), or high dose (1000 μg) injection of major house dust mite allergen (Der p1) adsorbed to 20:1 ratio of aluminium hydroxide ($Al(OH)_3$) solution (Pierce Biochemicals, Thermo Fisher Scientific (Hong Kong) Ltd, Shatin, Hong Kong). Fourteen days later, the mice were exposed daily to aerosolized Der p1 allergen for 7 consecutive days (days 15-21). The mice were individually placed in a 50-ml plastic tube and exposed to aerosolized Der p1 allergen for 30 min. The aerosol was produced by Pulmo-Aide E0570 compresor nebulizer (DeVilbiss Corp., Somerset, PA, USA), driven by compressed air at 5-9 L/min. Different concentrations (doses) of Der p1 solution were nebulized: sham (PBS), low dose (15 μg/mL), or high dose (1500 μg/mL) solutions. The illustration for the protocol can be seen in Figure 1.

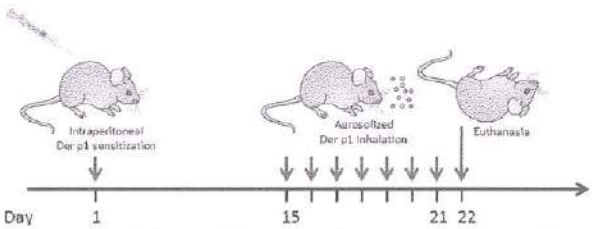

Figure 1. The scheme of our study protocol: mice intraperitoneal sensitization, aerosol inhalation exposure, and euthanasia.

Bronchoalveolar Lavage (BAL) and Measurement of IL-12

Mice were put under anesthesia at 24 h after the last challenge (day 22). BALF was obtained from the whole lungs by inserting a cannula into the surgically exposed trachea for cell analysis. BAL was performed via 3 intratracheal instillations with 0.3 ml Hank's balanced salt solution (HBSS) + 1% Bovine Serum Albumin (BSA), followed by 3 instillations with 1 ml of HBSS to collect cells for cytospin analysis as previously described [15,16]. The BAL fluid of the first three fractions was centrifuged and the supernatant was used for cytokine (IL-12) detection and measurement. IL-12 levels in BALF were measured using standard indirect ELISA method. After staining with May-Grünwald-Giemsa, total and differential cell counts were done using standard morphologic criteria.

Preparation of Lung and Lymph nodes

Immediately after BAL, the mice were killed by right cardiac puncture and blood aspiration using an 18 gauge polyurethane catheter (Becton Dickinson, Madrid, Spain). The collected blood was used for measurement of serum cytokines. Pulmonary and systemic circulation were then perfused with saline/EDTA to remove the intravascular pool of cells. Paratracheal and parathymic intrathoracic lymph nodes were collected in steril petri dishes on ice for other studies. Lungs were carefully separated from thymic and cardiovascular remnants and removed in toto, including the main brochi and trachea. Left lung was separated for histological and immunohistochemical examination. Right lung was thoroughly minced using iridectomy scissors and incubated for 30 min in digestion medium in a humidified incubator at 37°C and 5% CO_2, according to a modified protocol [17] and further used for other studies.

Histological examination

For histological examination, the left lung was removed and fixed in 4% paraformaldehyde. Then, the tissues were embedded in resin (Jung HistoResin Plus; Reichert/Jung, Heidelberg, Germany) and cut into 2-μm sections, which were stained with hematoxylin and eosin (H–E).

Measurement of Hsp70 expression by lung dendritic cells

For immunohistochemical analysis, some of the lung tissue sections were deparaffinized and underwent standard procedures for antigen retrieval[18]. Tissue sections were then double stained to detect the expression of Hsp70 by lung dendritic cells. Anti CD11c and anti-hsp70 monoclonal antibodies were used (Santa Cruz Biotechnology, Santa Cruz, CA, USA). CD11c positive dendritic cells were stained with 3,3'-diaminobenzidine (DAB), while Hsp70 were stained with light violet blue (LV blue). Methyl green was used as counterstaining. Tissue sections for this process were mounted using 5% gelatin. Examination and interpretation of Hsp70 expression by lung dendritic cells was done by two independent observers. Expression scoring was done according to modification of the method of Soini et al[19] and Pizem et al [20]. Scores from both observers were checked for consistency.

