日本財団補助金による
1998 年度日中医学協力事業報告書

－在留中国人研究者研究助成－

99年 3月 15日

財団法人 日中医学協会
理事長 中島 章 殿

研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

1．研究者氏名 梁 亦鄧

研究機関 名古屋大学医学部 研究指導者 祖父江 元 職名 教授
所在地 〒名古屋市昭和区鶴舞町65 電話 (052)741-2111 内線 2386

研究テーマ 神経変性疾患における神経栄養因子についての研究

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 (有) ・ 無 (学会名・内容)

第40回日本神経学会
多発筋炎（polymyositis PM)，皮膚筋炎(dermatomyositis DM)におけるcostimulatory 分子の発現

(2) 学会誌等に発表した論文 有 ・ (無) (雑誌名・論文名)

3．今後の研究計画

多発筋炎(PM)が発病している時にLymphotoxinは重要な役割が果たしているので、今は免疫組織化学方法で筋組織と浸潤細胞にその発現を確定していて、RT-PCR 及び　In situ hibridyzationを使って、筋組織にLymphotoxin　mRNAの発現および発現量を調べている途中です。

4．研究指導者の意見

梁亦鄧先生は現在名古屋大学大学院医学研究科に在学で、神経内科学を勉学している。勉学、研究に対する意欲は充分にあり、成果が期待できる。また、将来に対する構想もしっかりしており、日中の学術交流にも貢献するものと期待される。

研究指導者氏名 [illegible] 印

5．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

論文発表に当っては、日中医学協会－日本財団補助金による旨を明記して下さい。

多発筋炎(PM)、皮膚筋炎(DM)におけるcostimulatory分子の発現

梁　亦　鄧　中国医科大学附属第一臨床病院神経内科　医師
祖父江　元　名古屋大学医学部神経内科　教授

【目的】PM,DMの筋組織におけるcostimulatory 分子の発現率を定量的に検討する。

【対象と方法】PM20例とDM10例の生検筋。厚さ6μmの連続切片を作製し、H&E、免疫組織化学染色(in situ immunohistochemistry)を行ない、浸潤細胞におけるcostimulatory(CD80 and CD86)分子の発現率を定量的に検討した。

【結果】PM, DMとも浸潤細胞でCD80,CD86の発現があり、浸潤細胞にCD86の発現率（PMで35%+/-0.11，DMで32.12%+/-0.14）はCD80（PMで0.09.94%+/-0.05，DMで10.97%+/-0.06）より高い，明らかな差は見られなかった（P＞0.05）.

【結論】PM,DMにおけるcostimulatory 分子の発見は病気の発生機序に基づくと思われる.

KEY WORDS:Polymyositis, Dermatomyositis, CD80, CD86 in situ immunohistochemistry

Expression of costimulatory molecules, CD80 and CD86, on monocytes in muscle tissues from patients with polymyositis and dermatomyositis

INTRODUCTION

Autoimmune diseases are presumably mediated by activated, auto-antigen -reactive T cell (1, 2). Two distinct signals are required to induce differentiation from naive to activated T cell: one is antigen-specific signal mediated through the interaction between major histoconpatibity complex (MHC)-peptide complexes and T cell receptor , and the other is non-antigen specific that is calld "costimulatory" signal(3, 4). B7 molecules on antigen-presenting cells (APC) reacting with the CD28 receptor on T cells provide the most potent costimulatory signals to T cells (5,6). B7 is known as a family that have at least three distinct isoform molecules, namely B7-1 (CD80), B7-2 (CD86) and B7-3 (7). CD80 and CD86 bind to same countereceptor that is known CD28 and CTLA-4 and provide costimulatory signals. Blocking of the B7-CD28 pathway in vitro results in T cell anergy (8, 9, 10), whereas in vivo bloking the B7-CD28 pathway results in immunosuppression (11, 12,). CTLA-4 expressed on T cells after activation may regulate T cell function (13,). Blocking of the B7-CTLA-4 pathway results in augumentation of immunoresponse.

