IgA 腎症患者における自己リンパ球混合培養反応 (AMLR) に関する研究

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Deficiency of the autologous mixed lymphocyte reaction in patients with IgA nephropathy

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【Summary】

We studied the distribution of autologous rosette-forming cells (ARFC), the population of T cell subsets, autologous mixed lymphocyte reaction (AMLR) and allogeneic mixed lymphocyte reaction (allo-MLR) in the peripheral blood of 20 healthy adult donors and 16 patients with IgA nephropathy. The mean percentages of ARFC were markedly reduced ($p < 0.001$) in the patients compared with the healthy controls. OKT 3 (pan-T) cells and OKT 4 (helper/inducer T) cells were significantly reduced ($p < 0.001$) when compared to healthy controls. AMLR is impaired or absent in most patients with IgA nephropathy. The response of T cells to allogeneic normal non-T cells was diminished in patients when compared to normal cases.

Key words : AMLR,
ARFC,
IgA nephropathy

【概 要】

自己リンパ球混合培養反応 autologous mixed lymphocyte reaction (AMLR) は, *in vitro* で自己の非 T 細胞に対して自己の OKT4 T 細胞が反応する現象であり, その反応が免疫学的特異性と免疫記憶を具備していることからリンパ球の細胞間相互作用における免疫 network のモデルとして注目されている。

本論文では, IgA 腎症患者における AMLR, 健常人リンパ球との allogeneic MLR および自己赤血球ロゼット形成 autologous rosette formation (ARF) を調べるとともに, 患者末梢血中の T cell subsets と AMLR との相関につ

いて検討した。

対象および方法：IgA 腎症患者 16 名，健康人 20 名の末梢血より比重遠沈法にて単核球を採取したのち，その一部を plastic Petri dish に移し 37°C，60 分間温置して plastic 表面に付着する細胞と plastic 表面に付着しない非付着細胞を得た。

Petri dish に付着しなかった細胞群は，ヒツジ赤血球と rosette を形成させたのち，比重遠沈法により T 細胞を精製した。この T 細胞群は，OKT 8 monoclonal 抗体とウサギ補体で処理して OKT 4 細胞に分画し，AMLR，allogeneic MLR の反応細胞として，マイトマイシン処理された自己の非 T 細胞あるいは健康人の非 T 細胞と混合培養した。ARF は，末梢血リンパ球を Con A とともに 72 時間，前培養したのち，自己赤血球を加えてロゼット形成能を観察した。末梢血中の T cell subsets は，monoclonal 抗体の OK シリーズのうち補体結合能を有する OKT 3，T4，T8 を用い microcytotoxicity test により全細胞に対する死細胞を算定した。

成績：IgA 腎症患者の AMLR の反応性は，健康人の AMLR に比較して著明に低下した ($p < 0.001$)。

反応細胞に患者の OKT4⁺ 細胞，刺激細胞を健康人の非 T 細胞として MLR を実施したときの反応性は患者の AMLR の成績と有意差をみとめなかったが，逆の場合の MLR の反応は，健康人における allogeneic MLR の反応性と一致した。自己赤血球ロゼット形成率は，患者リンパ球で著しく低下していた ($p < 0.001$)。患者の末梢血 T cell subsets は，OKT3⁺，T4⁺ 細胞の著明な低下をみとめたが，OKT8⁺ 細胞に変化はみとめなかった。

結論：今回，IgA 腎症患者において AMLR の反応性の著明な低下，末梢血リンパ球中の OKT4⁺ 細胞および ARF 形成率の著明な低下がみとめられたが，AMLR は，その反応細胞が OKT4⁺ 細胞に限極されていること，自己赤血球ロゼット形成細胞が AMLR における反応細胞の一部である可能性も報告されていることから推測すると，IgA 腎症患者の免疫応答は，OKT4⁺ 細胞の反応系に異常があることが示唆された。

I. Introduction

IgA nephropathy is characterized by the presence of massive IgA-containing immune deposits within mesangial areas¹⁾. The origins of the glomerular immune complexes and role of IgA in the development of this renal disease are still unknown. A role of cell-mediated mechanisms in IgA nephropathy was suggested by Sakai et al.²⁾. They reported that IgA specific suppressor T cell activity is decreased in IgA nephropathy patients³⁾.