Statistical Analysis

Data were analyzed with the statistical package SPSS 16.0 (SPSS Inc., Chicago, IL). Values are expressed as mean ± SD. To check the consistency of Hsp70 expression scoring by two independent observers paired t test and Pearson's correlation were used. Kolmogorov-Smirnov test was used to check the normality of data distribution, and Levine's test was used to check the homogeneity of data variant. The differences between the groups were tested using ANOVA. For data with abnormal distribution Kruskal-Wallis A and Kendall Tau analysis were used. Pearson's test was used to determine correlation between Hsp70 expression scores and IL-12 levels. $P < 0.05$ was accepted as statistically significant.

Results

Lung Dendritic Cells Exposed to High Sensitizing and Inhalation Doses of Der p1 Allergen Express High Levels of Hsp70

The expression of Hsp70 in lung dendritic cells under different sensitizing and inhalation doses of Der p1 allergen is shown in Figure 2.

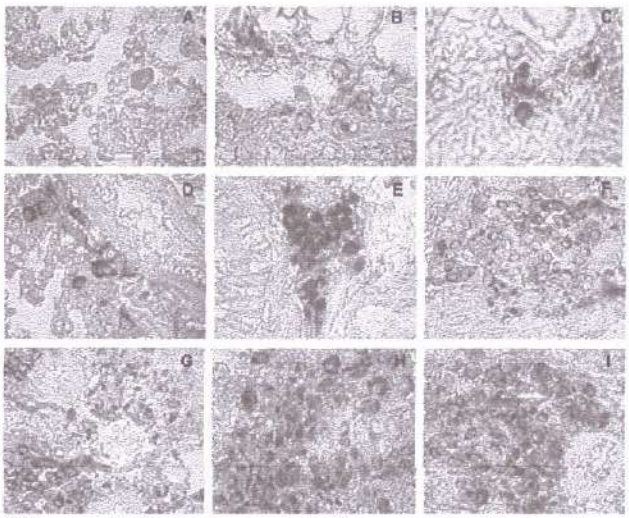

Figure 2. The representative expressions of heat shock protein Hsp70 by lung dendritic cells under different sensitizing and inhalation dose of Der p1 allergen.

The above figures shows the representative expressions of heat shock protein 70 (Hsp70) by lung dendritic cells in groups of mice (n=4-5) exposed to different sensitizing and inhalation

dose of Der p1 allergen as seen under light microscopy (1000x magnification) in immunohistochemistry (IHC) lung tissue sections. We use double staining technique to reveal lung dendritic cells (CD11c positive) which express Hsp70 (Hsp70 positive). (A: control mice, B: sham sensitization + low dose inhalation, C: sham sensitization + high dose inhalation, D: low dose sensitization + sham inhalation, E: low dose sensitization + low dose inhalation, F: low dose sensitization + high dose inhalation, G: high dose sensitization + sham inhalation, H: high dose sensitization + low dose inhalation, I: high dose sensitization + high dose inhalation).

Interpretation and scoring were done by two independent observers, both showed high consistency (t=1.922, p=0.063; r=0.974, p≤0.001). The relative expression scores for each group of interventions were measured and shown in Figure 3.

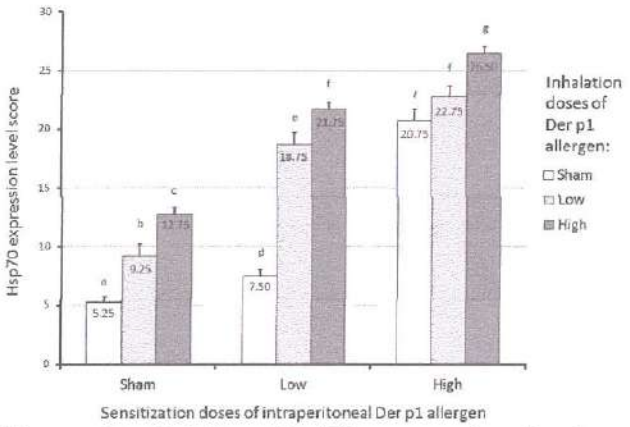

Figure 3. Relative Hsp70 expression by lung dendritic cells under different sensitizing and inhalation dose of Der p1 allergen.

Mean values of relative heat shock protein 70 (Hsp70) expression in each group of mice (n=4-5) and the respective standard error bars were presented. Groups marked with different alphabet (a,b,c,d,e,f, and g) showed significantly different expression score ($p<0.05$). Groups with the same alphabetical marks had non-significant difference in Hsp70 expression.

