(p=0.0079). Serum creatinine at renal biopsy was 3.57± −2.31 mg/dL in the dialysis-independent group, and this was significantly lower than the serum creatinine level of 9.10± −2.6 mg/dL of the dialysis group (p=0.000259). As for the renal pathological findings, the percentage of global sclerosis among all the glomeruli was 24.7± −19.9% in the dialysis-independent group vs. 68.5± −19.7% in the dialysis group, which shows the latter to be significantly higher (p=0.002).

Conclusion: CRP was significantly higher in the multi-organ-damaged group relative to the kidney-located type group. The percentage of global sclerosis determined by renal biopsy and the amount of serum creatinine at the renal biopsy were key factors in determining the renal prognosis. The absence of a significant correlation between the percentage of crescentic formation and the renal prognosis suggests the possibility of suppressing progress to global sclerosis.

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Key words：MPO-ANCA, crescentic glomerulonephritis, Birmingham Vasculitis Activity Score (BVAS), rapidly progressive glomerulonephritis, renal biopsy

緒　言

抗好中球細胞質抗体(ANCA)関連血管炎にはウェゲナー肉芽腫，顕微鏡的多発血管炎，Churg-Straus 症候群などが含まれる．欧米では ANCA 関連血管炎のうちウェゲナー肉芽腫が約 6 割を占めるという報告が多い[1]．本邦では，ウェゲナー肉芽腫に比し顕微鏡的多発血管炎が多いといわれ，ANCA 関連腎炎では PR3-ANCA に比し MPO-ANCA 陽性が欧米に比し圧倒的に多い[2]．

本邦では ANCA の測定が保険適用となり，ANCA 関連血管炎の早期発見が可能となり，検診で発見されるケースも増えてきている．最近では，高齢者の増加とともに ANCA 関連血管炎患者は徐々に増加し，早期診断治療も可能となってきた．MPO-ANCA 関連血管炎は，障害臓器として腎臓，肺が多く，急速進行性腎炎を示している．しかし，その多彩な病態，治療，腎予後についてはいまだ検討が少ない．

今回われわれは，急速進行性の腎障害を有し腎生検を施行した MPO-ANCA 関連血管炎の症例を対象とし，病理および臨床所見と予後との関係について検討した．

対象および方法

1．対象患者

対象は，1995 年から 2004 年までの 10 年間に当科に入院し，血清学的に MPO-ANCA 陽性で，臨床的には急速進行性腎炎を呈し，腎生検を施行した 18 例である．うち男性は 7 例，女性は 11 例で，年齢は 28～74 歳(平均 58.6 歳)である．

2．方　法

腎生検の実施時期は原則的に治療前とし，腎生検時の血管炎の活動性は Birmingham Vasculitis Activity Score (BVAS)，厚生労働省進行性腎障害研究班による臨床重症度分類を用いて評価した．BVAS は 1 全身症状，2 皮膚病変，3 粘膜・眼病変，4 耳鼻咽喉部病変，5 胸部病変，6 心血管病変，7 腹部病変，8 腎病変，9 神経系病変の 9 項目より成り，4 週間以内に新たに出現したか増悪したものを陽性所見としてスコア化したものである．臨床病態の解析のために，臨床病型は BVAS の項目を用いて行い，腎の項目のみ陽性であったものを腎限局型，腎の項目に加え腎以外の他の項目も陽性となったものを多臓器型と定義した．

腎予後については，1 年後の時点で維持透析に至った透析移行群と，透析離脱例も含めた腎機能が保存された腎生存群の 2 群に分けて検討した．寛解を BVAS 0 点とし，再発は新たに BVAS 2 点以上となった時点と定義した．

腎病理所見は腎生検のパラフィン切片を Periodic acid Schiff 染色，Hematoxylin Eosin 染色，Masson 染色，PAM 染色にて評価した．それぞれの糸球体は半月体(細胞性，線維細胞性，線維性)，硬化(分節性，全節性)を算出し，全糸球体の数で除して割合(%)を出した．尿細管間質病変は，間質の細胞浸潤と線維化を，"なしを 0，軽度(0%～25%)を 1，中等度(25%～50%)を 2，高度(50%～)を 3"とスコアリングした．間質に浸潤する細胞の種類，血管炎，動脈硬化は"なしを 0，ありを 1"とした．また，蛍光抗体法にて IgG，IgA，IgM，C3，C4，C1q を染色した．

臨床検査所見は腎生検時の尿検査(尿蛋白，尿沈渣における赤血球数)，血清クレアチニン値，CRP，MPO-ANCA を用いた．合併症状における肺病変の有無は胸部 X 線，胸部 CT で確認した．

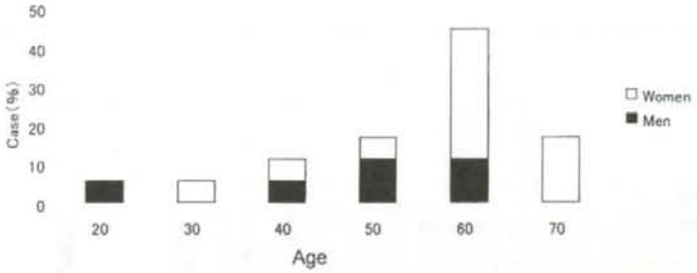

Fig. 1. Distribution of age at the time of renal biopsy in ANCA-associated vasculitis

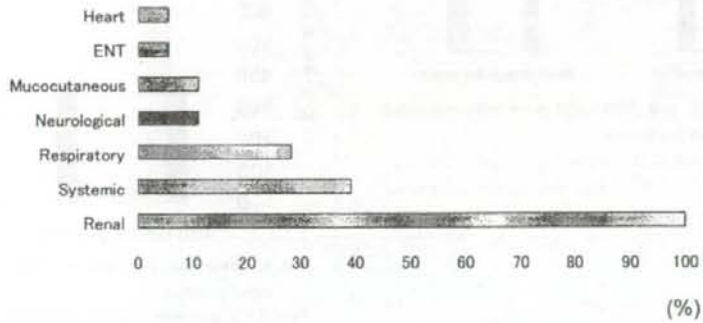

Fig. 2. Clinical symptoms in association with BVAS
ENT：ear, nose and throat

統計解析は Student *t* test および χ^2 検定を用いた。なお，測定値は平均値＋－標準偏差で表わした。

結　果

腎生検時の臨床所見は，平均年齢 58.6±13.9 歳，男女比はやや女性に多く，60 代が 44％(8 例 18 例)とピークであった(Fig. 1)。腎生検時の検査所見は，血清クレアチニン値 4.5±3.0 mg/dL，CRP 7.5±9.7 mg/dL，MPO-ANCA 379±291 EU であった。尿検査では尿蛋白が 1.46±1.41 g/日，尿沈渣では赤血球が平均 43±36 HPF であった。ネフローゼ症候群を示した症例は 2 例であった。

血管炎の重症度は，厚生労働省特定疾患対策研究事業「進行性腎障害に関する調査研究」による臨床学的重症度は，Grade Ⅰ：33％，Grade Ⅱ：56％，Grade Ⅲ：0％，Grade Ⅳ：11％であり，平均では 1.8±0.89 と比較的軽症例が多かった。血管炎の活動性は BVAS で評価し平均 14.8±3.2 であった。項目別合併頻度を Fig. 2 に示した。腎の項目はすべての症例で最大点数の 12 点であり，BVAS の大部分を占めた。腎の項目のみ陽性となった腎限局型は 22％(7 例)であった。腎以外の項目では全身症状が 39％，肺病変が 28％と多かった。肺病変のうち，間質性肺炎(4 例)が最も多く，肺出血は 1 例のみであった。

臨床病型と CRP，MPO-ANCA の関係を調べた。臨床病型は BVAS により腎限局型(39％，7 例)，多臓器型(61％，11 例)に分け，2 群間における CRP，MPO-ANCA の値に関連性があるかどうかを調べた。CRP は腎限局型では 1.2±1.4 mg/dL，多臓器型では 12.6±10.5 mg/dL と有意に腎限局型は低値であった($p=0.0079$)。一方，MPO-ANCA は腎限局型では 393±320 EU，多臓器型では 355±280 EU であり，両群の間に有意差はなかった($p=0.793$)(Fig. 3)。

腎予後は，1 年後に維持透析が必要でなかった腎生存群は 67％(12 例)，透析移行群は 33％(6 例)であった。この 2 群間における腎生検施行時の血清クレアチニン値は腎生存群では平均 3.57±2.31 mg/dL であったのに対し，透析移行群では 9.10±2.6 mg/dL と有意に腎生存群では低値であった($p=0.000259$)。また，MPO-ANCA は腎生存群では 368±305 EU，透析移行群では 380±285 EU と 2 群間に有意差は

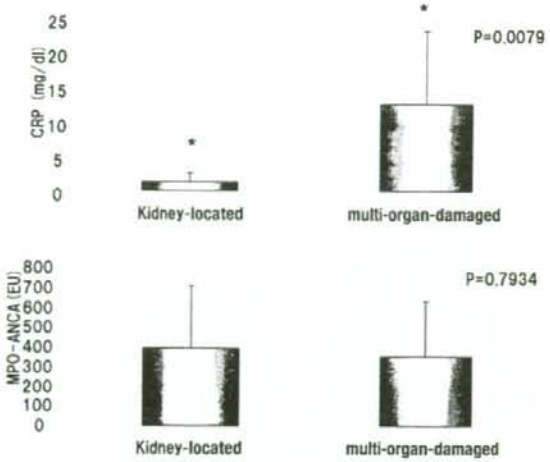

Fig. 3. Relation of CRP and MPO-ANCA to kidney-located or multi-organ-damaged group

*p<0.05 was considered statistically significant. CRP values were significantly higher in the multi-organ-damaged group than that in the kidney-located group.

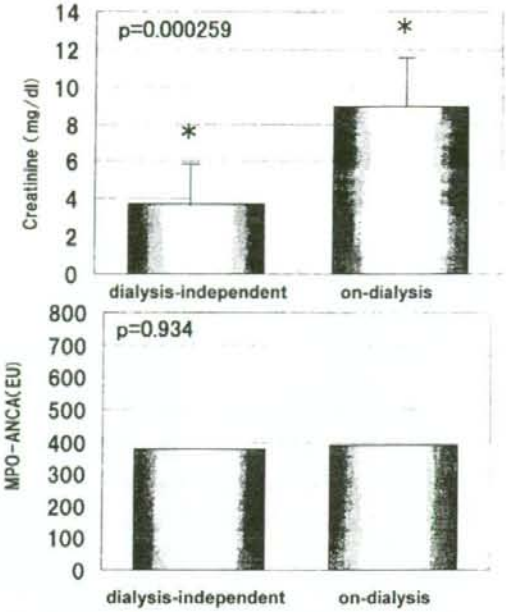

Fig. 4. Relation of serum creatinine and MPO-ANCA to renal prognosis

*p<0.05 was considered statistically significant. Creatinine levels were significantly higher in the dialysis group than in the dialysis-independent group.