Polymyositis (PM) and dermatomyositis (DM) is an autoimmune inflammatory diseasese characterized by lymphocyte infiltration within muscle fascicles or around blood vessels. The segmental myonecroses of all fiber types associated with myophagocytosis and muscle fiber regeneration were seen in biopsied muscle specimens (14, 15, 16).

To determine whether the costimulatory molecules, CD80 and CD86, affects the necrosis progression of polymyositis and dermatomyositis. we conducted quantification of the subsets of the B7-1and B7-2 in muscle specimens of polymyositis and dermatomyositis with immunohistochemical methods.

MATERIALS AND METHODS

Patients

Twenty patients with polymyositis and 10 patients with dermatomyositis were included in this study. The diagnosis of PM and DM met the clinical diagnosis criteria previously reported (14, 15, 16, 17). Each case showed progressive muslcle weakness, elivated creatine kinase (CK) levels without steroid therapy, and typical histopathological and Immunohistochemical findings. Table 1 listed the ages, sexes and creatine kinase (CK) levels et al. of the patients.

Musle Samples

The muscle tissue specimens were obtained from biceps brachii or quadriceps femoris muscles by open biopsy. Biopsied muscles were rappidly frozen in isopentane chilled in dry ice, and reserved at -80 °C untill use.

Histological, Histochemical studies

For histologic and histochemical analyses, consecutive 8-μm-thick cryostat sections were stained with hematoxylin-eosin (H & E), modified -Gomori, routin adenosine triphosphatse (ATPase, PH10.0), NADH-TR as desctibed elsewhere (18).

Immunohistochemistry.

The muscle specimens were cut at 6μM-thick in a cryostat at -25 °C onto silan-coating slides, fixed in cold acetone for 10 min, air dried and were blocked with normal horse serum at room temperature for 60 min. And they were incubated with mouse monoclonal immunogloblin G (IgG) antibody directed against CD4, CD8, CD19, CD 68 , and CD80, CD86 antigen diluted with phosphate-buffered saline (PBS PH 7.4) at 4 °C overnight, then we used the avidine-biotin-peroxidase complex (ABC) method (Vectastain elite ABC kit, Burlingame, USA), and the antibody binding was visulized with 3'3-diaminobenzidine (DAB). Fimmally, sections were counterstained with methylgreen and mounted in canada baresam.

Quantification of mononuclear cell subsets

In order to quantify mononuclear cell subsets, four scopes of each specimen were randomly chosen and analyzed. We observed at least 1,000 mononuclear cells in each specimen, and calculated the frequency of cells positive for CD80 and CD86. For each accumulation the total number of cells was counted in the H & E stained section. The B7-1, B7-2 positive reaction for a surface antigen consisted of a rim of reaction product around the cell were counted in the respective section in the series.

The student T test was used for statistical comparisons. Differences were considered significant at $P < 0.05$.

RESULT

Immunohistochemical detection of CD80 and CD86 in patients of PM and DM

There were no muscle fibers positive for CD80 and CD86 in the specimens of both PM and DM. In contrast, these molecules were expressed on the infiltrating cells between muscle fibers. CD86 were expressed on many monocytes, but CD86 were on only a few monocytes.

Quantitation of mononuclear cell subsets:

The quantitative analysis of CD80 and CD86 positive cells in PM and DM was demonstrated at table 2. Mononuclear cells positive for CD80 or CD86 were present in both diseased muscles. The frequency of CD80 positive cells was higher in DM than in PM, whereas that of CD86 positive cells was lower in DM. But, stastical analysis showed no significant difference of the frequency of CD80 and CD86 positive cells between both diseases.

DISCUSSION

Human T cells contain two distinct subsets, T-helper (Th) 1 and Th2 cells, that mediate cell-mediated and humoral immune response, respectively. Th1 cells produce mainly IL-2 and IFN-γ, and Th2 cells produce IL-4,5, and 10. Immunological disorders can cause a shift in the Th1/Th2 ratio, which could affect manifestations of different diseases. Naive T cell secreting both patterns of these cytokines have been called Th0 cells and this phenotype is thoght to be in transient stage along a defferentiation pathway to Th1 or Th2 cells(20).

CD80 (B7-1) is expressed on resting monocytes, dedritic cells, macrophage and activated, but not resting, T, NK and B lymphocytes (19). In the other hand, CD86 (B7-2) is expressed on macrophage, monocytes, and activated T and B cells (25). These two molecules expressed on antigen presenting cells were reported to promote T cell differentiation to Th1 and Th2 respectively (23).

It has been reported that cell-mediated immunity operates in polymyositis and humoral immunity in dermatomyositis. Consistent with this theory, Arahada et al reported the prominence of Th1-type cytokine in PM and Th2-type cytokine in DM (26).

In this study, we detected no difference of the frequencies of mononuclear cells bearing CD80 and CD86 molecules on their surfaces between these diseases. Therefore, these two molecules could not exert a pivotal role in the bifurcation of immunoresponces when clinical signs were evolved in these two diseases. We infered that CD80 and CD86 could promote the

differenciation from Th0 to Th1 or Th2 in early stage of disease evolution and have the same functions of simply augumentation of inflammation in that time. Alternatively, differenciation of naive T cells is not decided by only CD80 and CD86 molecules. The interaction of another adhesion molecles, that is CD40 and CD40-ligand, have been shown to be critical in the regulation of B7 expression during T-B-cell collaboration (24). In addition, the cooperation among cytokines of IL-12, IFN-γ ,IL-4 et al. may play an important role in T cell differenciation. The ralative roles of such interaction shold be further investigation.

REFERENCES

1.Powell,M.B.,D. Mitchelll, J. Lederman, S. Buckmeier, S. Zamvil,M. Graham,N.H. RUddle, and L.Steinman. Lymphotoxin and tumor nevrosis factor-alpha production by myelin basic protein-soecific T cell clones correlates with encephalitogenicity. Int.Immunol.1990,2:539-544

2.Kuchroo,V..J.,C.A.Martin,J.M.Greer, S. Ju,R.A.Sobel, and M.E. Dorf. Cytokines and adhetion molecules contribute to the ability of myelin proteolipid protein-specific I cell clones to mediate experimentsl allergic encephalomyelitis. J. Immunol.1993,151:4371-4382

3.Schwartz,R.H. A cell culture model for T lymphocyte clonal anergy. Science (Wash.DC).1990.248:1349-1356

4.Jenkins, M.K., C.Chen, G. Jung, D.L. Mueller, and R.H. Schwartz. Inhibition of antigen-specific proliferation of type 1 murine T cdll clones after stimulation with immunobilized anti-CD3 monoclonal antibody. J.Immunol,1990,144:16-22

5. Linsley PS, Ledbetter JA, The role of the CD28 receptor during T cell responses to antigen. Annu Rev Innunol 1993;11:191-211

6. Linsley PS, Brady W. Grosmaire L, Damle NK, Ledbetter JA. Binding of the B cell actibation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation.J Exp Med 1991;173:721-33.

7. Hathcock KS,Laszlo G,Dick HB,Bradshaw J Linsley P, Hodes RJ. Identification of an altermative CTLA-4 ligand costimulatory for T cell activation. Science 1993;262:905-7

8.Freeman,G.,F.Borriello,R.J.Hoder,H.Reiser,K.S. Hathcock,G. Laszlo,A.J.et al. Uncovering the functional alternative CTLA-4 counter-receptor in B7-deficient mice. Sience(Wash.DC),1993 262:709-909

9. Gimmi,C.D., G.J. Gribben,G.Grey, and L.M.Nadler.Human T-cell Clonal anergy is induced by antigen presentation in the abcence of B7 costimulation.Proc.Natl.Acad.Sci.USA. 1993.90:6586-6590

10.Turka,L.A.P.S.LInsley,H..lin,W.brady,J.M>leiden,R.Q.Wei, M.L.Gibson,X.G.Zheng,S.Myrdal, and D.Gordon. T-cell activation by the CD28 ligand B7 is rewuared for cardiac allograft rejection in vivo.Proc.Natl.Acad.Sci.USA. 1992,89: 11102-11105

11.Linsley,P.S.,P.M.Wallace,J.Johnson,M.G.Gibson,J.L.Greene, J.A.Ledbetter,C.Sigh, and M.A.Tepper. Immunosupression in vivo by a soluble form of the CTLA-4 T cell activation molecule. Science (Wash.DC)1992,257:792-795
12.Brunet, J.F.Denizot,M.F. Luciani,M.RouxDosseto,M.Suzan, M.G.Mattei,and P. Golstein. A new menber of the immunoglobulin superfamily-CTLA-4. Nature 328: 267-270
13.Jenkins, M.K. The ups and downs of T-cell costimulatory molecuti. Immunity. 1994, 1:443-446
14. Andrew G.Engel Clara Franzini-Armstrong . Myology. Second Edition .McGRAW-HILL, INC. HEALTH PROFESSIONS DIVISION
15 Anthony Bohan MD. and James B. Peter MD.PHD. Polymyositis and Dermatomyositis. The New England Journal of Medicine 1975,292 (No 7):345-347 and 1975,292 (No 8):403-407
16. inflammatory myopathy: A review of etiologic and pathogenetic factors. Muscle & Nerve 1982,5:573-592
17. Thomas A. Medsger,Jr.Chester V.oddis. Classification and Diagnosic Criteria from Polymyositis and Dermatomyositis. The Journal of Rheumatology 1995:22:4
18. Dubowitz V: Muscle Biopsy: A Practical Approch,2nd ed eastbourne,Bailliere Tindall,1985
19. Linsley,P.S. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc.Natl. Acad.Sci.USA. 1990:87:5031-5035
20. Abehsira.et al, 1992;148:3820-3829 J. immunol ,Monsmann
21. Jun,C.H. Bluestone, T.A.Nadler, L.M. and Thompson,C,B.1994 Immunology Today 15,321-331)
22. Paul M.Kaye Costimulatory and the regylation of antimicrobial immunity Immunology Today 1995,16(9) 423-427.
23. Paul M.Kaye Costimulatory and the regylation of antimicrobial immunity Immunology Today 1995,16(9) 423-427.
24. Durie,F.H., For,T.M.,Masters,S.R.,Laman,J.D.and Noelle,R.J. Immunology Today 1994,15.,406-411)
25. Nandi,D., Gross,J.a. and Allison ,J.P.J.Immunol. 1994,152,3361-3369
26. Kiichi Arahata and Andrew G Engel ,Monoclonal /antibody /analysis of Mononuclear Cells in Myopathies Annal Neurology 1984;13:193-208)

Table 1.

Diag.	No of cases	Sex		Age (years)		duration of illness (months)		Serum CK(u/l)	
		Male	Female	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
PM	20	9	11	21-74	58.65±.14.29	1-42	10.4 ± 11.70	474-13470	4049.55 ± 3197.68
DM	10	3	7	16-78	55.1 ± 17.73	1-36	10 ± 11.85	195-7653	3381.9 ± 2696.9

PM: polymyositis DM: dermatomyositis

Table 2 Quantitative analysis of B7 cell in PM and DM

Cell type or marker	PM	DM
B7-1 (CD80) (%)	0.1336 ± 0.020	0.1395 ± 0.028 *
B7-2 (CD86) (%)	0.3609 ± 0.025	0.2701 ± 0.027 *

*: $p<0.05$