Lung dendritic cells that were exposed to a higher intraperitoneal sensitizing doses of Der p1 allergen tend to express significantly higher levels of Hsp70 (Figure 3). This tendency was also seen if we modulate the inhalation dose of Der p1 allergen (low dose inhalation, grey bars, and high dose inhalation, dark grey bars). Coupling effects were seen, as the higher the sensitizing doses and the higher the inhalation doses, the higher the expressions of Hsp70. The non-significant difference of Hsp70 expression between sham inhalation and low inhalation in groups with high sensitization dose (20.75 vs. 22.75 respectively, $p>0.05$), indicates that the sensitizing dose is more important in determining the Hsp70 expression by lung dendritic cells than the inhalation dose.

Higher Level of IL-12 was Detected in BALF in Group of Mice Exposed to Higher Sensitizing and Inhalation Doses of Der p1 Allergen

Similar to the expression levels of Hsp70 by lung dendritic cells, higher intraperitoneal sensitizing doses of Der p1 allergen tend to result in increased levels of IL-12 in BALF (Figure 4). The same tendency was also seen if we modulate the inhalation dose of Der p1 allergen (low dose inhalation, grey bars, and high dose inhalation, dark grey bars). Coupling effects were also seen, as the higher the sensitizing doses and the higher the inhalation doses, the higher the levels of IL-12 in BALF, particulary in groups which received high sensitization doses of intraperitoneal Der p1 allergen (143.54, 204.46, and 328.04 respectively, $p<0.05$).

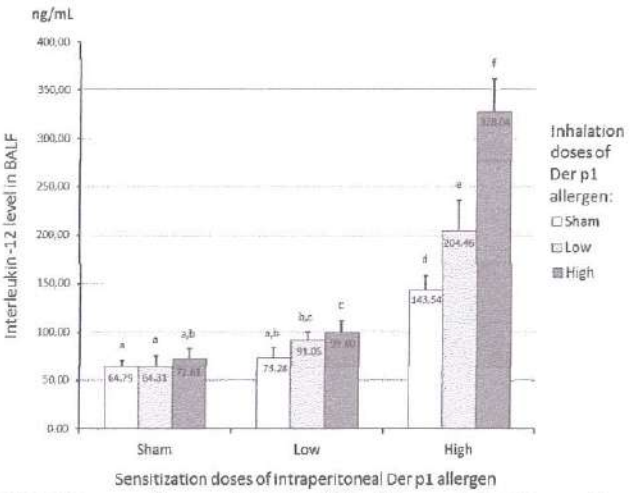

Figure 4. Interleukin-12 levels in bronchoalveolar lavage fluid under different sensitizing and inhalation dose of Der p1 allergen.

Mean values of interleukin-12 (IL-12) levels in each group of mice (n=4-5) and the respective standard error bars were presented. Groups marked with different alphabet (a,b,c,d,e, and f) showed significantly different IL-12 levels compared to other groups ($p<0.05$). Groups with the same alphabetical marks had non-significant difference in IL-12 levels.

The expression levels of Hsp70 by lung dendritic cells significantly correlated with IL-12 levels in BALF

There was a significant positive correlation between the levels of Hsp70 expression by lung dendritic cells with IL-12 levels in BALF ($r=0.581$; $p\leq0.001$; Figure 5). This result implies that the stressed lung dendritic cells expressed high levels of stress protein which also associated with the levels of IL-12, which in turn contribute to the induction of Th1 type immune response or a shift from the initial Th2 type immune response.

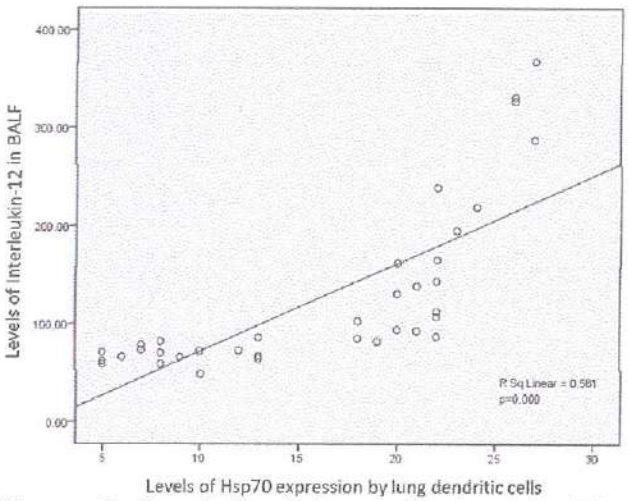

Figure 5. Correlation between levels of Hsp70 expression by lung dendritic cells with interleukin-12 levels in bronchoalveolar lavage fluid (BALF).