Recently, human T cells and T cell subsets have been defined with monoclonal antibodies^{4,5)}. OKT4 T cell contain cells with helper/inducer phenotype and OKT8 monoclonal antibody reacts with suppressor/cytotoxic phenotype cells^{6,7)}. Circulating T cells and T cell subsets have been extensively studied in patients with IgA nephropathy^{8,9)}.

The AMLR is an interesting immunological phenomenon in which OKT4 T cells proliferate upon stimulation with autologous non-T cells (consisting of B cells, null cells and macrophages)¹⁰⁻¹⁴⁾. This reaction has been shown to have both immunological memory and specificity. It has recently been demonstrated that a subset of T cells that can form rosettes

with autologous red blood cells is involved in the AMLR. ARFC have surface and functional characteristics of post-thymic precursors and among these characteristics there are some that have been identified in responsive cells of the AMLR¹⁵⁾. We have previously reported that ARFC is markedly decreased in patients with IgA nephropathy¹⁶⁾.

The impairment of AMLR has been documented in patients with systemic lupus erythematosus (SLE)¹⁷⁻¹⁹⁾, Hodgkin's disease²⁰⁾, Sjögren's syndrome²¹⁾, rheumatoid arthritis¹⁹⁾, infectious mononucleosis²²⁾, and chronic lymphocytic leukemia²³⁾. During the past decade, knowledge of immunopathogenetic mechanisms in renal diseases has expanded considerably. Our study was designed to investigate the AMLR in the peripheral blood of patients with IgA nephropathy as well as to characterize the cells involved in the depression of AMLR.

II. Materials and Methods

1. Patient studies

Twenty healthy adult donors and 16 patients with IgA nephropathy with various degrees of renal function impairment were studied. No patients had had previous treatment with steroids or other drugs at

the time of study. Diagnosis of the disease was confirmed histologically by renal biopsy.

2. Cell preparations

Mononuclear cells were isolated from fresh heparinized venous blood by Ficoll-Isopaque density gradient centrifugation. T and non-T cell fractions were separated as described by Smolen et al.²⁴⁾. Briefly, the cells were incubated on plastic Petri dishes for 60 min at 37°C. The nonadherent cells were mixed with a 1.5% neuraminidase treated sheep red blood cell (SRBC) suspension and were fractionated into T cells and nonadherent non-T cells (mainly B+null cells) by centrifugation on Ficoll-Isopaque as described above. E-rosette forming cells collected from the bottom of the tubes were treated by 0.83% NH₄Cl buffered with tris ammonomethane to lyse SRBC and washed three times. Before, their use as stimulator cells, the adherent cells (macrophages; M ϕ), which were recovered with a rubber policeman, and the B+null cells+M ϕ (1×10^6 cells/ml) were treated with 25 μ g of mitomycin-C for 20 min at 37°C.

Purification of T cell subsets was performed using the OKT8 monoclonal antibody (Ortho Pharmaceutical, Raritan, NJ). The method was analogous to one described by Smolen et al.²⁴⁾. Briefly, 5×10^6 T cells/20 μ l in Hank's BSS (HBSS) were incubated with an equal volume of a OKT8 monoclonal antibody for 15 min at 37°C. Then rabbit complement was added, and the incubation was continued for 45 min at 37°C. The cells were washed three times and resuspended in HBSS. To assess the completeness of killing, the residual cells were analysed using the OKT4 and OKT8 monoclonal antibodies. As analysed by trypan blue dye exclusion, the OKT8+complement pretreated population yielded >95% OKT4 T cells and <5% OKT8 T cells.