なかった(p=0.934)(Fig. 4)。

腎生検は基本的には治療前に施行したが，発熱など全身状態不良時やクレアチニン高値であった2例は，ステロイド治療を先行させ，その治療後 6～17 日後に施行した。腎生検方法は，経皮的針生検が 15 例，開放腎生検が 3 例であった。採取糸球体は平均 26.9±18.4 個であり，全例で半月体形成を認め，半月体の多くは全周性であった。間質細胞浸潤は中等度～高度に認め，浸潤細胞は単核球が多かったが，好中球や形質細胞を認めた例も多かった。壊死性血管炎は全体の 22 %(4 例)に認め，小葉間動脈レベルで 2 例，細動脈レベルで 1 例認められた。小葉間動脈にフィブリノイド壊死を認めた 2 例は，いずれも多臓器病変を有し CRP が高度に上昇していた(20.95～23.4 mg/dL)が，腎病変に関しては血清クレアチニンは軽度の上昇(2.12～2.22 mg/dL)にとどまり，尿蛋白や赤血球尿も軽微であった。蛍光抗体法では pauci-immune 型と考えられる免疫グロブリンや補体の沈着がない例は全体の 56 %(10 例)であり，その他の症例は IgG，IgM，IgA，C3 などに軽度の沈着を認めたが，いわゆる IgA 腎症や膜性腎症，免疫複合体腎炎を示唆する病変は光顕上もなかった。

腎病理所見と腎予後との関連を調べるために，腎生存群，

Table. Pathological findings of patients with ANCA-associated vasculitis

Pathology	Dialysis-independent(%)	On-dialysis(%)	p value
Crescent	47.8±16.6	48.7±30.0	0.934
cellular	28.0±25.9	16.4±11.0	0.315
fibrocellular	13.7±14.8	15.6±15.7	0.803
fibrous	6.1±10.7	16.7±40.8	0.399
Global sclerosis	25.2±20.2	64.3±18.7	0.002
Interstitial infiltration(0/1/2/3)	2.4±0.8	3.0±0.0	0.115
Interstitial fibrosis(0/1/2/3)	2.3±0.8	3.0±0.0	0.090
Vasculitis(0/1)	0.3±0.4	0.2±0.4	0.709
Arteriolosclerosis(0/1)	0.9±0.7	0.5±0.7	0.517

透析移行群の両群間における腎病理所見の比較を Table に示した。全糸球体における全節性硬化糸球体率は，腎生存群が 24.7±19.9 %，透析移行群が 68.5±19.7 %と透析移行群は有意に高値であった(p=0.002)。全糸球体における細胞性半月体，線維細胞性半月体，線維性半月体の割合は両群間に有意差はなかった。間質の線維化は透析移行群のほうが高度である傾向があったが，有意差はなかった。

初期治療は進行性腎障害研究班，難治性血管炎研究班による治療指針に基づいて施行した。ステロイドパルス療法(メチルプレドニゾロン 1 g×3 日間)が 5 例，ステロイドセミパルス療法(メチルプレドニゾロン 0.5 g×3 日間)が 6 例，プレドニゾロンは体重当たり平均 0.76 mg/kg 使用した。免疫抑制薬の併用は 4 例であり，シクロホスファミドパルス療法 1 例，シクロホスファミドの内服が 3 例(体重当たり 1〜1.25 mg/kg)であった。MPO-ANCA の高値であった例や多臓器病変を認めた症例に対し，血漿交換療法(PE 2 例，DFPP 2 例)を併用した。腎予後と初期治療の関係については，腎生存群，透析移行群におけるステロイド投与量，投与期間，シクロホスファミド・ミゾリビンなどの免疫抑制薬の併用の有無について調べた。その結果，両群間の治療において有意差はいずれにも認めなかった。ただ，血漿交換療法については，透析移行群では施行例がゼロであったのに対し，腎生存群は 12 例中 4 例に施行しており，その有効性についてはさらに検討する必要があると思われた。

初期治療開始後 1 カ月目には，全例で BVAS の新規に悪化した点数が 0 点となり寛解に至った。初期治療後 1 カ月間における透析移行例は 6 例(33 %)であった。そのうち 2 例は初期治療により透析から離脱したが，別の 2 例が新たに透析導入となり，1 年後の時点で維持透析となった症例は 6 例(33 %)であった。初期治療後 1 カ月間における死亡例はなかったが，1 例は初期治療から半年後にサイトメガロウイルス感染にて死亡した。

治療による CRP，MPO-ANCA の推移としては，CRP は初期治療前が 7.5±9.7 mg/dL であったが，1 カ月後には 0.17±0.28 mg/dL と著明な低下を認め，最も鋭敏に治療効果を反映した。MPO-ANCA は治療前が 379±291 EU であったのに対し 1 カ月後には 241±288 EU と緩やかな低下傾向を認めた。

本症例群においては，その後 54±36 カ月のフォローアップを行ったところ，1 例において 6 年後に MPO-ANCA の上昇を伴った RPGN の再燃を認めた。

考　察

血管炎症候群は Jenette を中心とし，Chapelhill 会議により血管径を基準とする分類がなされた[1,3]。小型血管に炎症をきたすもののうち，顕微鏡的多発血管炎(MPA)，ウェゲナー肉芽腫，アレルギー性肉芽腫性血管炎(AGA)は結節性多発動脈炎より分離されたもので，その傷害血管は細動脈，毛細血管，細静脈である。ANCA はその病因の一つとされ，MPA，AGA，ウェゲナー肉芽腫は ANCA 関連血管炎とよばれている。本邦では MPO-ANCA 関連血管炎が多いが，その病態や組織に関する解析は十分とは言えない。

MPA の診断基準は 1998 年厚生労働省の難治性血管炎班会議により作成され，腎臓病変，間質性肺炎や肺出血などの肺病変，その他の臓器症状を満たす場合と定義されている[6]。臓器病変は腎，肺に多いが，多臓器に多彩な臨床症状を示すこともあり，その把握が困難なことも多い。Birmingham Vasculitis Activity Score(BVAS)はヨーロッパにおいて主にウェゲナー肉芽腫を対象に作られた血管炎の活動性評価の指標であり，本邦ではまだ広く普及しているとは言えない。しかし，MPA においても BVAS は血管炎の多彩な全身症状を評価し病型診断をする際に有用な手段となると考えられ，われわれは BVAS を用いて腎生検施行時の臓器病変も評価した[7,8]。腎の項目のうち血尿，蛋白尿，急速進行性腎障害はすべての症例で認め，次に全身症状の発熱，体重減少，筋肉痛などが続いた。肺の項目はほとんどが間質性肺炎であった。間質性肺炎には無症状で胸部 X 線では検出できない軽微なものもあり，KL-6 によるスクリーニングや胸部 CT による評価が必須であった。

今回われわれの検討では，腎限局か多臓器型かを最も反映した血清パラメーターは CRP であった。多臓器型では CRP が有意に高いが，腎限局型では CRP は有意に低値であり，自覚症状もなかった。腎生検時の血清クレアチニン値は腎限局型では 6.22±3.99 mg/dL，多臓器型では 4.8±3.01 mg/dL と有意差はなかったものの，腎限局型のほうが高い傾向にあった。これは，腎限局型では健診などで早期に発見されるケースと，尿毒症症状が出るまで見過ごされるケースがあり，無症状であるがゆえに診断が遅れやすい傾向にあった。検尿異常や GFR 低下の早期発見が課題と考える。

腎生検所見は，全例が半月体形成性腎炎を呈し，尿細管間質病変は高度であった。わが国では厚生省進行性腎障害に関する調査研究の RPGN 分科会による半月体形成率，半月体病期，尿細管・間質病変の程度を基にした病理組織学

所見スコア[9]や，1993 年に急速進行性腎炎症候群における Shigematsu らによる糸球体・間質病変の組織学的表記法[10]が作成されているが十分な検討はなされていない．一方ヨーロッパにおいては，European Vasculitis Study Group (EUVAS)により病理組織の定量的な評価法が示され，予後との関連についての検討がなされている．de Lind van Wijngaarden らは 18 カ月後の腎機能予後と関連する因子として，正常糸球体数，尿細管萎縮，上皮細胞内細胞浸潤をあげ，なかでも正常糸球体数と腎予後との相関を強調している[8]．これらの EUVAS の研究は，MPA 単独の評価ではなくウェゲナー肉芽腫の比率も高いことから，本邦に多い MPA や MPO-ANCA 関連腎炎における検討が求められる[11~18]．今回われわれの MPO-ANCA 関連腎炎の検討でも，硬化糸球体の割合は腎予後と強い相関を示したが，間質細胞浸潤，線維化は有意な予後悪化因子とはなりえなかった．硬化糸球体の割合は，今回の検討では全節性硬化糸球体の割合としており，正確には虚血や虚脱による変化を除外する必要があろう．今回の母集団は平均年齢が 58.6 歳であり，加齢による変化は無視できず，発症前の既往歴や潜在的な腎機能低下も考慮する必要があるかもしれない．また，半月体形成率と腎予後とには有意な相関がなく，これは EUVAS の結果と同様であった．

結　語

腎限局型に比し，多臓器型では有意に CRP が高かった．腎生検による硬化糸球体の割合は腎予後と強い相関を示し，血清クレアチニン値と腎予後は関連していた．半月体形成率と腎予後とには有意な相関がなく，早期発見，早期治療の重要性が示唆された．

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文　献

1. Jenette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CG, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum 1994；37：187-192.
2. Fujimoto S, Uezono S, Hisanaga S, Fukudome K, Kobayashi S, Suzuki K, Hashimoto H, Nakao H, Nunoi H. Incidence of ANCA-associated primary renal vasculitis in the Miyazaki prefecture：The first population-based, retrospective, epidemiologic survey. Clin J Am Soc Nephrol 2006；1：1016-1022.
3. Jenette JC, Falk RJ. Clinical and pathological classification of ANCA-associated vasculitis：what are the controversies ? Clin Exp Immunol 1995；101(Suppl 1)：18-22.
4. Janette JC, Falk RJ. Pathogenesis of the vascular and glomerular damage in ANCA-positive vasculitis. Nephrol Dial Transplant 1998；13(Suppl 1)：16-20.
5. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, Jennette JC. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest 2002；110：955-963.
6. Nakabayashi K, Hashimoto H. Microscopic polyangitis. In：Hashimoto H (ed). Clinical manual for vasculitis. Tokyo：Research Group of Intractable Vasculitis, Ministry of Health, Labor, and Welfare of Japan, 2002：24-26.
7. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, Savage C, Adu D. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. QJM 1994；87：671-678.
8. Lane SE, Watts RA, et al. Primary systemic vasculitis：clinical features and mortality. QJM 2005；98：97-111.
9. 米田雅美，山口　裕，山本　泉，小倉　誠，宇都宮保典，佐中　孜，細谷龍男．MPO-ANCA 関連腎炎の重複腎生検による腎病理推移についての組織学的検討．日腎会誌 2007；49：438-445.
10. Shigematsu H, Yamaguchi N, Koyama A. Glomerulointerstitial events in rapidly progressive nephritic syndrome, with special reference to histologic grade and stage on the renal lesions. Clin Exp Nephrol 1998；2：330-338.
11. Bajema IM, Hagen EC, Hansen BE, Hermans J, Noel LH, Waldherr R, et al. The renal histopathology in systemic vasculitis：an international survey study of inter- and intra-observer agreement. Nephrol Dial Transplant 1996；11：1989-1995.
12. Bajema IM, Hagen EC, Hermans J, Noel LH, Waldherr R, Ferrario F, van der Woude FJ, Bruijin JA. Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. Kidney Int 1999 56：1751-1758.
13. Hauer HA, Bajema IM, Van Houwelingen HC, Ferrario F, Noel LH, Waldherr R, Jayne DR, Rasmussen N, Bruijin JA, Hagen EC：European Vasculitis Study Group (EUVAS). Determinants of outcome in ANCA-associated glomerulonephritis：a prospective clinico-histopathological analysis of 96 patients. Kidney Int 2002；62：1732-1742.
14. Hauer HA, Bajema IM, Hagen EC, Noel LH, Ferrario F, Waldherr R, van Houwelingen HC, Lesavre P, Sinico RA, van der Woude F, Gaskin G, Verburgh CA, de Heer E, Bruijin JA. Long-term renal injury in ANCA-associated vasculitis：an analysis of 31 patients with follow-up biopsies. Nephrol Dial Transplant 2002；17：587-596.
15. Hauer HA, Bajema IM, Van Houwelingen HC, Ferrario F, Noel LH, Waldherr R, Javne DR, Rasmussen N, Bruijin JA, Hagen EC：European Vasculitis Study Group (EUVAS).