The higher the heat shock protein 70 (Hsp70) expression levels by lung dendritic cells, the higher the IL-12 levels in BALF ($r=0.581$, $p\leq0.001$).

Discussion

One of the mechanisms by which dendritic cells drive the differentiation of T lymphocytes can be explained through the 'cellular stress concept'. As has been shown in the present study, dendritic cells that exposed to a high sensitization and inhalation dose of allergen (Der p1) are indeed under stress, as demonstrated by high level of 'stress protein' (Hsp70) expression compared to dendritic cells that exposed to 'usual' or low sensitization and inhalation dose.

'Cellular stress concept' stated that any cell exposed to a variety of environmental stressors, including extremes of temperature, ultraviolet radiation, toxins, or infections undergo some damage in the cellular component which interfere with cellular essential functions. In response to this, the 'stressed' cell utilize certain cellular stress response that serve as an adaptive purpose to protect itself from unfavourable environemental conditions, both through short term mechanisms that minimize acute damage to the cell's overall integrity, and through longer term mechanisms which provide the cell a measure of resiliency against similar adverse conditions. One of the mechanisms that has been extensively studied is the expression heat shock or heat stress proteins (HSPs)[6]. Traditionally, HSPs are regarded as intracellular molecules which only released into extracellular compartments upon necrotic cell death. However, recently it has been shown that HSPs can be actively released extracellularly in response to a number of stressful conditions[20]. Our study result showed that exposure to high dose of allergen can be viewed as one of the stressful conditions.

Functionally, stress-inducible proteins can be grouped into seven classes. The predominant class of HSPs, the molecular chaperones, comprises five major and broadly conserved families-Hsp100s, Hsp90s, Hsp70s, Hsp60s, and small HSPs[6]. One of the most highly conserved chaperones is the Hsp70 protein. Under physiological conditions, this protein are involved in the de novo folding of proteins, but during and following stressful conditions, Hsp70 functions as a chaperone which enable the cell to cope with harmful aggregations of denaturated proteins. Thus, its extracellular expression confers protection against stresses that induce protein damage[21]. These phenomenon were observed in cultured cells, animal models, and human tissues, resulting from various stressful stimuli [22,23]. The mechanisms of active HSP release are somewhat controversial because initially researchers cannot identify any signal peptide

that targets this protein for classical secretion. Recent findings suggest that HSP was released by a non-classical protein transport pathway that requires intact membrane lipid rafts for efficient release. Others also demonstrated that HSP were secreted within exosomes[24–26].

One of the proposed functions of extracellular HSP is immune activity modulation. Many of these effects are mediated by cell surface receptors such as c-type lectin receptors and scavenger receptors expressed on a wide range of cell types[27]. Secreted Hsp70 were shown to induce significant cytokines release including IL-1β, TNF-α, and IL-12 by macrophages and dendritic cells[28–30]. Our study confirmed these findings and showed that lung dendritic cells exposed to a higher dose of Der p1 allergen not only express higher level of Hsp70 but also secrete higher level of IL-12. Our study found a significant positive correlation between the levels of Hsp70 expression by lung dendritic cells with IL-12 levels in BALF. It is of particular interest that other studies also observed a significant increase in Hsp70 (and thus, antibodies against Hsp70) in subjects who were exposed to environmental stresses such as carbon monoxide, heat, and dust [31]. Although not specifically mentioned in their report, we know that house dust mite like *Dermatophagoides pteronyssinus* is the dominant component of dust in the living environment [32]. Hsp70 was also reported to be present in the skin of allergic contact hypersensitivity model[33], indicating that Hsp70 is also an important mediators of allergic inflammation. All of those findings, either directly or indirectly, supported our study results.

As stated previously, extracellular HSP had been reported to activate macrophages, and dendritic cells via a receptor-mediated process [28,34]. Activated and mature macrophages or dendritic cells then express some costimulatory signals that are needed for naïve T lymphocytes priming and activation[35]. In our study, the expression of Hsp70 and the secretion of IL-12 can be viewed as the third signals that drive T lymphocyte differentiation.

