日本財団 助成事業
The Nippon Foundation

２００１年度日中医学協会共同研究等助成事業報告書

－在留中国人研究者研究助成－

2002年 3月 8日

財団法人 日中医学協会
理事長 殿

ユ セツヒョー
研究者氏名 兪 雪氷 ㊞
所属機関名 順天堂大学皮膚科
指導責任者氏名 小川 秀興 (印)
職名 教授
所在地 〒113-8421 東京都文京区本郷2-1-1
電話 03-3813-3111 内線 4448

1．研究テーマ

日本人軟癖患者におけるレトロウイルス様粒子の発見とその局在について

2．本年度の研究業績

(1) 学会・研究会等における発表 (有)・無 （学会名・演題）

日本研究皮膚科学会第26回年次学術大会・総会.
2001年9月7-8日. 愛媛県.松山市.

演題：日本人軟癖患者におけるレトロウイルス様粒子の発現とその局在について

(2) 学会誌等に発表した論文 (有)・無 （雑誌名・論文名）

<The Journal of Investigative Dermatology >> Vol. 117. No. 6
Dec. 2001. 1654-6.

Mutations of ATP2C1 in Japanese Patients with Hailey-Hailey Disease: Intrafamilial and Interfamilial Phenotype Variations and Lack of Correlation with Mutation Patterns.

3．今後の研究計画

1）本抗体（anti pso p27）が乾癬患者中のレトロウイルス様粒子に反応することを確認する。そのために、乾癬患者尿を採取し、密度勾配遠心分離法とウエスタンブロット法により、レトロウイルス様粒子を分離精製する。その後免疫電顕にて確認を行う。

2）本抗体がsquwamous cell carcinoma antigen (SCCA)と交差反応性を有するか検討する。pso p27 の部分アミノ酸配列が SCCA と相同性を有するためである。方法は、SCCA の mRNA を RT-PCR にて増幅、pCMV expression vector に挿入する。それを COS1 細胞に遺伝し導入し、Flag つきリコンビナント蛋白を回収し、anti pso p27 抗体で染色する。

3）最終的に、pso p27 をコードする遺伝子を同定する。本レトロウイルス様粒子は表皮肥満細胞に発現しているため、肥満細胞セルライン（本大学アトピー疾患研究センター）に本レトロウイルス様粒子を感染さ、それより cDNA ライブラリーを作製する。本抗体を用いて、expression スクリーニングを行う。

4．指導責任者の意見

乾癬は、罹患率が国民の約 0.2～0.3％と推定されており、数百万人の患者が存在すると考えられている。また原因不明で、根本的治療がないため、患者の QOL のみならず、医療経済的にも社会的問題となっている。本症の一因としてレトロウイルスの関与とその対策方法が明らかになれば、新治療法開発につながるだけでなく、社会的にも大変有意義と考えられる。俞医師は、勤勉かつ積極的に研究を行っており、今後上記に予定されている課題を早急にまとめることが可能と思われる。

指導責任者氏名 小川秀興 小川

5．研 究 報 告 書

別紙報告書作成要領により、添付の用紙で研究報告書を作成して下さい。

研究発表中または研究中の本人のスナップ写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

※発表に当っては、*日中医学協会助成金による*旨を明記して下さい。

日中医学協会助成事業

日本人乾癬患者におけるレトロウイルス様粒子の発見とその局在について

研究者氏名　　兪　雪氷
中国所属機関　北京航天医学研究所
日本研究機関　順天堂大学皮膚科
指導責任者　　教授　小川　秀興
共同研究者名　池田　志斐

Abstract

Retrovirus-like particles Pso p27 in psoriatic skin lesion were initially discovered in 1983. A monoclonal antibody (moAb) against internal protein, pso p27, of the particles was established in 1988. And the antibody has been reported to react to the stratum comeum cells, some spinous cells and some tryptase-positive dermal infiltrating cells of the psoriatic skins. In this study, we examined the reactivity of the moAb to the skins from Japanese patients with psoriasis by immun-fluorescent (IF) and immuno-electron microscopy (IEM). Frozen sections and paraffin sectiona were prepared from the lesion skins of 24 unrelated Japanese psoriatic patients, then proceeded by standard IF procedure. The skin reacted to the moAb by IF was immersed into parafomaldehyde and serially diluted sucrose solutions, then proceeded for IEM by standard procedure using gold conjugated goat anti mouse IgG. The results showed that positive reactivity was detected on the stratum comeum cells in 12 out of 24 patients lesion skins. By IEM, vesicles-like structure and surface of stratum comeum cells, as moAb, however, further confirmation by using immuno-gold labeled antibody should be required to exclude the effect of endogenous peroxidase from the cells such as neutrophils and mast cells. Conclusion: retrovirus-like particles present in the skin of some Japanese patients with psoriasis. And the retrovirus-like particles may participate in the pathophysiology of the disease.