Renal histology in ANCA-associated vasculitis : differences between diagnostic and serologic subgroups. Kidney Int 2002 ; 61 : 80-89.

16. Vergunst CE, van Gurp E, Hagen EC, van Houwelingen HC, Hauer HA, Noel LH, Waldherr R, Ferrario F, van der Woude FJ, Bruijin JA, Bajema IM ; EC BCR Project for ANCA-Assay Standardisation. An index for renal outcome in ANCA-associated glomerulonephritis. Am J Kidney Dis 2003 ; 41 : 532-538.
17. de Lind van Wijngaarden RA, Hauer HA, Wolterbeek R, Jayne DR, Gaskin G, Rasmussen N, Noel LH, Ferrario F, Waldherr R, Hagen EC, Bruijin JA, Bajema IM. Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis : A prospective analysis of 100 patients with severe renal involvement. J Am Soc Nephrol 2006 ; 17 : 2264-2274.
18. de Lind van Wijngaarden RA, Hauer HA, Wolterbeek R, Jayne DR, Gaskin G, Rasmussen N, Noel LH, Ferrario F, Waldherr R, Bruijin JA, Bajema IM, Hagen EC ; EUVAS. Chances of renal recovery for dialysis-dependent ANCA-associated glomerulonephitis. J Am Soc Nephrol 2007 ; 18 : 2189-2197.

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REVIEW ARTICLE

Anti-glomerular basement membrane antibody disease in Japan: part of the nationwide rapidly progressive glomerulonephritis survey in Japan

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Abstract Anti-glomerular basement membrane (anti-GBM) antibody disease is a rare, but well characterized cause of glomerulonephritis. It is defined by the presence of autoantibodies directed at specific antigenic targets within the glomerular basement membrane. This pattern of rapidly progressive glomerulonephritis and alveolar hemorrhage is often referred to as Goodpasture's syndrome. The prognosis for patients with anti-GBM antibody disease is poor. In Japan, to improve the prognosis of patients with rapidly progressive glomerulonephritis (RPGN), we conducted a nationwide survey of patients with RPGN and investigated the initial symptoms, laboratory findings including renal biopsy findings, treatment methods, and outcomes. Among patients with RPGN, patients with anti-GBM antibody disease were rare: 6.6% (47/715). Alveolar hemorrhage (Goodpasture's syndrome) was observed in 23.4% of patients with anti-GBM antibody disease. Most patients with anti-GBM antibody disease had renal failure at the time of diagnosis. The mean serum creatinine level of patients with renal-limited anti-GBM antibody disease was 7.07 ± 4.21 mg/dl and that of patients with Goodpasture's syndrome was 7.99 ± 4.31 mg/dl. The mean level of crescent formation was 78.99 ± 23.54% in patients with anti-GBM antibody disease, and a cellular crescent form was observed in 63.2% of those patients. The prognosis for patients with anti-GBM antibody disease is poor; the renal survival rate at 6 months after onset was 20.9%, and the mortality at 6 months after onset was 23.3%. To improve the prognosis for anti-GBM antibody disease, it may be necessary to detect this disease in the early stages and to treat it without delay.

Keywords Anti-glomerular basement membrane antibody disease · Goodpasture's syndrome · Epidemiology · Treatment · Prognosis

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Introduction

Anti–glomerular basement membrane (anti-GBM) antibody disease is a rare autoimmune disorder characterized by rapidly progressive glomerulonephritis (RPGN) with diffuse crescentic formation on renal biopsy. In 1919, Goodpasture first described the autopsy findings of an 18-year-old boy with acute renal failure and massive hemoptysis during an influenza virus infection [1], and Stanton and Tange reported nine cases with alveolar hemorrhage associated with glomerulonephritis. It was proposed that this condition be called Goodpasture's syndrome in 1958 [2]. On the other hand, the nephrotoxic serum nephritis model, first described by Masugi in 1934 [3], was induced in rats by a single injection of heterologous anti-kidney sera, and Ortega and Mellors described the staining of anti-IgG antibodies along glomerular capillary walls in rats with

nephrotoxic nephritis in 1956 [4]. In 1964, Scheer and Grossman reported linear staining of anti-human IgG antibodies in patients with Goodpasture's syndrome, similar to what was observed in rats with nephrotoxic nephritis [5]. Lerner et al. showed that antibodies eluted from the kidneys of patients with Goodpasture's syndrome could bind to the GBM of squirrel monkeys when injected in vivo and could elicit a disease pattern similar to that of anti-GBM antibody disease [6]. Therefore, anti-GBM antibody disease is defined by the presence of autoantibodies directed at specific antigenic targets within the glomerular and/or pulmonary basement membrane.

Anti-GBM antibody disease has an estimated incidence of one case per 2 million per year in European Caucasoid populations [7]. It is responsible for 1 to 5% of all types of antibody-induced glomerulonephritis [8] and is the cause of 10 to 20% of cases of crescentic glomerulonephritis [9, 10]. The disease occurs across all racial groups, but is most common in European Caucasoids. In Japan, to improve the prognosis of patients with RPGN, we conducted a nationwide survey of patients with RPGN in 365 hospitals between 1989 and 2000, and investigated the initial symptoms, laboratory findings, including renal biopsy findings, treatment methods, and outcomes [11]. In this review, the part of this nationwide survey of patients with RPGN that pertains to anti-GBM antibody disease is reported.

Epidemiological investigation

RPGN is defined by the World Health Organization as an abrupt or insidious onset of hematuria, proteinuria, anemia, and rapidly progressing renal failure [12]. In this nationwide survey, subjects who had rapid deterioration of renal function within several weeks and showed hematuria, proteinuria, and/or cellular casts upon urinalysis were regarded as patients with RPGN. Anti-GBM antibody disease was defined as the presence of serum anti-GBM antibody or a linear binding of IgG as detected by direct immunofluorescence (IF) in patients with RPGN. RPGN patients with anti-GBM antibody disease were divided into two types: anti-GBM antibody disease without alveolar hemorrhage was regarded as renal-limited anti-GBM antibody disease and that with alveolar hemorrhage was defined as Goodpasture's syndrome.

Based on this nationwide RPGN survey in Japan, 715 patients with RPGN were registered [11]. The most frequent primary disease was primary pauci-immune crescentic glomerulonephritis ($n = 283$, 39.6%; median age: 65; age range: 6–91); the second most frequent was microscopic polyangiitis (MPA; $n = 127$, 17.8%; median age: 68; age range: 5 to 91), and the third most frequent was anti-GBM antibody disease ($n = 47$, 6.6%). Among the subjects with anti-GBM antibody disease, those with alveolar hemorrhage (Goodpasture's syndrome) made up 1.5% of the RPGN patients in Japan. In comparison with foreign countries, the Japanese rate of anti-GBM antibody disease in RPGN was the lowest; Couser reported that 20% of the RPGN patients had anti-GBM antibody in the USA [9]. Anaganco et al. reported that the proportion of anti-GBM-associated RPGN in Europe was 12% [13], and Jennette reported that 15% of the RPGN patients had anti-GBM antibody [14].

All age groups are affected, but the peak incidence of anti-GBM antibody disease is in the 3rd decade in young men, with a second peak in the 6th and 7th decades affecting men and women equally [7, 15, 16]. Alveolar hemorrhage is more common in younger men, while isolated renal disease is more frequent in the elderly with a near-equal gender distribution. In this survey, the mean age at onset of renal-limited anti-GBM antibody disease was 52.6 ± 17.0 years and that for Goodpasture's syndrome was 49.4 ± 14.4 years. There was only one peak incidence of anti-GBM antibody disease in the 5th and 6th decades (Fig. 1). In comparison with MPA, the age at onset of anti-GBM antibody disease was younger; the mean age at onset of primary pauci-immune crescentic glomerulonephritis was 61.3 ± 15.8 years, and the mean age at onset of MPA was 65.6 ± 11.1 years. The gender distribution was nearly equal in renal-limited anti-GBM antibody disease (male:female = 1:0.94), but Goodpasture's syndrome was more common in females (male:female = 1:1.75).

Clinical symptoms

General malaise, weight loss, fever, or arthralgia may be the initial features of anti-GBM antibody disease in a

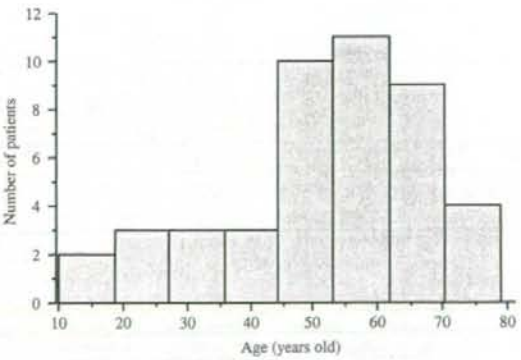

Fig. 1 The age distribution of patients with anti–GBM antibody disease in Japan. The histogram shows the number of patients with anti–GBM antibody disease classified by the patient's age at the onset of this disease

pattern similar to, but much less prominent than in systemic vasculitis. Symptoms relating to anemia may also occur even in the absence of significant hemoptysis. The principal clinical features relate to the development of renal failure due to RPGN or alveolar hemorrhage. In this survey, hemodialysis therapy had already been initiated in 59.6% (28/47) of the anti-GBM patients before the start of immunosuppressive treatments. Although one-third to two-thirds of patients with anti-GBM antibody disease demonstrate alveolar hemorrhage in general, in this survey 23.4% (11/47) of patients with anti-GBM antibody disease suffered from alveolar hemorrhage.