In conclusion, using our approach with differential sensitizing and inhalation doses of allergen, we elucidate one of the mechanisms by which dendritic cells sensing the 'danger' or the 'stressful condition' in the microenvironment, and make use the 'stress protein' they express or secrete to alert naïve T lymphocytes dan drive their appropriate direction of differentiation and ultimately determine the immune response toward immune tolerance to allergen. With this 'cellular stress concept', we can prove that the more 'stressed' the dendritic cells, the more 'stress protein' they secrete, and.

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Declaration of Interest

All of the authors declare that there are no potential conflicts of interest in relation to this manuscript writing and publication.

References

1. Woodfolk JA. High-dose allergen exposure leads to tolerance. Clinical reviews in allergy & immunology. 2005;28(1):43-58.
2. Michallet M-C, Saltel F, Flacher M, Revillard J-P, Genestier L. Cathepsin-Dependent Apoptosis Triggered by Supraoptimal Activation of T Lymphocytes: A Possible Mechanism of High Dose Tolerance. The Journal of Immunology. 2004;172(9):5405-14.
3. Nurieva RI, Liu X, Dong C. Molecular mechanisms of T-cell tolerance. Immunological reviews. 2011;241(1):133-44.
4. Kadowaki N. Dendritic Cells—A Conductor of T Cell Differentiation—. Allergology International. 2007;56(3):193-9.
5. Diebold SS. Determination of T-cell fate by dendritic cells. Immunology & Cell Biology. 2008;86(5):389-97.
6. Richter K, Haslbeck M, Buchner J. The Heat Shock Response: Life on the Verge of Death. Molecular Cell. 2010;40(2):253-66.
7. Bakthisaran R, Tangirala R, Rao CM. Small heat shock proteins: Role in cellular functions and pathology. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics. 2015;1854(4):291-319.
8. Fulda S, Gorman AM, Hori O, Samali A. Cellular Stress Responses: Cell Survival and Cell Death. International Journal of Cell Biology. 2010;2010:23.
9. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-κB pathway. International immunology. 2000;12(11):1539-46.
10. Panjwani NN, Popova L, Srivastava PK. Heat Shock Proteins gp96 and hsp70 Activate the Release of Nitric Oxide by APCs. The Journal of Immunology. 2002;168(6):2997-3003.
11. Wang Y, Kelly CG, Singh M, McGowan EG, Carrara A-S, Bergmeier LA, et al. Stimulation of Th1-Polarizing Cytokines, C-C Chemokines, Maturation of Dendritic Cells, and Adjuvant Function by the Peptide Binding Fragment of Heat Shock Protein 70. The Journal of Immunology. 2002;169(5):2422-9.