3. Analysis of lymphocyte subpopulations

Quantitation of T cell and T cell subsets were done by microcytotoxic assay²⁵⁾. One microliter of OKT3, OKT4 and OKT8 monoclonal antibodies suspended into each well of the Terasaki plates (Falcon Plastics, Los Angeles, Ca.) which had first been flooded with

liquid paraffin. One microliter of target lymphocytes suspension (concentration 2×10^6 cells/ml) dispensed into each well was then incubated for 30 min at 37°C. Subsequently, 5 μ l of rabbit complement was added. After incubation at 37°C for 60 min, the percentage of dead cells was determined by trypan blue dye exclusion.

4. Autorosette formation

Peripheral blood lymphocytes (1×10^5 cells/0.2 ml in the medium) were incubated for 72 hr at 37°C in a humidified atmosphere of 5% CO₂ in air with 10 μ g of Concanavalin A (Con A). Following incubation, the lymphocytes were treated with 0.1 M α -methyl-D-mannoside in HBSS for 60 min at room temperature, washed twice, and then tested for their ability to form rosettes with autologous red blood cells. One $\times 10^5$ lymphocytes in 0.1 ml of HBSS, 0.1 ml of fetal calf serum (FCS), and 0.1 ml of 1.5% autologous red blood cells in HBSS were mixed thoroughly and centrifuged at 450 g for 3 min and then incubated on ice water for 120~180 min. The cells were gently resuspended and one drop of the cell suspension was placed on a microscopic slide with one drop of 1% brilliant cresyl blue. Lymphocyte rosetting with three or more erythrocytes was considered as ARFC¹⁶⁾.

5. AMLR and allo-MLR

AMLR and allo-MLR were performed in duplicate using flat-bottomed microplates with 1×10^5 responder (OKT4 T) cells and 1×10^5 mitomycin C-treated autologous or allogeneic stimulator non-T cells (B+null+M ϕ) in a total volume of 0.2 ml of complete medium^{26,27)}, which consisted of TC199-Hepes supplemented with 100 U penicillin/ml, 100 μ g streptomycin/ml and heat inactivated 20% human AB serum. Cultures were performed for 7 days at 37°C in a humidified atmosphere of 5% CO₂ in air. One μ Ci of ³H-thymidine (³H-TdR) (5 Ci/mmol; Amersham Corp., Ill.) was added to each well for the last 20 hr of the culture. Results were expressed as mean counts per minute $\pm$ l.s.d. of duplicate cultures minus mean counts per minute of culture containing T cells alone (4cpm).

The statistical analysis was done by Student's

Fig. 1 Proportions of OKT3⁺ (pan-T cells), OKT4⁺ (helper/inducer phenotype) and OKT8⁺ (suppressor/cytotoxic phenotype) T cells in IgA nephropathy patients.

Fig. 2 Percentage of Con A activated autorosette forming cells in IgA nephropathy patients.

Fig. 3 AMLR in patients with IgA nephropathy and healthy controls.

t-test.

III. Results

Results of the proportions of T cell and T cell subsets are shown in Fig. 1. The population of OKT8 T cells in the patient group (25.31 ± 22.92%; mean ± 1 s.d., n=16) was comparable to simultaneously studied controls (28.60 ± 13.86%, n=20). The population of OKT3 (27.06 ± 21.81%) and OKT4 (21.81 ± 14.20%) T cells in the patient group was decreased as compared to healthy controls. These differences were statistically significant ($p < 0.001$).

Fig. 2 shows that the percentage of Con A stimulated ARFC in patients had mean percentages below the normal range (9.50 ± 8.60%). These values were significantly lower ($p < 0.001$) than those of healthy individuals.

Results of the AMLR in IgA nephropathy and control are shown in Fig. 3. Normal controls responded with a mean response ranging from 985 to 13,896 Δcpm (5,746 ± 3,433 Δcpm). There was no significant differences in the control AMLR on the

Table 1 Allogeneic MLR between OKT4⁺ T cells and non-T cells from patients with IgA nephropathy and healthy controls.