Laboratory examinations

Upon urinalysis, all patients with anti-GBM antibody disease had microscopic hematuria. Proteinuria is modest, but can be heavier when the disease has a more subacute course. In this survey, the mean 24-h excretion of urinary protein in renal-limited anti-GBM antibody disease was 2.1 ± 3.0 g and that of Goodpasture's syndrome was 3.7 ± 3.2 g (Table 1). Unfortunately, most patients with anti-GBM antibody disease had renal failure at the time of diagnosis, and the mean serum creatinine (s-Cr) level in renal-limited anti-GBM antibody disease was 7.07 ± 4.21 mg/dl, while that in Goodpasture's syndrome was 7.99 ± 4.31 mg/dl. Anemia was observed in most patients with anti-GBM antibody disease, and the mean hemoglobin concentration in renal-limited anti-GBM antibody disease was 8.8 ± 1.7 g/dl, while that in Goodpasture's syndrome was 7.5 ± 1.1 g/dl. The mean erythrocyte sedimentation rate (ESR) in renal-limited anti-GBM antibody disease was 105 ± 44 mm/h, and that in Goodpasture's syndrome was 82 ± 45 mm/h. The mean serum C-reactive protein (CRP) level in renal-limited anti-GBM antibody disease was 8.5 ± 7.2 mg/dl and that in Goodpasture's syndrome was 8.2 ± 8.1 mg/dl. In

Table 1 Characteristics of anti–glomerular basement membrane antibody disease in Japan

	Anti-GBM antibody disease		Microscopic polyangiitis ($n = 127$)	Wegener's granulomatosis ($n = 18$)
	Renal-limited ($n = 36$)	Goodpasture's syndrome ($n = 11$)		
Age (years)	52.6 ± 17.0	49.4 ± 14.4	65.6 ± 11.1	44.1 ± 15.5
Sex (M:F)	19:17	4:7	53:66	9:8
Urinalysis				
Proteinuria (g/day)	2.1 ± 3.0	3.7 ± 3.2	1.9 ± 3.1	0.8 ± 0.5
Hematuria	100 (26/26)	100 (10/10)	97.5 (118/121)	100 (14/14)
Blood cell counts				
WBC (/μl)	8,805 ± 3,609	10,436 ± 3,448	11,547 ± 4,880	9,431 ± 4,082
Hemoglobin (g/dl)	8.8 ± 1.7	7.5 ± 1.1	8.3 ± 1.7	9.2 ± 1.9
Platelet ($\times 10^4$/μl)	33.6 ± 12.8	29.4 ± 17.0	32.7 ± 13.6	35.9 ± 19.4
Chemistry				
Total protein (g/dl)	6.39 ± 1.14	6.43 ± 1.21	6.54 ± 0.84	6.64 ± 1.06
Albumin (g/dl)	2.94 ± 0.75	2.78 ± 0.51	2.83 ± 0.56	3.10 ± 0.39
Urea nitrogen (mg/dl)	55.9 ± 30.4	59.9 ± 26.5	50.8 ± 29.1	35.9 ± 26.3
Creatinine (mg/dl)	7.07 ± 4.21	7.99 ± 4.31	4.54 ± 3.13	3.84 ± 3.24
ESR (mm/h)	105 ± 44	82 ± 45	95 ± 40	92 ± 28
Serology				
CRP (mg/dl)	8.5 ± 7.2	8.2 ± 8.1	8.8 ± 7.9	9.6 ± 11.1
ANA (%)	11.8 (4/34)	27.3 (3/11)	36.8 (46/125)	11.8 (2/17)
A-DNA (%)	0 (0/34)	22.2 (2/9)	6.6 (7/106)	0 (0/16)
A-GBM (%)	100 (32/32)	100 (11/11)	1.2 (1/83)	0 (0/11)
MPO-ANCA (%)	10.3 (3/29)	20.0 (2/10)	95.5 (105/110)	18.8 (3/16)
PR3-ANCA (%)	0 (0/29)	10.0 (1/10)	3.6 (4/110)	68.8 (11/16)
Kidney size by abdominal US/CT				
Atrophy (%)	9.7 (3/31)	20.0 (2/10)	8.5 (10/118)	16.7 (3/18)
Swelling (%)	35.5 (11/31)	0 (0/10)	19.5 (23/118)	16.7 (3/18)
Normal (%)	54.8 (17/31)	80.0 (8/10)	72.0 (85/118)	66.7 (12/18)

Anti-GBM anti-glomerular basement membrane, *WBC* white blood cell, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *ANA* anti-nuclear antibody, *A-DNA* anti-DNA antibody, *A-GBM* anti-glomerular basement membrane antibody, *MPO* myeloperoxidase, *ANCA* anti-neutrophil cytoplasmic antibody, *PR3* proteinase-3, *US* ultrasonography, *CT* computed tomography

comparison with other forms of RPGN, such as MPA and Wegener's granulomatosis (WG), there was no difference in inflammation markers, such as leukocyte count, ESR, and serum CRP. However, in patients with anti-GBM antibody disease, the mean level of s-Cr at the time of diagnosis was higher than that in patients with MPA (4.54 ± 3.13 mg/dl) or WG (3.84 ± 3.24 mg/dl). Therefore, early diagnosis of anti-GBM antibody disease is very important.

The diagnosis of anti-GBM antibody disease is dependent on the detection of anti-GBM antibodies either in the circulation or in kidney tissue. These serum antibodies are usually detected using an enzyme-linked immunosorbent assay or radioimmunoassay method. The antibodies have not been reported to occur in the absence of disease, and false negatives are rare when appropriate checks are performed. In this survey, 91.5% (43/47) of patients with anti-GBM antibody disease were diagnosed via the detection of serum anti-GBM antibodies. In serological examinations, other autoantibodies were not usually detected. However, in this survey, anti-nuclear antibodies were detected in 11.8% of renal-limited anti-GBM antibody disease and in 27.3% of patients with Goodpasture's syndrome. Anti-DNA antibody was not detected in renal-limited anti-GBM antibody disease, but it was detected in 22.2% of patients with Goodpasture's syndrome. Moreover, anti-neutrophil cytoplasmic antibodies (ANCA) were detected in 12.8% (5/39) of patients with anti-GBM antibody disease; a perinuclear pattern was detected in all five anti-GBM antibody disease patients with ANCA, and a cytoplasmic pattern was detected in one. The coexistence of anti-GBM antibody and ANCA occurred in 15-50% of cases of anti-GBM antibody disease described in the previous literature [17–21]. In addition, previous studies revealed that patients with double-positive antibodies were MPO-ANCA predominant, older, and male predominant [17–20]. In this survey, the age at onset of patients with double-positive antibodies was higher (the mean age was 52.6 years), but female dominant (male:female = 1:4). The prognosis of patients with double-positive antibodies varied; the renal and patient survivals of patients with double-positive antibodies were reported to be better [17–18], not significantly different [19], or worse [20–21] than those of patients with anti-GBM antibody alone. In this survey, the prognosis of patients with double-positive antibodies was poor; two of them died, and the remaining three patients required maintenance hemodialysis. Alveolar hemorrhage was observed in two of five patients with double-positive antibodies, and three of them had interstitial pneumonitis.

Kidney sizes were usually normal or enlarged due to inflammation. In this survey, ultrasonography showed that 61.0% of patients with anti-GBM antibody disease had kidneys of normal size, while atrophic kidneys were observed in 12.2% of patients, and enlarged kidneys were observed in 26.8%.

Histopathological findings

A renal biopsy is essential in suspected anti-GBM antibody disease to confirm the diagnosis and to assess the renal prognosis. The histological pattern of disease starts with mesangial expansion and hypercellularity and progresses to focal and segmental glomerulonephritis with infiltration by leukocytes accompanied by segmental necrosis with prominent breaks in the GBM. Later, glomeruli develop an extensive crescent formation composed of parietal epithelial cells and macrophages in association with the destruction of the GBM. In this survey, renal biopsy or autopsy was performed in 40 of 47 patients with anti-GBM antibody disease; 2 of these patients were excluded from the analysis of glomerular lesions because their renal specimens included 5 or fewer glomeruli (Table 2). The mean percentage of glomeruli showing crescent formation was $78.99 \pm 23.54\%$ in patients with anti-GBM antibody disease. The mean percentage of glomeruli showing crescent formation in patients with anti-GBM antibody disease was significantly higher than that in MPA, another form of RPGN. The percentage of patients with anti-GBM antibody disease who had more than 50% crescentic glomeruli was 89.5% (34/38). Crescent patterns are usually divided into three groups: cellular, fibrocellular, or fibrous crescents. In 63.2% of anti-GBM antibody disease, the most dominant crescent pattern was cellular. The percentage of patients with cellular crescentic glomeruli in anti-GBM antibody disease was higher than in MPA and WG.

Interstitial inflammation is usually present and may relate to the binding of antibodies to the basement membrane of distal convoluted tubules. In this survey, severe tubulointerstitial damage was present in 45.0% of cases of anti-GBM antibody disease, and moderate damage was present in 40.0%.

Linear binding of IgG is universally detected by direct IF. Linear C3 is found in 60 to 70% of kidney biopsies, but does not influence the severity of the renal lesion [22].

Pulmonary lesions are usually observed to be hemorrhages, with numerous hemosiderin-containing macrophages, deposits of fibrin and alveolar cell hyperplasia, histologically. Necrosis of alveolar walls with polymorphonuclear cell infiltration is also recognized. On IF examination, linear binding of IgG is usually detected along the alveolar basement membrane. In this survey, histopathological data of pulmonary lesions were not accumulated.

Table 2 The histologic pattern of anti-glomerular basement membrane antibody disease in Japan

		Anti-GBM antibody disease ($n = 40$)	Microscopic polyangiitis ($n = 103$)	Wegener's granulomatosis ($n = 18$)
Percentage of patients with adequate specimens		100 (40/40)	98.1 (101/103)	94.4 (17/18)
Percentage of patients with more than five glomeruli		95.0 (38/40)	91.3 (94/103)	77.8 (14/18)
Mean number of glomeruli		21.53 ± 12.87	21.28 ± 14.12	22.47 ± 20.62
Mean percentage of glomeruli showing crescent formation		78.99 ± 23.54	58.74 ± 27.66	76.76 ± 20.90
Percentage of patients with more than 50% crescentic glomeruli		89.5 (34/38)	60.6 (57/94)	78.6 (11/14)
Most dominant crescent pattern (%)	Cellular	63.2 (24/38)	50.0 (47/94)	35.7 (5/14)
	Fibrocellular	21.1 (8/38)	37.2 (35/94)	42.9 (6/14)
	Fibrous	15.8 (6/38)	9.6 (9/94)	21.4 (3/14)
Percentage of patients with vasculitis		5.0 (2/40)	46.5 (54/101)	0 (0/17)
Grade of tubulointerstitial damage (%)	None	7.5 (3/40)	2.0 (2/101)	11.8 (2/17)
	Mild	7.5 (3/40)	9.9 (10/101)	11.8 (2/17)
	Moderate	40.0 (16/40)	48.5 (49/101)	52.9 (9/17)
	Severe	45.0 (18/40)	39.6 (40/101)	23.5 (4/17)

Anti-GBM anti-glomerular basement membrane

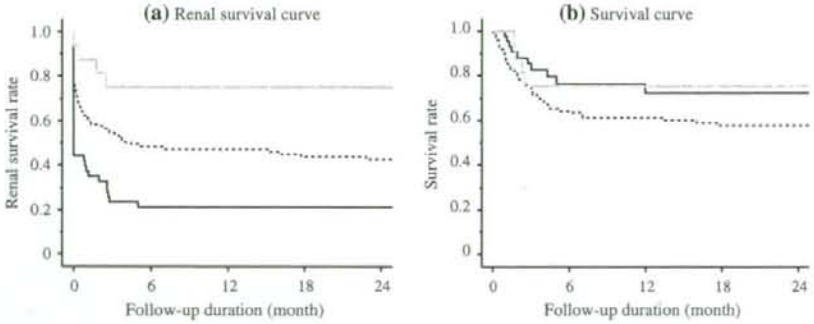

Fig. 2 The survival curves in patients with anti-GBM disease in Japan. The patients' renal survival curve (Kaplan-Meier method) is shown on the left (**a**), and their survival curve is shown on the right (**b**). The *straight line* is the survival curve of patients with anti-GBM antibody disease, the *dotted line* is the survival curve of patients with microscopic polyangiitis, and the *shaded line* is the survival curve of patients with Wegener's granulomatosis