12. Li Z, Menoret A, Srivastava P. Roles of heat-shock proteins in antigen presentation and cross-presentation. Current Opinion in Immunology. 2002;14(1):45-51.
13. Murshid A, Gong J, Calderwood SK. The role of heat shock proteins in antigen cross presentation. Frontiers in immunology. 2012;3:63-.
14. Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 Is a Common Receptor for Heat Shock Proteins gp96, hsp90, hsp70, and Calreticulin. Immunity. 2001;14(3):303-13.
15. Mähler M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Laboratory Animals. 2014;48(3):178-92.
16. Tournoy, Kips, Schou, Pauwels. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. Clinical & Experimental Allergy. 2000;30(1):79-85.
17. Moerloose KB, Robays LJ, Maes T, Brusselle GG, Tournoy KG, Joos GF. Cigarette smoke exposure facilitates allergic sensitization in mice. Respiratory research. 2006;7(1):49-.
18. Hove CLV, Maes T, Joos GF, Tournoy KG. Prolonged Inhaled Allergen Exposure Can Induce Persistent Tolerance. American Journal of Respiratory Cell and Molecular Biology. 2007;36(5):573-84.
19. Misharin AV, Morales-Nebreda L, Mutlu GM, Budinger GRS, Perlman H. Flow cytometric analysis of macrophages and dendritic cell subsets in the mouse lung. American journal of respiratory cell and molecular biology. 2013;49(4):503-10.
20. D'Amico F, Skarmoutsou E, Stivala F. State of the art in antigen retrieval for immunohistochemistry. Journal of Immunological Methods. 2009;341(1):1-18.
21. Soini Y, Pääkkö P, Lehto VP. Histopathological evaluation of apoptosis in cancer. The American journal of pathology. 1998;153(4):1041-53.
22. Pizem J, Coer A. Detection of apoptotic cells in tumour paraffin sections. Radiology and Oncology. 2003;37(4):225-32.
23. Liao D-F, Jin Z-G, Baas AS, Daum G, Gygi SP, Aebersold R, et al. Purification and Identification of Secreted Oxidative Stress-induced Factors from Vascular Smooth Muscle Cells. Journal of Biological Chemistry. 2000;275(1):189-96.
24. Daugaard M, Rohde M, Jäättelä M. The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. FEBS Letters. 2007;581(19):3702-10.
25. Tang D, Khaleque MA, Jones EL, Theriault JR, Li C, Wong WH, et al. Expression of heat shock proteins and heat shock protein messenger ribonucleic acid in human prostate carcinoma in vitro and in tumors in vivo. Cell stress & chaperones. 2005;10(1):46-58.
26. Rajdev S, Hara K, Kokubo Y, Mestril R, Dillmann W, Weinstein PR, et al. Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. Annals of Neurology. 2000;47(6):782-91.
27. Bausero MA, Gastpar R, Multhoff G, Asea A. Alternative mechanism by which IFN-gamma enhances tumor recognition: active release of heat shock protein 72. Journal of immunology (Baltimore, Md : 1950). 2005;175(5):2900-12.
28. Lancaster GI, Febbraio MA. Exosome-dependent Trafficking of HSP70: A NOVEL SECRETORY PATHWAY FOR CELLULAR STRESS PROTEINS. Journal of Biological Chemistry. 2005;280(24):23349-55.
29. Vega VL, Rodríguez-Silva M, Frey T, Gehrmann M, Diaz JC, Steinem C, et al. Hsp70 Translocates into the Plasma Membrane after Stress and Is Released into the Extracellular Environment in a Membrane-Associated Form that Activates Macrophages. The Journal of Immunology. 2008;180(6):4299-307.
30. Murshid A, Theriault J, Gong J, Calderwood SK. Investigating receptors for extracellular heat shock proteins. Methods in molecular biology (Clifton, NJ). 2011;787:289-302.
31. Colaco CA, Bailey CR, Walker KB, Keeble J. Heat Shock Proteins: Stimulators of Innate and Acquired Immunity. BioMed Research International. 2013;2013:11.
32. Somensi N, Brum PO, de Miranda Ramos V, Gasparotto J, Zanotto-Filho A, Rostirolla DC, et al. Extracellular HSP70 Activates ERK1/2, NF-kB and Pro-Inflammatory Gene Transcription Through Binding with RAGE in A549 Human Lung Cancer Cells. Cellular Physiology and Biochemistry. 2017;42(6):2507-22.
33. Mansilla MJ, Costa C, Eixarch H, Tepavcevic V, Castillo M, Martin R, et al. Hsp70 Regulates Immune Response in Experimental Autoimmune Encephalomyelitis. PLOS ONE. 2014;9(8):e105737.
34. Wu T, Tanguay RM. Antibodies against heat shock proteins in environmental stresses and diseases: friend or foe? Cell stress & chaperones. 2006;11(1):1-12.
35. Kim J, Lee S, Woo S-y, Han Y, Lee JH, Lee I-Y, et al. The indoor level of house dust mite allergen is associated with severity of atopic dermatitis in children. Journal of Korean medical science. 2013;28(1):74-9.
36. Yusuf N, Nasti TH, Huang C-M, Huber BS, Jaleel T, Lin H-Y, et al. Heat shock proteins HSP27 and HSP70 are present in the skin and are important mediators of allergic contact hypersensitivity. Journal of immunology (Baltimore, Md : 1950). 2009;182(1):675-83.
37. Paltsev M, Severin S, Danilevskii M, Moskaleva EY. Regulation of the physiological functions of human dendritic cells by recombinant heat shock protein Hsp70. Neuroscience and Behavioral Physiology. 2011;41(1):74-82.
38. Sallusto F, Lanzavecchia A. The instructive role of dendritic cells on T-cell responses. Arthritis research. 2002;4 Suppl 3(Suppl 3):S127-S32.