Responder cells (OKT4 ⁺ T cells)	Stimulator cells (non-T cells)	Response (Δ cpm; mean $\pm$ 1 s.d.)
patients	unrelated patients	1,859 $\pm$ 1,382 (n=16)
patients	controls	743 $\pm$ 536 (n=16)
controls	patients	6,769 $\pm$ 4,102 (n=20)
controls	unrelated controls	8,131 $\pm$ 5,517 (n=20)

basis of sex and age. In contrast, the AMLR response of patients with IgA nephropathy showed impairment with the average being $1,164 \pm 744$ Δ cpm. This mean value is significantly different from that of normal controls ($p < 0.001$).

The results of allo-MLR are shown in Table 1. Allo-MLR response in patients with IgA nephropathy was significantly lower ($1,859 \pm 1,382$ Δ cpm) than that normal allo-MLR response ($8,131 \pm 5,517$ Δ cpm). In contrast to the impairment of the allo-MLR response between patient responder cells and control stimulator cells, the response between control responder cells and patient stimulator cells are normal.

IV. Discussion

Opeltz et al.¹⁰⁾ using the fractionated T and non-T cells from normal donors observed that, in AMLR, T cell proliferation occurred when stimulated with non-T cells. This phenomenon shows two important characteristics of an immune system, specificity and immunological memory. Recently it has been demonstrated that OKT4 T cells are the major responder in AMLR^{11,13)}. However, the nature of the stimulator cells in AMLR has been controversial. In our experiments, the stimulator, non-T cell population contained B cells, null cells, and M ϕ .

In the present study, we have defined an abnormality of immune response in patients with IgA nephropathy: cultures containing OKT4 T cells and autologous non-T cells have a decreased proliferative response in the AMLR, whereas OKT4 responder cells from patients with IgA nephropathy had a decreased response in cultures containing allogeneic normal non-T cells. Additionally, T cells from healthy donors have a normal proliferative response in cultures containing non-T cells from patients with IgA nephro-

pathy. Further, we found significant depletion of OKT4 T cell populations in patients with IgA nephropathy. In contrast, the population of OKT8 T cells were similar to those found in healthy controls.

A number of investigators have reported decreased populations of T cells and T cell subsets as measured by monoclonal antibodies in patients with IgA nephropathy^{8,9)}. The analysis of circulating T cells and T cell subsets, using monoclonal antibodies are still controversial^{8,9)}. Our data of T cell subsets do not confirm the results of other investigators who demonstrated depletion of OKT8 T cell populations in patients with IgA nephropathy. The discrepancies between our data and those reported by other investigators could be due to technical differences as they used a more sensitive assay than the method in the present study. Smolen et al.²⁴⁾ and others^{11,13)} have observed that purified OKT4 T cells, an inducer cells subset, but not purified OKT8 T cells, a suppressor/cytotoxic cell subset, respond to autologous non-T cells. Therefore, the impaired response in the AMLR in IgA nephropathy agrees with a depletion of OKT4 T cells IgA nephropathy. A defect of autologous stimulator cells has been described in patients with SLE¹⁷⁾, infectious mononucleosis²²⁾, and CLL²³⁾. The defect in Hodgkin's disease appears to be in the responder T cell population rather than in the stimulator cells²⁰⁾. Our data of AMLR and allo-MLR suggested a defective response of responder cells. In the AMLR, OKT4 T cells produced interleukin 2 (IL-2)²⁹⁾. We observed lack of production of IL-2 in all eight patients with IgA nephropathy that were examined for IL-2 production (data not shown). Studies are in progress in our laboratory to clarify this point.

We found depletion of pan-T cells as defined with

OKT3 monoclonal antibody. A subpopulation of human T cells and about 40% of peripheral blood T cells form rosettes with autologous red blood cells after stimulation with Con A¹⁶⁾. These cells have been termed autologous rosette-forming cells (ARFC). It has been demonstrated that ARFC proliferative response to autologous non-T cells in AMLR¹⁵⁾. AMLR has been performed between ARFC and autologous non-T cells of cancer patients³⁰⁾. After enrichment of ARFC, there was no difference in the AMLR of normal blood and blood of cancer patients. The number of ARFC was lower in peripheral blood T cells of IgA nephropathy patients ($p < 0.001$) than in the peripheral blood of normal individuals. These

results indicate that the deficiency of AMLR in patients with IgA nephropathy is due to a reduction in the number of autoreactive T cells and not to a defect of autologous stimulator non-T cells.

The AMLR may represent interaction between T cells and non-T cells. Decreased interaction of these cells may induce aberrations of immunoregulation leading to autoimmune diseases. Conceivably, IgA nephropathy might result from a failure of immunoregulation. Further understanding of the mechanism by which the AMLR occurs in normal subjects and in patients with abnormal immune responses may provide further insight into the pathogenesis of this as well as other autoimmune diseases.

References

- 1) McCoy, R.C., Abramowsky, C.R., Tisher, C.C.: IgA nephropathy. *Am. J. Pathol.*, 76 : 123~144, 1974.
- 2) Sakai, H., Endoh, M., Arimori, S.: Decrease of IgA specific suppressor T cell activity in patients with IgA nephropathy. *Clin. Exp. Immunol.*, 38 : 243~248, 1979.
- 3) Endoh, M., Sakai, H., Nomoto, Y., Tomino, Y., Kaneshige, H.: IgA specific helper activity of T cells in human peripheral blood. *J. Immunol.*, 127 : 2612~2613, 1981.
- 4) Kung, P.C., Goldstein, G., Reinherz, E.L., Schlossman, S.F.: Monoclonal antibodies defined distinctive human T cell surface antigens. *Science*, 206 : 347~349, 1979.
- 5) Reinherz, E.L., Kung, P.C., Goldstein, G., Schlossman, S.F.: Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA*, 76 : 4061~4065, 1979.
- 6) Reinherz, E.L., Kung, P.C., Goldstein, G., Schlossman, S.F.: A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by a heteroantisera termed TH₂. *J. Immunol.*, 124 : 1301~1307, 1980.
- 7) Reinherz, E.L., Schlossman, S.F.: Regulation of the immune response inducer and suppressor T lymphocyte subsets in human beings. *New Engl. J. Med.*, 303 : 370~373, 1980.
- 8) Rothschild, E., Chatenoud, L.: T cell subset modulation of immunoglobulin production in IgA nephropathy and membranous glomerulonephritis. *Kidney Int.*, 25 : 557~564, 1984.
- 9) Linne, T., Wasserman, J.: Lymphocyte subpopulation and immunoglobulin production in IgA nephropathy. *Clin. Nephrol.*, 23 : 109~111, 1985.
- 10) Opelz, G., Kuchi, M., Takasugi, M., Terasaki, P.I.: Autologous stimulation of human lymphocyte subpopulations. *J. Exp. Med.*, 142 : 1327~1333, 1975.
- 11) Damle, N.K., Hansen, J.A., Good, R.A., Gupta, S.: Monoclonal antibody analysis of human T lymphocyte subpopulation exhibiting autologous mixed lymphocyte reaction. *Proc. Natl. Acad. Sci. USA*, 78 : 5096~5098, 1981.
- 12) Van Wauwe, J., Goossens, J.: Monoclonal anti human T lymphocyte antibodies. Enumeration and characterization of T cell subsets. *Immunology*, 42 : 157~164, 1981.
- 13) Kozak, R.W., Moody, C.E., Staiano-Coico, L., Weksler, M.E.: Lymphocyte transformation induced by autologous cells. XII. Quantitative and qualitative differences between human autologous and allogeneic reactive T lympho-

- cytes. *J. Immunol.*, 128 : 1723~1727, 1982.
- 14) Reinherz, E.L., Morimoto, C., Fitzgerald, K.A., Huzzey, R.E., Daley, J.F., Schlossman, S.F. : Heterogeneity of Human T₄ inducer T cells defined by a monoclonal antibody that delineates two distinct functional subpopulations. *J. Immunol.*, 128 : 463~468, 1982.
 - 15) Palacios, R., Llorente, L., Alaróon-Segovia, D., Ruiz-Arguelles, A., Diaz-Jouanen, E. : Autologous rosette-forming T cells as the responding cells in human autologous mixed-lymphocyte reaction. *J. Clin. Invest.*, 65 : 1527~1530, 1980.
 - 16) Kuramochi, T., Ohnuki, T., Inoue, T., Ishida, M. : Autologous rosette-forming cells in patients with renal diseases. *Clin. Exp. Immunol.*, 54 : 765~770, 1983.
 - 17) Sakane, T., Steinberg, A.D., Green, I. : Failure of autologous mixed lymphocyte reaction between T and non-T cells in patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA*, 75 : 3464~3468, 1978.
 - 18) Kuntz, M.M., Innes, J.B., Weksler, M.W. : The cellular basis of the impaired autologous mixed lymphocyte reaction in patients with systemic lupus erythematosus. *J. Clin. Invest.*, 63 : 151~153, 1979.
 - 19) Smith, J.B., Knowlton, R.P., Koons, L.S. : Different autologous mixed lymphocyte reaction correlate with disease activity in systemic lupus erythematosus and rheumatoid arthritis. *Clin. Exp. Immunol.*, 48 : 155~162, 1982.
 - 20) Engleman, E.G., Benike, C.J., Hoppe, R.T., Kaplan, H.S. : Autologous mixed lymphocyte reaction in patients with Hodgkin's disease. Evidence for a T cell defect. *J. Clin. Invest.*, 66 : 149~158, 1980.
 - 21) Miyasaka, N., Sauvezie, B., Pierce, D.A., Daniels, T.E., Talal, N. : Decreased autologous mixed lymphocyte reaction in Sjögren's syndrome. *J. Clin. Invest.*, 66 : 928~933, 1980.
 - 22) Moody, C.E., Casazza, P.A., Christensen, W., Weksler, M.E. : Lymphocyte transformation induced by autologous cells. VIII. Impaired autologous mixed lymphocyte reactivity in patients with acute infectious mononucleosis. *J. Exp. Med.*, 150 : 1448~1455, 1979.
 - 23) Smith, J.B., Knowlton, K.P., Koons, L.S. : Immunologic studies in chronic lymphocytic leukemia : defective stimulation of T cell proliferation in autologous mixed lymphocyte culture. *J. Natl. Cancer Inst.*, 58 : 579~585, 1977.
 - 24) Smolen, J.S., Chused, T.M., Novotny, E.A., Steinberg, A.D. : The human autologous mixed lymphocyte reaction. III. Immune circuits. *J. Immunol.*, 129 : 1050~1053, 1982.
 - 25) Mittal, K.K., Mickey, M.R., Singal, D.P., Terasaki, P.I. : Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation*, 6 : 913~927, 1968.
 - 26) Smolen, J.S., Sharrow, S.O., Reeves, J.P., Boegel, W.B., Steinberg, A.D. : The human autologous mixed lymphocyte reaction. I. Suppression of macrophages and T cells. *J. Immunol.*, 127 : 1987~1993, 1981.
 - 27) Smolen, J.S., Luger, T.A., Chused, T.M., Steinberg, A.D. : Responder cells in the human autologous mixed lymphocyte reaction. *J. Clin. Invest.*, 68 : 1601~1604, 1981.
 - 28) Gupta, S. : Autologous mixed lymphocyte reaction in health and disease states in man. *Vox Sang.*, 44 : 264~288, 1983.
 - 29) Palacios, R., Möller, G. : HLA-DR antigens rendering resting R cells sensitive to interleukin 2 and induced production of the growth factor in the autologous mixed lymphocyte reaction. *Cell. Immunol.*, 63 : 143~153, 1981.
 - 30) Uchida, A., Micksche, M. : Autologous mixed lymphocyte reaction in the peripheral blood and pleural effusions of cancer patients. *J. Clin. Invest.*, 70 : 98~104, 1982.
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