Treatments and prognosis

Without treatment, the prognosis for patients with anti-GBM antibody disease is poor. In this survey, 4 of 47 patients with anti-GBM antibody disease had an undefined clinical course, and the mean follow-up period was 20.3 ± 24.3 months. Excluding the above four patients, the renal survival rate at 6 months after onset was 20.9% (9/43), but the mortality rate at 6 months after onset was 23.3% (10/43, Fig. 2). In comparison with MPA and WG, there was no difference in the survival rate, but the renal survival rate of anti-GBM antibody disease was significantly lower ($P < 0.001$ by the Logrank Mantel-Cox test). Wilson and Dixon reported that 25 of 53 patients died, and only 7 retained independent renal function [8]. It appeared that neither corticosteroids nor immunosuppressive agents had an influence on the renal outcome. However, it is difficult to normalize circulating anti-GBM antibody titers in the short term by the combination therapy of corticosteroids and immunosuppressive agents alone. The demonstration that anti-GBM antibodies were pathogenic provided a rationale for the current approach to treatment using therapeutic plasma exchange combined with immunosuppressive agents. The effectiveness of this therapeutic approach for improving renal function has been reported (Table 3). Renal function improves in 15–75% of patients

Table 3 Previous investigations of treatments for anti–glomerular basement membrane antibody disease

Authors	Ref. No.	Year	Treatment	*N*	Alveolar hemorrhage (%)	1 year (%)	
						Patient survival	Renal survival
Benoit et al.	[23]	1963	No treatment	52	100	4	2
Proskey et al.	[24]	1970	IS	56	100	77	23
Wilson and Dixon	[8]	1973	IS	53	60	53	13
Beirne et al.	[25]	1977	IS	29	54	42	17
Teague et al.	[26]	1978	IS + PE	29	100	64	31
Briggs et al.	[27]	1979	IS	18	61	84	22
Peters et al.	[28]	1982	IS + PE	41	56	76	39
Walker et al.	[29]	1985	IS + PE	22	62	59	45
Savage et al.	[30]	1986	IS + PE	108	52	78	20
Johnson et al.	[31]	1986	OCS + CYC	9	NA	89	22
			OCS + CYC + PE	8	NA	100	75
Herody et al.	[32]	1993	OCS + CYC + AZA	29	50	93	41
Merkel et al.	[16]	1994	OCS + CYC + PE	35	57	89	29
Daly et al.	[33]	1996	IS + PE	40	67	NA	20
Li et al.	[34]	2004	IS + PE	10	40	70	15
Cui et al.	[35]	2005	IS + PE	97	58	92	22

Ref reference, *N* number of patients, *IS* immunosuppressants (including methylprednisolone pulse therapy, oral corticosteroids, cyclophosphamide or azathioprine), *PE* plasma exchange, *OCS* oral corticosteroids, *CYC* cyclophosphamide, *AZA* azathioprine, *NA* not available

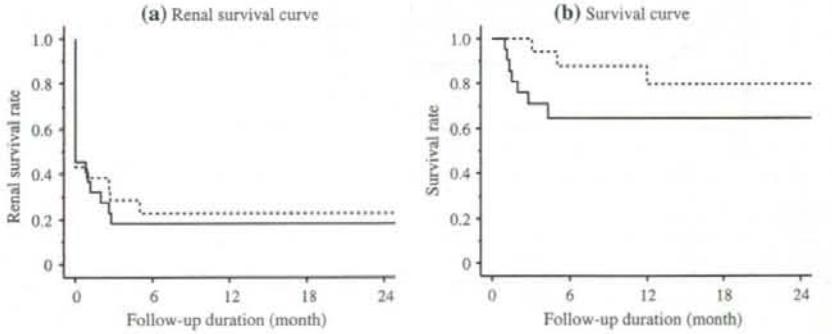

Fig. 3 The survival curves of patients with anti–GBM antibody disease: Difference between the presence and absence of plasma exchange. The patients' renal survival curve (Kaplan-Meier method) is shown on the left (**a**), and their survival curve is shown on the right (**b**). The *straight line* is the survival curve of anti–GBM antibody disease patients treated with plasma exchange, and the *dotted line* is the survival curve of those treated without plasma exchange

with anti-GBM antibody disease through the combination of plasma exchange with corticosteroids and immunosuppressive agents, while the renal survival rates of anti-GBM antibody disease patients treated with immunosuppressive agents alone ranged from 2–22% [7, 8, 16, 23–35]. Improvement of renal function is usually evident within days of the start of plasma exchange. However, it should be emphasized that the regimen has never been properly assessed by a prospective randomized controlled trial because of the rarity and acuteness of the condition. The only reported randomized controlled trial was very small and used lower doses of both plasma exchange and cyclophosphamide than those that are used generally [31].

In this survey, 55.3% (26/47) of patients with anti-GBM antibody disease were treated with plasma exchange. However, there was no significant difference in the renal survival rate between anti-GBM antibody disease patients treated with and without plasma exchange ($P = 0.683$ by the log-rank Mantel-Cox test, Fig. 3a). Moreover, there was no significant difference in mortality between anti-GBM antibody disease patients treated with and without plasma exchange ($P = 0.109$, Fig. 3b). Renal function

Table 4 Numbers of patients with end-stage renal failure or death among patients with anti-glomerular basement membrane antibody disease: a comparison of various treatments

Prognosis		ESRD or death				Death			
Plasma exchange		Absent		Present		Absent		Present	
OCS	Absent	100%	(5/5)	100%	(2/2)	0%	(0/5)	0%	(0/2)
	Present	68.8%	(11/16)	80.0%	(16/20)	18.8%	(3/16)	35.0%	(7/20)
MP	Absent	100%	(6/6)	100%	(4/4)	16.7%	(1/6)	0%	(0/4)
	Present	66.7%	(10/15)	77.8%	(14/18)	13.3%	(2/15)	38.9%	(7/18)
CYC	Absent	77.8%	(14/18)	83.3%	(10/12)	5.6%	(1/18)	16.7%	(2/12)
	Present	66.7%	(2/3)	80.0%	(8/10)	66.7%	(2/3)	50.0%	(5/10)
IVCY	Absent	75.0%	(15/20)	80.0%	(16/20)	10.0%	(2/20)	35.0%	(7/20)
	Present	100%	(1/1)	100%	(2/2)	100%	(1/1)	0%	(0/2)
Total		76.2%	(16/21)	81.8%	(18/22)	14.3%	(3/21)	31.8%	(7/22)

ESRD end-stage renal disease, *OCS* oral corticosteroids, *MP* methylprednisolone pulse therapy, *CYC* cyclophosphamide, *IVCY* intravenous cyclophosphamide

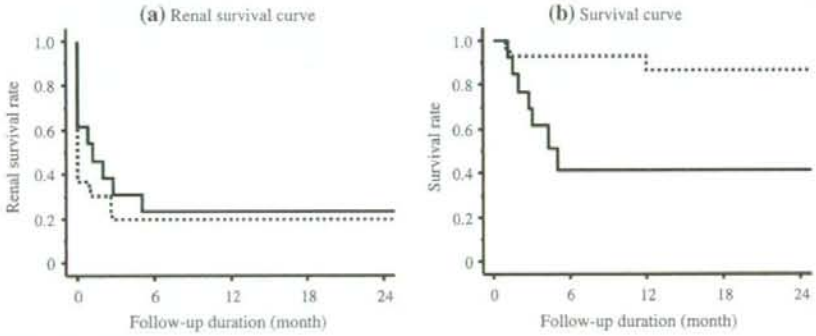

Fig. 4 The survival and renal survival rates in patients with anti-GBM antibody disease: Difference between the presence and absence of cyclophosphamide. The patients' renal survival curve (Kaplan-Meier method) is shown on the left (**a**), and their survival curve is shown on the right (**b**). The *straight line* is the survival curve of anti-GBM antibody disease patients treated with cyclophosphamide, and the *dotted line* is the survival curve of those treated without cyclophosphamide

improves coincident with the introduction of plasma exchange in about 80% of patients with a s-Cr less than or equal to 6.8 mg/dl (600 μmol/l), but in far fewer of those with higher s-Cr levels or those who require dialysis [36]. This result may suggest that in patients with a s-Cr level over 6.8 mg/dl (600 μmol/l) and an absence of alveolar hemorrhage, the benefits of treatment are outweighed by the risks [37]. There have been a number of anecdotal reports of recovery in such patients [30, 38, 39] who usually have a short history with rapidly declining renal function and who usually show a recent onset of the disease with possibly extensive crescent formation without evidence of scarring on renal biopsy. In this survey, unfortunately, 72.3% (34/47) of patients with anti-GBM antibody disease had s-Cr levels higher than 6 mg/dl at the time of diagnosis, and the mean percentage of crescent formation was high in anti-GBM antibody disease patients. Therefore, in most patients with anti-GBM antibody disease in this survey, the time of diagnosis may have been too late to improve the renal function by combination therapy. Although the efficacy of this regimen of therapeutic plasma exchange and immunosuppressive agents was not confirmed in this survey, aggressive treatment may sometimes be justified in particular cases, even in the presence of severe renal failure.

Alveolar hemorrhage is usually responsive to treatment with this regimen and may even respond to the injection of methylprednisolone [40]. As an immunosuppressive therapy, 85.1% (40/47) of patients with anti-GBM antibody disease were treated with oral corticosteroids, 78.7% (37/47) were treated with methylprednisolone pulse therapy, 29.8% (14/47) were treated with oral cyclophosphamide, and 6.4% (3/47) were treated with intravenous cyclophosphamide (IVCY) therapy. Excluding four patients with

unknown outcomes, the final outcome of each treatment is shown in Table 4. Although there was no significant difference in the renal survival rate between anti-GBM antibody disease patients treated with and without cyclophosphamide ($P = 0.495$, Fig. 4a), the survival rate of anti-GBM antibody disease patients treated with cyclophosphamide was significantly lower than that of anti-GBM antibody disease patients treated without cyclophosphamide ($P = 0.003$, Fig. 4b). However, in anti-GBM antibody disease patients treated with cyclophosphamide, the percentage with alveolar hemorrhage was significantly higher than in those treated without cyclophosphamide (50.0% vs. 14.3%, $P = 0.02$). Moreover, the mean dose of oral corticosteroids in anti-GBM antibody disease patients treated with cyclophosphamide was higher (0.96 ± 0.25 mg/kg/day vs. 0.76 ± 0.23 mg/kg/day, $P = 0.07$) than that in anti-GBM antibody disease patients treated without cyclophosphamide. In addition, the operation rate of plasma exchange in anti-GBM antibody disease patients treated with cyclophosphamide was significantly higher than that in patients treated without cyclophosphamide (85.7% vs. 52.4%, $P = 0.04$). Therefore, in this survey, the condition of the anti-GBM antibody disease patients treated with cyclophosphamide may have been more severe than that of the patients treated without cyclophosphamide, resulting in a poor survival rate. On the other hand, the renal survival of the patients treated with cyclophosphamide was not poor, regardless of their poor survival rate. Considering the results of this survey and those described in the previous literature, we can conclude that cyclophosphamide may be a useful immunosuppressive therapy even considering its adverse effects.