Key words: psoriasis; pso p27 antigen; immunofluorescence; IEM.

Introduction

The psoriasis-associated antigen, pso p27, can be isolated from psoriasis scale and is present in complement-activating immune complexes in psoriatic scale, and in serum from patients with psoriasis. pso p27 antigen was localized to a sub-fraction of dermal cells and in the endothelial lining of some of dermal vessels. The antigen is produced by tryptase-positive cells in the skin lesions and is shown to be a major antigen in the immune reactions in psoriasis.

The infiltration of inflammatory cells, which is an initial event in the development of skin lesions in psoriasis, strongly suggests that immune reaction play a major role in the pathogenesis of this condition.

Immunological analyses have shown that a protein present in psoriatic scale, pso p27, participates in the generation of complement-activating immune complexes, both in the psoriatic plaques (1 ^ 3) and in synovial liquid of patients with psoriasis arthritis (1). Recently, we presented evidence that the pso p27 antigen is expressed by mast cells in the skin lesion (4). The expression of the antigen was reduced or omitted when the lesions were in remission, even if the mast cells were still pre-sent. Indicating a direct correlation between the inflammatory and the expression of pso p27 antigen.

In this study we followed 24 patients with extensive psoriasis in order to investigate the percent of the expression of pso p27 antigen at the psoriasis patients and to observe the expression by immuno-electron microscopy (IEM).

-------The main pathological changes in psoriasis are a hyperproliferation of epidermal keratinocytes and an inflammatory reaction in the dermis with infiltration of lymphocytes and macrophages. In established lesions polymorphonucler leukocytes are regularly noted in the epidermis, often present in the characteristic Munro micro abscesses.

Material and method

Specimens

Punch biopsies (3-4mm) were obtained from skin lesions of 24 patients suffering from static chronic plaque psoriasis and 4 normal persons. The specimens were frozen with Tissue-Tek O.C.T. Compound in liquid nitrogen and stored at –80C or embedded in paraffin after fixation in ethanol. [1] [2]. Thin sections (4 - 5 mm) of the skin biopsies were analyzed with respect to pso p27 antigen using monoclonal anti-pso p27 antibodies.

Immunofluorescence analysis

Frozen sections (4-5 um thick) and deparaffined sections (5-6um) were prepared for immunofluorescence staining. Sections were fixed in 70% methyl alcohol plus 0.3%

hydrogen peroxide for ten minutes and blocked with 1% BSA, 5% skim milk and 0.1% Tween 20 of PBS for 30 minutes. Then, sections were incubated with mouse monoclonal anti-pso p27 antibodies (10ug/ml) 50ul each section overnight at 4°C. (monoclonal anti-pso p27 antibodies was provided by Pro. Ole-Jan Iversen. Departments of Microbiology, University Hospital, Norway.). After 5 times washes in 0.1% Tween 20 of PBS, sections were incubated with anti-mouse immunoglobulins FICT kaninchen F (ab') 2 IgG (DAKO. Denmark) for 1 hour at 37C. Normal skin sections following another 5 washes in PBS, sections were overlapped using antifade solution - FluoroGuard ant fade reagent (Bio-Red Laboratories. CA.USA.). Finally, sections were examined using a Leitz fluorescence microscope. Normal sink sections were as negative control and same psoriatic sections were omitted anti pso p27 antibody as positive control.

Detection pso p27 antigen by Immunological electric microscopy

Immouno-glod method:

1. Prepare sections for EM

Dissect the skin specimens to the smallest possible size for handling and good penetration. Lightly fix tissue in 2% paraformaldehyde 0.1% glutaraldehyde for 30 minutes. Wash tissue in PBS 30 minutes. Then tissues were cut to thin sections. [3]

2. Immunological staining

Then pre-embedding sections of skin tissue were blocked by 2% BSA-PBS for 1 hour. After washing in PBS, sections were incubated with the primary antibody-- mouse monoclonal anti-pso p27 antibodies (10ug/ml) 50ul each section overnight at 4°C. Following 5 washes in PBS, sections were incubated with-- anti mouse IgG: Gold conjugated (dilution to 1:50 and 1:25) for 3 hours at room temperature. Sections were washed in PBS with 1% --serum, 1% BSA, 0.1% Tween 20 and 0.1% sodium azide thoroughly and then washed in PBS for 5 times. Fixing sections in 1% glutaraldehyde for 10 minutes and washing in water 30 minutes. [4] [12].

RESULTS

Fluorescence was extension in the stratum corneum and in the scale. Fig.1. And bright fluorescence localized to a subfraction of dermal cells and at the endothelial lining of some of the dermal vessels. Fig.2. There were not meaningful fluorescence was detected both at normal skin sections and the control psoriatic sections. Fig. 3,4. The positive expression ratio of the pso p27 antigen at skin lesions of psoriasis was 50%.

Conclusion

1. Retrovirus-like particles present in the half of the skins from Japanese patients with psoriasis.
2. The retrovirus-like particles may participate in the patho-physiology of the disease.

References

1. Morten Dalaker, Trond Jacobsen, Hilde Lysvand, Iversen O-J. Expression of the Psoriasis-associated Antigen, Pso p27, is inhibited by Cyclosporin A. *Acta Derm Venereol.* 1999; 79: 281-284.
2. Iversen OJ.K. Bergh and H. Lysvand. Use of scale antiboies for the detection of antigens in psoriatic lesions. *Acta Derm Venereol.* 1993; 73:31-34.
3. Humbel BM, de Jong MD, Muller WH, Verkleij AJ. Pre-embedding immumolabeling for electron microscopy: an evaluation of permeabilization methods and markers. *Microsc Res Tech.* 1998 Jul 1; 42 (1):43-58.
4. Zwart JA, Iversen OJ, Sand T, Dale LG, Unsgard G. Higher levels of antibodies against the psoriasis-associated antigen pso p27 in cerebrospinal fluid from patients with low back pain and sciatica. Spine. 1999 Feb 15;24(4):373-7.
5. Jacobsen T, Lie BA, Lysvand H, Wiig M, Pettersen HB, Iversen OJ. Detection of psoriasis-associated antigen pso p27 in sarcoidosis bronchoalveolar lavage fluid using monoclonal antibodies. Clin Immunol Immunopathol. 1996 Oct;81(1):82-7.
6. Iversen OJ, Lysvand H, Bergh K, Eriksen J, Elsayed S. The N-terminal amino acid sequence of the psoriasis-associated antigen, pso p27. Arch Dermatol Res. 1995;287(8):761-3. No abstract available.
7. Iversen OJ, Lysvand H, Jacobsen T, Bergh K, Lie BA. The psoriasis-associated antigen, pso p27, is expressed by tryptase-positive cells in psoriatic lesions. Arch Dermatol Res. 1995;287(5):503-5. No abstract available.
8. Iversen OJ, Bergh K, Lysvand H. Use of scale antibodies for the detection of antigens in psoriatic lesions. Acta Derm Venereol. 1993 Feb;73(1):31-4.
9. Asbakk K, Rodahl E, Lysvand H, Iversen OJ. An antigenic determinant is shared by psoriasis-associated p27 antigen and the Fc part of human IgG. APMIS. 1991 Jun;99(6):551-6.
10. Iversen OJ. The expression of retrovirus-like particles in psoriasis.
J Invest Dermatol. 1990 Nov;95(5):41S-43S. Review.

11. Asbakk K, Bergh K, Iversen OJ. The psoriasis-associated antigen, pso p27, participates in the formation of complement activating immune-complexes in psoriatic scale. APMIS. 1990 Feb;98(2):143-9.
12. Rodahl E, Asbakk K, Iversen OJ. Participation of antigens related to the psoriasis associated antigen, pso p27, in immune complex formation in patients with ankylosing spondylitis. Ann Rheum Dis. 1988 Aug;47(8):628-33.

注：本研究は、2001年9月8日“日本研究皮膚科学会第２６回学術大会総会”にて発表。
作成日：２００２年３月８日