Relapse or recurrence of anti-GBM antibody disease with antibody production has been reported, but is quite rare. Recurrences may occur many years after the initial presentation with or without evidence of either renal or pulmonary disease [41–44]. These episodes may occur spontaneously or may be precipitated by infection or exposure to a toxic agent. In this survey, relapse or recurrence was also rare in patients with anti-GBM antibody disease (13.9%) in comparison with patients with ANCA-associated vasculitis, such as WG (29.4%) and MPA (29.3%). Therefore, remission induction therapy is more important in anti-GBM antibody disease.

Conclusion

In the nationwide RPGN survey in Japan, the incidence of anti-GBM antibody disease in RPGN was not high. However, most patients with anti-GBM antibody disease unfortunately had renal failure and had a high percentage of crescent formation at the time of diagnosis. Consequently, in most patients with anti-GBM antibody disease, it may already be too late at the time of diagnosis to perform the combination therapy of therapeutic plasma exchange and immunosuppressive agents, resulting in poor renal survival. Thus, it is important to detect anti-GBM antibody disease in the early stages and to treat it without delay.

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References

1. Goodpasture EW. The significance of certain pulmonary lesions in relation to the etiology of influenza. Am J Med Sci. 1919;158:863–70.
2. Stanton MC, Tange JD. Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). Aust N Zeal J Med. 1958;7:132–44.
3. Masugi M. Über die experimentelle glomerulonephritis durch das spezifische antinieren serum. Ein beiträg zur pathogenese der diffusen glomerulonephritis. Beitr Pathol Anat. 1934;92:429–66.
4. Ortega LG, Mellors RC. The role of localized antibodies in the pathogenesis of nephrotoxic nephritis in the rat. J Exp Med. 1956;104:151–70.
5. Scheer RL, Grossman MA. Immune aspects of the glomerulonephritis associated with pulmonary hemorrhage. Ann Intern Med. 1964;60:1009–21.
6. Lerner RA, Glassock RJ, Dixon FJ. The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. J Exp Med. 1967;126:989–1004.
7. Turner AN, Rees AJ. Antiglomerular basement membrane disease. In: Davison AM, Cameron JS, Grünfeld J-P, Kerr DNS, Ritz E, Winearls CG, editors. Oxford textbook of nephrology. 2nd ed. Oxford: Oxford University Press;1998. pp. 645–66.
8. Wilson CB, Dixon FJ. Anti-glomerular basement membrane antibody-induced glomerulonephritis. Kidney Int. 1973;3:74–89.
9. Couser WG. Rapidly progressive glomerulonephritis: classification, pathogenetic mechanisms, and therapy. Am J Kidney Dis. 1988;11:449–64.
10. Andrassy K, Kuster S, Waldherr R, Ritz E. Rapidly progressive glomerulonephritis: Analysis of prevalence and clinical course. Nephron. 1991;59:206–12.
11. Sakai H, Kurokawa K, Koyama A, et al. The management of rapidly progressive glomerulonephritis. Jpn J Nephrol. 2002;44:55–82 (in Japanese).
12. Churg J, Bernstein J, Glassock RJ. Classification of glomerular disease. In: Churg J, Bernstein J, Glassock RJ, editors. Renal disease. Classification and atlas of glomerular diseases, 2nd ed. New York, Tokyo: Igaku-Shoin;1995. pp. 1–13.
13. Angangco R, Thiru S, Esnault VL, Short AK, Lockwood CM, Oliveira DB. Does truly 'idiopathic' crescentic glomerulonephritis exist? Nephrol Dial Transplant. 1994;9:630–6.
14. Jennette JC. Crescentic glomerulonephritis. In: Jennette JC, Olson JL, Schwartz MM, Silva FG, editors. Heptinstall's pathology of the kidney, 5th ed. Philadelphia, New York:Lippincott-Raven;1998. pp. 625–56.

15. Segelmark M, Butkowski R, Wieslander J. Antigen restriction and IgG subclasses among anti-GBM autoantibodies. Nephrol Dial Transplant. 1990;5:991–6.
16. Merkel F, Pullig O, Marx M, Netzer KO, Weber M. Course and prognosis of anti-basement membrane antibody (anti-BM-Ab)-mediated disease: Report of 35 cases. Nephrol Dial Transplant. 1994;9:372–6.
17. Jayne DR, Marshall PD, Jones SJ, Lockwood CM. Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. Kidney Int. 1990;37:965–70.
18. Bosch X, Mirapeix E, Font J, Borrellas X, Rodriguez R, López-Soto A, Ingelmo M, Revert L. Prognostic implication of anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in anti-glomerular basement membrane disease. Clin Nephrol. 1991;36:107–13.
19. Yang G, Tang Z, Chen Y, Zeng C, Chen H, Liu Z, Li L. Antineutrophil cytoplasmic antibodies (ANCA) in Chinese patients with anti-GBM crescentic glomerulonephritis. Clin Nephrol. 2005;63:423–8.
20. Rutgers A, Slot M, van Paassen P, van Breda Vriesman P, Heeringa P, Tervaert JW. Coexistence of anti-glomerular basement membrane antibodies and myeloperoxidase-ANCAs in crescentic glomerulonephritis. Am J Kidney Dis. 2005;46:253–62.
21. Levy JB, Hammad T, Coulthart A, Dougan T, Pusey CD (2004) Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. Kidney Int 66:1535–1540.
22. Turner AN, Rees AJ Antiglomerular basement disease. In: Davison AM, Cameron JS, Grunfeld J-P, Ponticelli C, Ritz E, Winearls CG, van Ypersele C, editors. Oxford textbook of clinical nephrology, 3rd ed. Oxford: Oxford University Press;2005. pp. 579–600.
23. Benoit FL, Rulon DB, Theil GB, Doolan PD, Watten RH. Goodpasture's syndrome: A clinicopathologic entity. Am J Med. 1963;58:424–44.
24. Proskey AJ, Weatherbee L, Easterling RE, Greene JA Jr, Weller JM. Goodpasture's syndrome. A report of five cases and review of the literature. Am J Med. 1970;48:162–73.
25. Beirne GJ, Wagnild JP, Zimmerman SW, Macken PD, Burkholder PM. Idiopathic crescentic glomerulonephritis. Medicine. 1977;56:349–81.
26. Teague CA, Doak PB, Simpson IJ, Rainer SP, Herdson PB. Goodpasture's syndrome: an analysis of 29 cases. Kidney Int. 1978;13:492–504.
27. Briggs WA, Johnson JP, Teichman S, Yeager HC, Wilson CB. Antiglomerular basement membrane antibody-mediated glomerulonephritis and Goodpasture's syndrome. Medicine. 1979;58:348–61.
28. Peters DK, Rees AJ, Lockwood CM, Pusey CD. Treatment and prognosis in antibasement membrane antibody-mediated nephritis. Transplant Proc. 1982;14:513–21.
29. Walker RG, Scheinkestel C, Becker GJ, Owen JE, Dowling JP, Kincaid-Smith P. Clinical and morphological aspects of the management of crescentic anti-glomerular basement membrane antibody (anti-GBM) nephritis/Goodpasture's syndrome. Q J Med. 1985;54:75–89.
30. Savage COS, Pusey CD, Bowman C, Rees AJ, Lockwood CM. Antiglomerular basement membrane antibody mediated disease in the British Isles 1980–4. Br Med J. 1986;292:301–4.
31. Johnson JP, Moore JJ, Austin HA, Balow JE, Antonovych TT, Wilson CB. Therapy of anti-glomerular basement membrane antibody disease: Analysis of prognostic significance of clinical, pathologic and treatment factors. Medicine. 1985;64:219–27.
32. Herody M, Bobrie G, Gouarin C, Grunfeld JP, Noel LH. Anti-GBM disease: Predictive value of clinical, histological and serological data. Clin Nephrol. 1993;40:249–55.
33. Daly C, Conlon PJ, Medwar W, Walshe JJ. Characteristics and outcome of anti-glomerular basement membrane disease: a single-center experience. Ren Fail. 1996;18:105–12.
34. Li FK, Tse KC, Lam MF, Yip TP, Lui SL, Chan GS, et al. Incidence and outcome of antiglomerular basement membrane disease in Chinese. Nephrology. 2004;9:100–4.
35. Cui Z, Zhao MH, Xin G, Wang HY. Characteristics and prognosis of Chinese patients with anti-glomerular basement membrane disease. Nephron Clin Pract. 2005;99:c49–55.
36. Levy JB, Pusey CD. Still a role for plasma exchange in rapidly progressive glomerulonephritis? J Nephrol. 1997;10:7–13.
37. Flores JC, Taube D, Savage CO, Cameron JS, Lockwood CM, Williams DG, Ogg CS. Clinical and immunological evolution of oligoanuric anti-GBM nephritis treated by haemodialysis. Lancet. 1986;1:5–8.
38. Cohen LH, Wilson CB, Freeman RM. Goodpasture syndrome: Recovery after severe renal insufficiency. Arch Intern Med. 1976;136:835–7.
39. Maxwell AP, Nelson WE, Hill CM. Reversal of renal failure in nephritis associated with antibody to glomerular basement membrane. Br Med J. 1988;297:333–4.
40. de Torrente A, Popovtzer MM, Guggenheim SJ, Schrier RW. Serious pulmonary hemorrhage, glomerulonephritis, and massive steroid therapy. Ann Intern Med. 1975;83:218–9.
41. Dahlberg PJ, Kurtz SB, Donadio JV, Holley KE, Velosa JA, Williams DE, Wilson CB. Recurrent Goodpasture's syndrome. Mayo Clin Proc. 1978;53:533–7.
42. Mehler PS, Brunvand MW, Hutt MP, Anderson RJ. Chronic recurrent Goodpasture's syndrome. Am J Med. 1987;82:833–5.
43. Hind CR, Bowman C, Winearls CG, Lockwood CM. Recurrence of circulating anti-glomerular basement membrane antibody three years after immunosuppressive treatment and plasma exchange. Clin Nephrol. 1984;21:244–6.
44. Levy JB, Lachmann RH, Pusey CD. Recurrent Goodpasture's disease. Am J Kidney Dis. 1996;27:573–8.

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総　説

CAWS血管炎惹起の分子メカニズム

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要　旨

高度化医療の進歩に従い，易感染患者の数が増加し，深在性真菌症の増加がみられている．*Candida albicans* は深在性真菌症の起因菌の一つであるが，我々は*C. albicans* NBRC 1385を培養し，その上清より可溶性多糖画分（*C. albicans* water soluble fraction; CAWS）を得，その活性を評価してきた．その結果，マウスへのCAWS静脈内投与により致死活性が発現すること，腹腔内投与により大動脈起始部および冠状動脈に血管炎を誘導すること，そして数種の異なる系統のマウスにCAWSを投与することにより，この血管炎の重症度は，用いるマウスの系統によって大きく異なることを明らかにしてきた．そこで，さらにさまざまな系統のマウスを用いて，CAWS血管炎感受性について検討した．その結果，ほとんどの系統で血管炎が惹起されたことから，CAWS血管炎は普遍的なモデルであることが強く示唆された．また，系統ごとに強弱はさまざまであり，複数の遺伝子が血管炎惹起と調節に関わっていると考えられた．

Key words：*C. albicans*由来可溶性多糖画分（*C. albicans* water soluble fraction; CAWS），血管炎（arteritis），マウス系統差（strain difference），モデルマウス（animal model）

1　はじめに

CAWSは*C. albicans* NBRC 1385を完全合成培地（C-limiting medium）で培養すると，培養上清に産生される可溶性の多糖画分である．1970年代より，東邦大学付属大橋病院の村田，直江らは，川崎病患児由来*C. albicans*の菌体アルカリ抽出画分（CADS）をマウスに投与することにより川崎病類似の冠状動脈炎を誘発できることを報告してきた[1-3]．同様のプロトコールによりCAWSの血管炎誘発活性を検討したところ，重症の血管炎が誘発されることが明らかとなった[4]．さらにCAWSは，急性致死毒性[5,6]，好中球の活性化作用[7]，マウスの耳が壊死する重症の炎症の惹起[8]，など種々の活性を有することが明らかとなってきた．本稿では，CAWSの血管炎誘発活性のマウスの系統による感受性の違いを中心に報告する．

2　近交系マウスにおけるCAWS血管炎

従来からのCADS血管炎誘発プロトコール（Fig. 1）に従い，CAWSをC3H/HeN，DBA/2，CBA/J，C57BL/6の4系統のマウスに投与し，大動脈起始部周辺のHE染色像を観察した．その結果，血管炎の重症度に差はあるものの，すべての系統のマウスに血管炎が観察された（Fig. 2）．中でも，DBA/2とC57BL/6は重症の血管炎を発症し，それと比較して，C3H/HeNは軽症だった．CBA/Jは更に軽症であり，DBA/2，C57BL/6，C3H/HeNは観察したすべてのマウスで血管炎が観察されたのに対し，CBA/Jの血管炎発症率は10％だった．

3　その他の近交系マウスにおけるCAWS血管炎

はじめに検討した4系統のマウスにおいて，CAWS血管炎の感受性に差が観察されたことから，更にさまざまな系統のマウスを用いてCAWS血管炎感受性を検討した．CAWSの投与プロトコールは，Fig. 1のプロトコールでは結果判定まで2ヵ月以上の期間が必要で，投与に必要なCAWSもマウス1匹当たり40 mgとかなり大量であったため，短縮のプロトコールを考案した（Fig. 3）．このプロトコールを用いて，DBA/1，C3H/HeJ，Balb/c，A/J，AKR/N，CBA/Nの6系統のマウスにCAWSを投与し，大動脈起始部周辺のHE染色像を観察した（Fig. 4）．その結果，DBA/2と同じ起源で，Littleによって薄いチョコレート色の毛色のlineから作成された亜系の1つであるDBA/1は，DBA/2同様の重症の血管炎を発症した．Toll-like Receptor 4遺伝子に点突然変異を持ち，LPS低感受性のC3H/HeJは，C3H/HeNと同様にCAWS血管炎の感受性は低かったが，血

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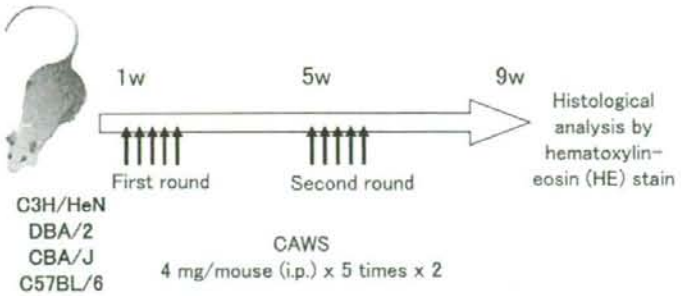

Fig. 1. Experimental schedule of coronary arteritis induction.

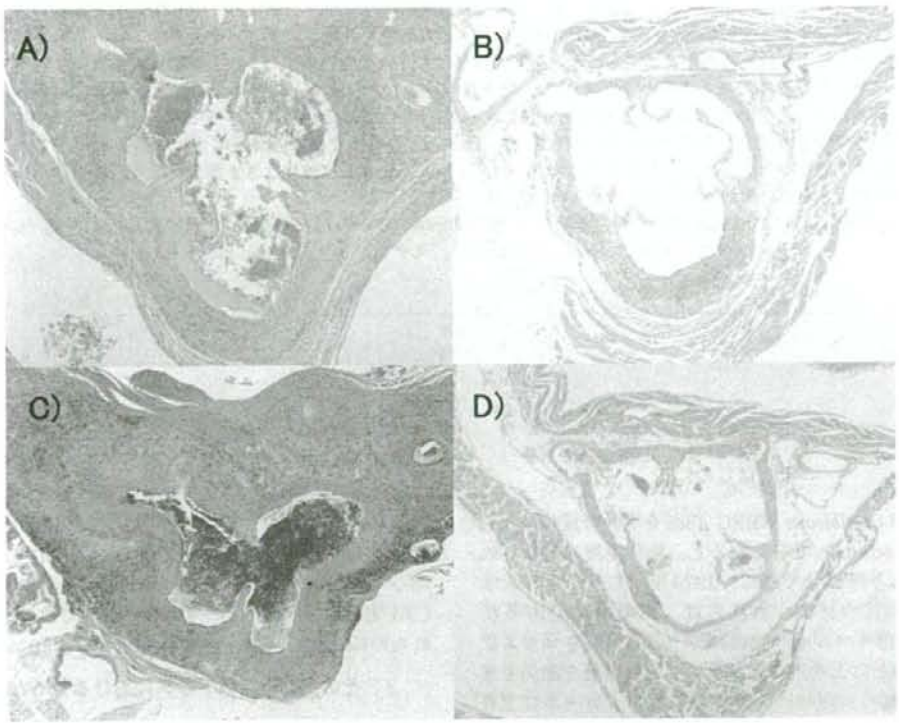

Fig. 2. Histological observations of coronary arteritis. CAWS (4 mg/mouse) was administered i.p. to DBA/2, C3H/HeN, C57BL/6, CBA/J mice for five consecutive days in the 1st and 5th week. In the 9th week, mice were sacrificed and prepared sections were stained with hematoxylin-eosin. A) DBA/2, B) C3H/HeN, C) C57BL/6, D) CBA/J

管の一部が腫れ炎症性の細胞が集積していた．抗体産生能が高く，Th2応答を誘導されやすい系統であるBalb/c，DBA/2と同様に補体C5に欠損のあるA/J，AKR/Nもまた，重症の血管炎を発症した．CBA/Jと同じ起源であり，そのBtk遺伝子に変異がありB細胞の分化不全を起こすCBA/NもCAWS血管炎が誘導された．

4 その他のマウスにおけるCAWS血管炎誘発活性

4.1. クローズドコロニー

ある集団内の動物間のみで繁殖が継続されているクローズドコロニーでは，個体間に遺伝的な差がある．このクローズドコロニーであるICRを用いてCAWS血管炎の発症を検討した（Fig. 5A）．その結果，観察したすべてのマウスにCAWS血管炎の発症が認められた．

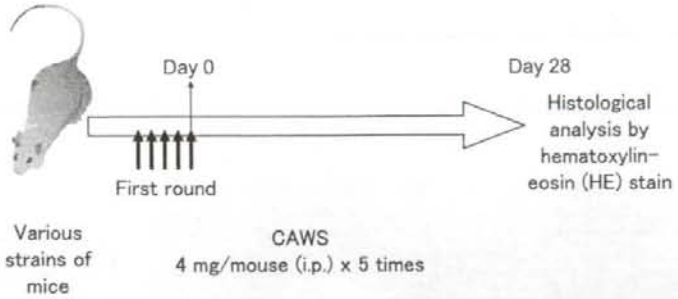

Fig. 3. New experimental schedule of coronary arteritis induction.

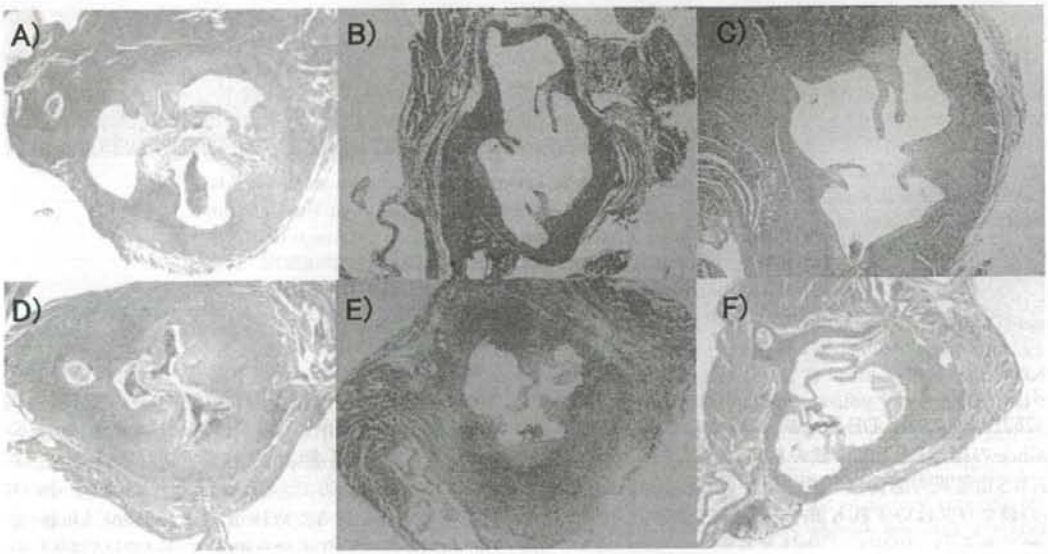

Fig. 4. Histological analysis of several strains of mice administered CAWS. CAWS (4 mg/mouse) was administered i.p. to DBA/1, C3H/HeJ, Balb/c, A/J, AKR/N, CBA/N mice for five consecutive days. Twenty-eight days later, the aorta with the coronary arteries of these mice were stained with hematoxylin-eosin.
A) DBA/2, B) C3H/HeN, C) Balb/c, D) A/J, E) AKR/N, F) CBA/N

Fig. 5. Histological analysis of several strains of mice administered CAWS. CAWS (4 mg/mouse) was administered i.p. to closed a colony or F1 mice for five consecutive days. Twenty-eight days later, the aorta with the coronary arteries of these mice were stained with hematoxylin-eosin. A) ICR, B) BDF1, C) CDF1

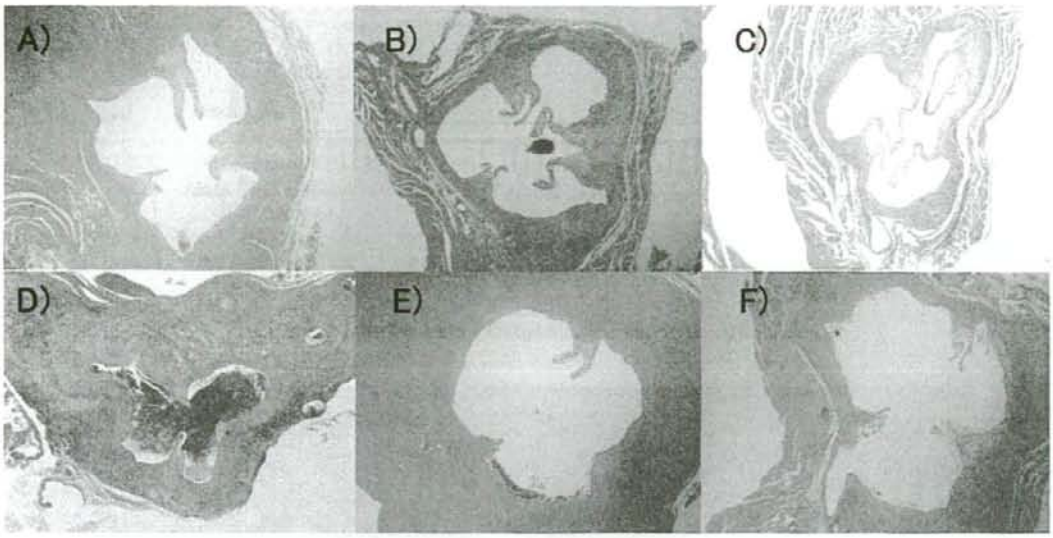

Fig. 6. Histological analysis of several strains of mice administered CAWS. CAWS (4 mg/mouse) was administered i.p. to various mice for five consecutive days. Twenty-eight days later, the aorta with the coronary arteries of these mice were stained with hematoxylin-eosin.
A) Balb/c, B) Balb/c *nu/nu*, C) C.B.-17/Icr-*scid/scid*, D) C57BL/6, E) C57BL/6J Ham Slc-*bg*, F) WBB6F1-*W/W*^{v}

4.2. F1マウス

C57BL/6の雌とDBA/2の雄のF1であるBDF1と，Balb/cの雄とDBA/2の雌のF1であるCDF1を用いてCAWS血管炎の感受性を検討した（Fig. 5B, C）．これらの親マウスはいずれも重症のCAWS血管炎を起こす系統であるが，BDF1，CDF1どちらのマウスにも血管炎が誘発された．

4.3. ミュータントマウス

Balb/cをバックグラウンドとするBalb/c *nu/nu*は，T細胞分化の場としての胸腺を欠くため，胸腺依存の免疫不全を特徴とするマウスである．一方，C.B.-17/Icr-*scid/scid*は，同じくBalb/cをバックグラウンドとし，T細胞，B細胞の両方を欠損するモデルマウスとして汎用されている．これらのマウスのCAWS血管炎について検討すると，Balb/c *nu/nu*もC.B.-17/Icr-*scid/scid*も，Balb/cと比較すると感受性はやや弱い傾向にあった（Fig. 6 A-C）．

また，C57BL/6をバックグラウンドとし，NK細胞の機能が低下しているC57BL6J Ham Slc-*bg*や，C57BL/6-W^{v}/＋の雄とWB－W/＋の雌のF1マウスでありmast細胞を欠損しているWBB6F1-*W/W*^{v}を用いてCAWS血管炎について検討した結果，重症のCAWS血管炎が誘導されることが明らかとなった（Fig. 6D-F）．

5 考察

種々の系統のマウスを用いて検討した結果，重症度に差はあるもののほとんどの系統でCAWS血管炎が惹起されることが明らかとなった．検討したマウス系統において，CAWS血管炎に抵抗性を示した系統は，CBA/Jのみであった．一方で，CBA/Jと同じ起源で，そのBtk遺伝子に変異があるためBruton's tyrosine kinaseを欠損し，B細胞の分化不全を起こすCBA/Nは顕著な血管炎を発症した．CBA/Nの血管炎は，重症の血管炎を発症する系統であるDBA/2で報告されているような[9]，冠状動脈起始部付近の大動脈における弾性繊維の損傷を伴っていた（data not shown）．大動脈は伸縮性に富む弾性型動脈に属する動脈であり，この伸縮性は，中膜に多くの弾性層を形成していることにより与えられるものである．CAWS血管炎が発症する大動脈起始部は特に弾性繊維が多く存在し，この部分に損傷が起こることによってさらに炎症が拡大している可能性が考えられた．

更に我々はCBA/JのCAWS血管炎への抵抗性に着目し，CBA/Jの脾臓細胞を*in vitro*でCAWS刺激し，そのサイトカイン産生について検討した．その結果，CBA/JはCAWS刺激に対して，その他の系統のマウス（C3H/HeN，DBA/2，C57BL/6）と比較して，IL-10の産生を強く誘導することが明らかとなった[4]．IL-10は抗炎症性のサイトカインであり，血管炎の抑制に関与している可能性があるのではないかと考えている．

微生物成分による血管炎誘導の報告として，*Lactobacillus casei*を用いたものがある[10]．*Lactobacillus casei* cell wallをマウスに投与すると，川崎病様の冠状動脈炎，動脈瘤が生じ，この発症にはNOが関与しているとされ

ている．一方，マウス腹腔マクロファージのIFN-γ存在下でのNO産生能には，系統差があることが報告されている[11]．この中で，重症のCAWS血管炎の起こるDBA/2のNO産生能は比較的低く，NO産生の系統差とCAWS血管炎の重症度との間には直接的な相関はなかった．しかしながら，DBA/2は真菌多糖（β-glucan）によりIFN-γ産生が強く誘導される系統であり[12]，さらにCAWS刺激によりIFN-γを誘導することから[13]，IFN-γによって誘導されたNOがCAWS血管炎の重症化に関与している可能性も考えられた．

重症の血管炎を誘発する系統では，IL-6やTNF-αなどの炎症性サイトカインの産生も上昇していることが明らかとなっている[4]．さらに，Balb/c *nu/nu*やC.B-17/Icr-*scid/scid*のCAWS血管炎は比較的軽症だが，血管壁の肥厚は観察され，完全な抑制には至らなかった．これらのことから，CAWS血管炎の誘発，重症化にはさまざまな細胞由来の因子が複雑に関わっているものと考えられたが，CAWS血管炎は多くのマウス系統に普遍的に，そして簡便に誘発することができる．このことから，血管炎モデルマウスとしての使用が期待されており，血管炎誘発，抑制に関わる更なる詳細な解析が望まれる．

一方で，CAWSの物性の解析も行ってきた．これまでの検討により，CAWSの組成は，糖78±6.6%，蛋白15±6.3%の糖タンパク質であり，マンナン・グルカン比（mannnan/glucan）は6.3±1.3，特定のmannan構造を認識する因子血清のNo.11，13，13bと高い反応性を示し，Limulus G因子を活性化し，エンドトキシンのコンタミは2 ng/mg以下であることが明らかとなっている．

*C. albicans*を異なる条件下で培養すると，細胞構造の変化が起こる．そこで，異なる培養条件でCAWSを作成し，物性や生理活性の違いを解析した．10種類の因子血清を用いて得られたCAWSの反応性を検討したところ，違いが観察されたことから，培養条件はCAWSのmannan構造に著しい影響を与えることが明らかとなった．さらに，27℃，pH 5.2で*C. albicans*を培養した培養上清由来のCAWS 27℃-pH 5.2は，致死活性も血管炎誘発活性も著しく低く，培養液の温度とpHの双方が活性に影響を与えていることが明らかとなった[14]．

CAWSは*C. albicans*の増殖過程において菌体から放出される多糖画分である．*C. albicans*の培養条件を変えることにより得られるCAWSの構造や活性が異なることから，感染部位により真菌症患者に与える真菌由来多糖画分の影響は異なってくる可能性も考えられる．さらに，深在性真菌症患者血中にはβ-glucanが検出され，真菌症の診断に利用されている．多くの臨床データより真菌症が治癒すると血中β-glucan濃度は速やかに減少するが，宿主の体内にはβ-glucanを含む菌体が長期間にわたって存在し続ける[15]．このような観点からも，今後更に，*C. albicans*由来の多糖画分の構造や活性について詳細に解析してゆきたい．

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文　献

1) Murata H: Experimental candida-induced arteritis in mice. Relation to arteritis in the mucocutaneous lymph node syndrome. Microbiol Immunol **23**: 825-831, 1979.
2) Murata H, Naoe S: Experimental *Candida*-induced arteritis in mice--relation to arteritis in Kawasaki disease. Prog Clin Biol Res **250**: 523, 1987.
3) Takahashi K, Oharaseki T, Wakayama M, Yokouchi Y, Naoe S, Murata H: Histopathological features of murine systemic vasculitis caused by *Candida albicans* extract－an animal model of Kawasaki disease. Inflamm Res **53**: 72-77, 2004.
4) Nagi-Miura N, Shingo Y, Adachi Y, Ishida-Okawara A, Oharaseki T, Takahashi K, Naoe S, Suzuki K, Ohno N: Induction of coronary arteritis with administration of CAWS (*Candida albicans* water-soluble fraction) depending on mouse strains. Immunopharmacol Immunotoxicol **26**: 527-543, 2004.
5) Nagi-Miura N, Shingo Y, Kurihara K, Adachi Y, Suzuki K, Ohno N: Involvement of platelet activating factor, histamine and serotonin in acute lethal shock induced by *Candida albicans* water-soluble extracellular polysaccharide fraction (CAWS) in mice. Biol Pharm Bull **30**: 1354-1357, 2007.
6) Okada N, Asai S, Hotta A, Miura N, Ohno N, Farkas I, Hau L, Okada H: Increased inhibitory capacity of an anti-C5a complementary peptide following acetylation of N-terminal alanine. Microbiol Immunol **51**: 439-443, 2007.
7) Ishida-Okawara A, Nagi-Miura N, Oharaseki T, Takahashi K, Okumura A, Tachikawa H, Kashiwamura S, Okamura H, Ohno N, Okada H, Ward PA, Suzuki K: Neutrophil activation and arteritis induced by *C. albicans* water-soluble mannoprotein-beta-glucan complex (CAWS). Exp Mol Pathol **82**: 220-226, 2007.
8) Hoshino A, Nagao T, Ito-Ihara T, Ishida-Okawara A, Uno K, Muso E, Nagi-Miura N, Ohno N, Tokunaka K, Naoe S, Hashimoto H, Yasuhara M, Yamamoto K, Suzuki K: Trafficking of QD-conjugated MPO-ANCA in murine systemic vasculitis and glomerulonephritis model mice. Microbiol Immunol **51**: 551-566, 2007.
9) Hirata N, Ishibashi K, Ohta S, Hata S, Shinohara H, Kitamura M, Miura N, Ohno N: Histopathological examination and analysis of mortality in DBA/2 mouse vasculitis induced with CAWS, a water-soluble extracellular polysaccharide fraction obtained from *Candida albicans*. Yakugaku Zasshi **126**: 643-650, 2006.
10) Zídek Z, Franková D, Boubelík M: Genetic variation in *in-vitro* cytokine-induced production of nitric oxide by murine peritoneal macrophages. Pharmacogenetics **10**: 493-501, 2000.
11) Adewuya O, Irie Y, Bian K, Onigu-Otite E, Murad F: