研究成果の刊行に関する一覧表

書籍							
著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
鈴木和男	血管炎発症のメカニズム		annual review「腎臓」	中外医学社	東京	2012年1月	43-57
鈴木和男	MPO-ANCA関連血管炎モデルの血管内皮細胞傷害機構		『炎症と免疫』	先端医学社	東京	2011	VOL.19(6)577-584
小林茂人、藤元昭一、鈴木和男	「難治性血管炎調査研究班」研究から一血管炎の分類に関する世界的動向		「脈管学」	メディカルトリビューン社	東京	2011年4月	51巻1号 73-77
小林茂人, 藤元昭一, 鈴木和男	血管病理　ANCA関連血管炎　欧州リウマチ学会/アメリカリウマチ学会による新しい血管炎の分類・診断基準の作成		病理と臨床	文光堂	東京	2011	29巻3号245-248
宇野賀津子	インターフェロン-α, β, γ	宇野賀津子	広範囲血液・尿化学検査　免疫学検査—その数値をどう読むか一［第7版］	日本臨床	東京	2010	p.96-101
河内正治	呼吸不全用栄養剤	丸山道生, 小川哲史	PDN Lectures Chapter-2 経腸栄養	PEGドクターズネットワーク	東京	2011	http://peg.or.jp/
河内正治	吸入麻酔薬	天羽敬祐	麻酔科学レビュー2011	総合医薬社	東京	2011	62-69
武曾惠理	急速進行性糸球体腎炎診療ガイドQ&A第７章 RPGNの治療　急速進行性糸球体腎炎症候群 第２版ダイジェスト版	監修　松尾清一、編集　山縣邦弘	進行性腎障害診療指針　急速進行性糸球体腎炎　診療ガイド	診療と診断社	東京	2011	105-109
武曾惠理	血管炎の腎病変　全身性疾患に伴う腎障害の診断と治療の実際		総合臨床2011 vol.60(6)	永井書店	東京	2011	1371-1377
武曾惠理	免疫グロブリン大量療法　特集 免疫疾患と腎障害		腎と透析	東京医学社	東京	2011	121-123
今井圓裕、安田宜成、松尾清一	CKD運動の意義とCKD患者の動向		臨牀透析	日本メディカルセンター	東京	2011	9-16
今井圓裕、松尾清一	慢性腎症の抗原		腎臓	日本腎臓財団	東京	2011	1-310
今井圓裕	慢性腎症		医学と薬学	自然科学社	東京	2011	149-155

今井圓裕、友雅司	第55回日本透析医学会・第53回日本腎臓学会共催シンポジウムより『透析導入基準』		日本透析医学会雑誌	日本透析医学会	東京	2011	123-136
今井圓裕	疫学と疾患としての重要性		progress in Medicine	ライフ・サイエンス	東京	2011	389-394
今井圓裕	海外の腎性貧血ガイドライン		腎と透析	東京医学社	東京	2011	236-239
今井圓裕	ステロイドパルス療法		医学のあゆみ	医歯薬出版	東京	2011	301-302
今井圓裕	ネフローゼ症候群診療指針		綜合臨牀	永井書店	大阪	2011	1320-1328
湯村和子	ループス腎炎	槇野博史、秋澤忠男	腎疾患・透析最新の治療2011-2013	南江堂	東京	2011	143-148
湯村和子	AAVの活動性の評価方法であるBVASについて教えてください	監修 松尾清一 編集 山縣邦弘	急速進行性糸球体腎炎　診療ガイドQ&A	診断と治療社	東京	2011	64-66
	AAVの臓器傷害の評価方法であるVIDについて教えてください						67-69
湯村和子	第4章診断　Q26	監修　松尾清一　編集　富野康日己、川村哲也	IgA腎症　診療ガイドQ&A	診断と治療社	東京	2011	61-63
有村義宏（分担執筆）	免疫血清検査—自己抗体	中原一彦	パーフェクトガイド 検査値事典	総合医学社	東京	2011	412-421
有村義宏（分担執筆）	チャーグ・ストラウス症候群	井村裕夫	症候群ハンドブック	中山書店	東京	2011	460-461
臼井丈一，山縣邦弘	急速進行性糸球体腎炎，ANCA関連腎炎	富野康日己，柏原直樹、南学正臣	EBM腎臓病の治療2011-2012	中外医学社	東京	2011	42-46
松尾清一，山縣邦弘他		松尾清一，山縣邦弘	急速進行性糸球体腎炎診療ガイドQ&A	診断と治療社	東京	2011	1-154
藤元昭一	特発性半月体形成性腎炎	槇野博史，秋澤忠男	腎疾患・透析最新の治療 2011-2013	南光堂	東京	2011年	136-139
佐地勉	急性期川崎病への抗サイトカイン療法（抗TNFα製剤Infliximab）		Annual Review 循環器 2011	中外医学社	東京	2011	331-336

MPO-ANCA 関連血管炎モデルの血管内皮細胞傷害機構

鈴木和男*

抗好中球細胞質抗体(anti-neutrophil cytoplasmic autoantibodies：ANCA)の標的分子にはミエロペルオキシダーゼ(MPO)と proteinese(PR)3 があり，顕微鏡的多発血管炎(microscopic polyangiitis：MPA)患者血清中には MPO-ANCA が多く検出される．ANCA は，活性化好中球表面に表出する MPO，PR-3 に反応し，局所に浸潤した好中球をさらに活性化し傷害性分子を放出して自己組織・細胞を傷害する．一方，ANCA は，腎糸球体血管内皮細胞にも直接作用して好中球の誘導・接着に関与する．その標的分子は moesin であることが明らかになった．加えて，moesin は，MPO の H 鎖の N 末端近くのアミノ酸配列と相同性を示す部分が存在する．その自己抗体が MPO-ANCA 関連血管炎患者の血中に存在することから，新たな ANCA 抗体としての可能性が示唆され，臓器特異的な発症機構の解明につながるものと期待されている．また，SCG/Kj などのモデルマウスは，発症機構の解析や病態解明および治療法の開発に必須である．

はじめに

ANCA は顕微鏡的多発動脈炎(microscopic polyangiitis：MPA)，多発血管炎性肉芽腫症(granulomatosis with polyangiitis：GPA［旧名：Wegener 肉芽腫症］)，アレルギー性肉芽腫性血管炎/Churg-Strauss 症候群(allergic granulomatous angiitis：AGA/CSS)で代表される血管炎症候群において陽性を示し，診断の補助検査として重要な抗好中球細胞質抗体(anti-neutrophil cytoplasmic autoantibodies)で，好中球細胞質に存在する分子(対応抗原)に対する自己抗体の総称である．ANCA が自己抗体であることで[1]，好中球の染色状態により，pANCA と cANCA と命名され(第 2 回国際 ANCA ワークショップ(1989 年 5 月，オランダ))，その後，その対応抗原がそれぞれ，好中球に存在するミエロペルオキシダーゼ(MPO)と proteinase(PR)3 であることが報告された[2]．

〔キーワード〕
MPO-ANCA associated vasculitis (AAV)
endothelial cells
neutrophils
novel ANCA
moesin

＊Suzuki Kazuo/千葉大学大学院医学研究院 免疫発生学・炎症制御学

1．ANCA が反応する標的細胞・分子

感染症や炎症によって活性化された好中球は，細胞内分子の MPO，PR3 やほかの分子を好中球細胞表面に表出し，MPO-ANCA や PR3-ANCA 抗体の標的となる．MPO が MPO-ANA の対応抗原であることは，MPO 欠損マウスを用いて裏付けられている[3]．これらの抗体の Fab が標的分子と結合することに呼応して Fc 受容体とも反応して好中球をさらに活性化すると推定されている．事実，Fc 受容体欠損マウスでは，血管炎を発症し

ない.

1）好中球の活性化による血管傷害にかかわるMPO-H_2O_2系の作動

MPO-ANCAの対応抗原のMPOは，H_2O_2を基質としてOCl^-を産生し細胞傷害に関与する酵素で，血管炎の発症機構に関与していると考えられている．MPO-H_2O_2系を誘導する好中球は，本来一義的には，殺菌，殺真菌，殺ウイルスなど感染防御にはたらいているが，条件・状況によって生体側に不利な細胞傷害を引き起こす．実際，感染によってMPOやPR3などが血液中に放出され，炎症がおさまると同時にクリアされ，C-reactive protein(CRP)と同様の血中レベルの変動を示す．血管炎や川崎病の患者の血中には，高MPO活性とともに活性化好中球が循環していることが明らかになっている．この様にして，生体の防御に加えて，生体側に不利な自己の細胞の傷害を引き起こす好中球の活性化にも，MPO-ANCAやPR3-ANCAが深くかかわっている．

2．血管内皮細胞傷害にかかわるサイトカイン・ケモカインの産生

感染によって腫瘍壊死因子(tumor necrosis facter：TNF)-αやインターロイキン(IL)-8などのサイトカイン・ケモカインレベルが上昇する．これらのサイトカイン・ケモカインは，MPO-ANCA関連血管炎では好中球の活性化状態を継続させることにかかわっている可能性もあり，それが発症要因の1つと考えられている．すなわち，感染時に好中球・マクロファージが急速に組織に動員され，長期にわたる感染によって過剰に活性化された場合には細胞傷害を引き起こす．このように，異常に活性化された好中球が血管炎の発症と進行に深く関与しているものと考えられている．一方，TNF-αで刺激された好中球は，MPOを細胞膜上に表出し，MPO-ANCAがそれを認識して好中球を活性化する[4)~6)]．また，血中のMPO分子が好中球表面に結合し，それに対してMPO-ANCAが結合することも好中球活性化の原因の1つと考えられている[7)]．活性化好中球は自身のインテグリンと血管内皮細胞に発現するintercellular adhesion molecule(ICAM)-1などの接着分子を介して血管内腔に接着し，そこでO_2^-，H_2O_2，OCl^-などの活性酸素種や，elastase，metalloproteaseなどのライソゾーム酵素を細胞外へ放出することで近傍の血管内皮細胞を傷害する．さらに，血管外へ遊走した後に同様の機序で組織傷害を引き起こすことも考えられる．Xiaoら[8)]は，抗マウスMPO抗体の単独投与によるpauci-immune様(IgGや補体C3の沈着が糸球体内にほとんどみられない)糸球体腎炎を報告しており，MPO-ANCAが糸球体腎炎の発症において重要な役割を担っていることが証明されつつある．しかしながら，糸球体腎炎発症における血管内皮細胞のバイオロジーは不明な点が多く，*in vitro*，*in vivo*の両面から詳細に検討されはじめている．

3．ANCAのエピトープ部分とMPO-ANCAが内皮細胞に直接反応する新分子moesin

1）ANCAのエピトープ解析

血管炎疾患患者血清中のANCA抗体価の変動は，必ずしも疾患の病態と連動していない場合もあり，病態と密接に関与するANCAとはなにかとの疑問が提示されている．このため，ANCAの抗原との反応部位(エピトープ)と病態との関係を解析することが必要になっている．欧米ではPR3-ANCAが多いことから，PR3-ANCAのエピトープは，米国のSpecksら(Mayo Clinic, USA)のグループによる解析法が，日本に多いMPO-ANCAのエピトープは，解析用パネルデータを報告したSuzukiらのグループによる解析法[9)]が，それぞれEuropean Vasculitis Study Group(EUVAS)会議で採用が決定された(Parma，2010年6月)．MPO-ANCAエピトープ解

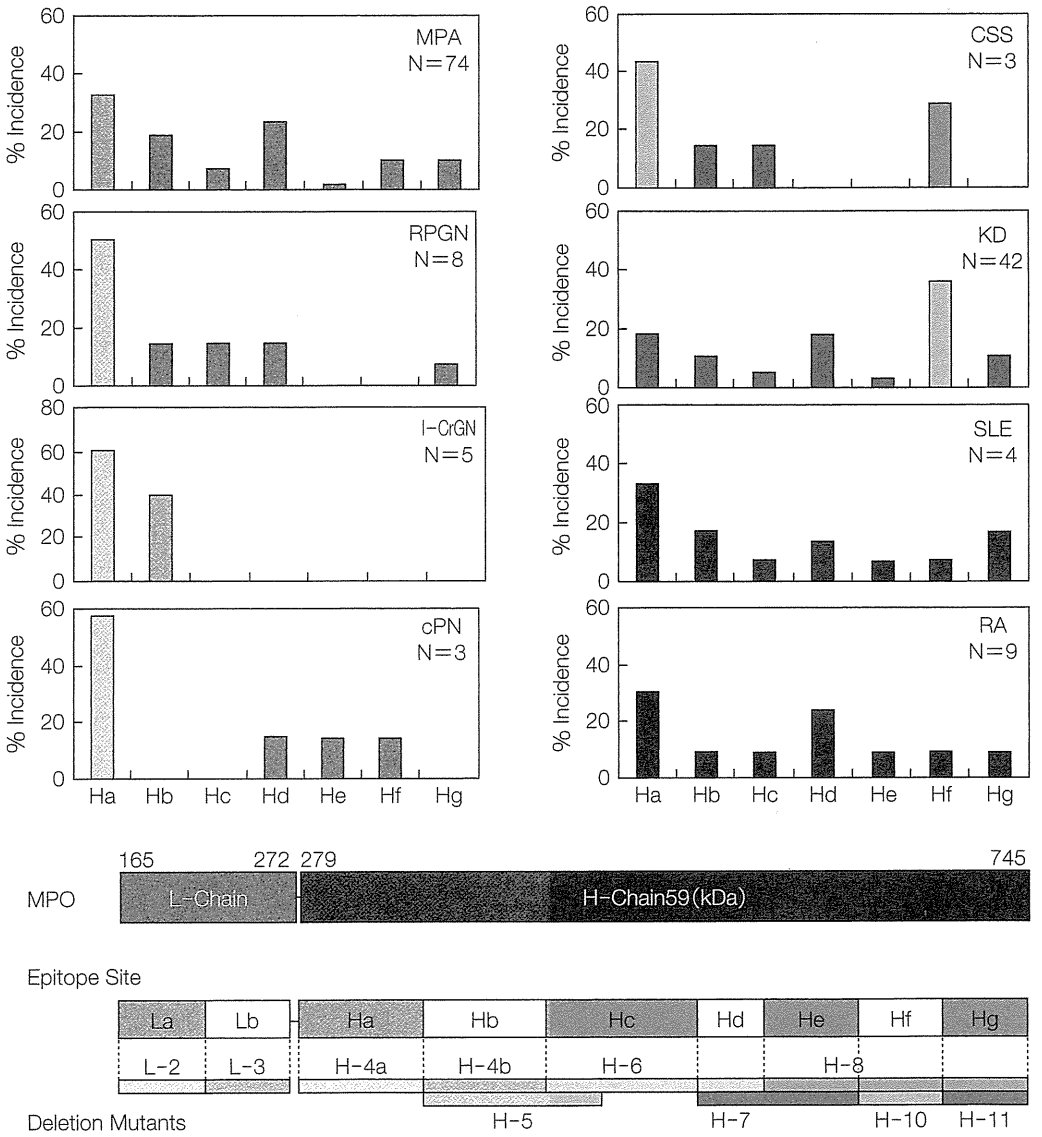

図 1. エピトープ解析による患者血清の反応(Suzuki K *et al*, 2007[10]より改変引用)

析について簡単に解説すると，MPO を 7 部分に分けて，*E. coli* にて産生したリコンビナントフラグメントからなるパネルセットを作製し，血管炎関連患者血清の MPO-ANCA 抗体のエピトープを解析したとき，MPO の L 鎖とは全く反応せず，主として H 鎖の N および C 末端に単独で反応するエピトープをもつ MPO-ANCA 抗体が重症化と関連している．さらに，「厚生省・難病血管炎班(橋本博史班長)2004-2006」において，種々の血管炎患者の MPO-ANCA 陽性を認めた 176 例の血管炎患者血清における MPO-ANCA のエピトープを解析し，とくに，MPA 患者の血清は，H 鎖の N および C 末端に単独で反応するエピトープを示した(**図 1**)[10].

2）MPO-ANCA の腎糸球体血管内皮細胞表面の moesin への結合

前項でも述べたが，血管炎疾患患者血清中の ANCA 抗体価の変動は，必ずしも疾患の病態と連動していない場合もあり，病態と密接に関与する自己抗体の存在も指摘されている．しかし，傷害される血管内皮細胞についての詳細な報告はな

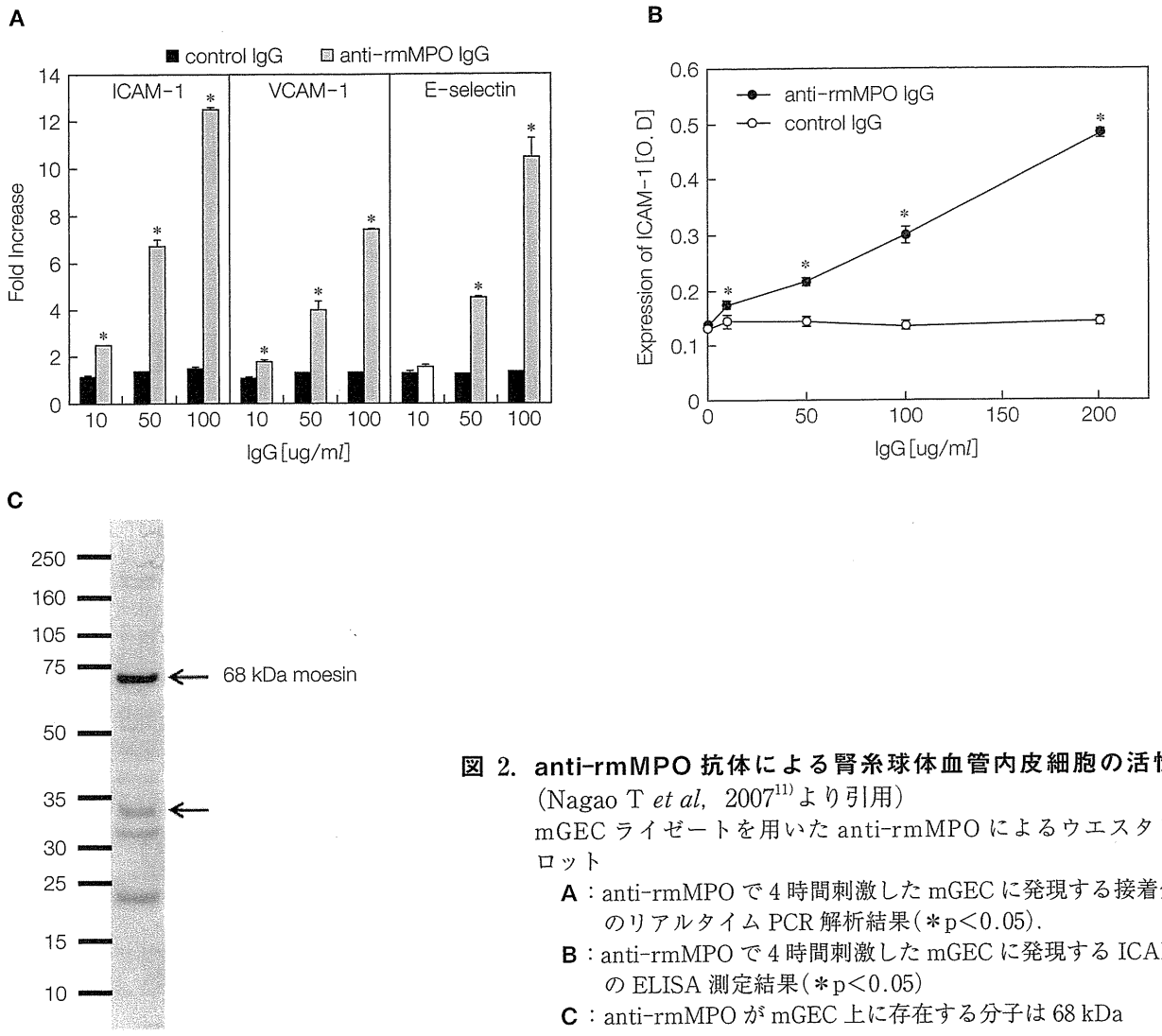

図 2. anti-rmMPO 抗体による腎糸球体血管内皮細胞の活性化
(Nagao T *et al*, 2007[11]より引用)
mGEC ライゼートを用いた anti-rmMPO によるウエスタンブロット
A：anti-rmMPO で 4 時間刺激した mGEC に発現する接着分子のリアルタイム PCR 解析結果(＊$p<0.05$).
B：anti-rmMPO で 4 時間刺激した mGEC に発現する ICAM-1 の ELISA 測定結果(＊$p<0.05$)
C：anti-rmMPO が mGEC 上に存在する分子は 68 kDa

いため，筆者ら[11]は MPO-ANCA の腎糸球体血管内皮細胞に対する影響を調べ，MPO-ANCA 単独で腎糸球体血管内皮細胞を「直接」活性化し，接着分子の発現が誘導されることを報告した[11]．C57BL/6 マウスより単離した腎糸球体血管内皮初代培養細胞 (mouse glomerular endothelial cells：mGEC) に，recombinant MPO IgG (anti-rmMPO) を加えて培養し，接着分子発現を解析した結果，anti-rmMPO 抗体によって接着分子 (ICAM-1, vascular cell adhesion molecule (VCAM)-1, E-selectin) の発現は anti-rmMPO 濃度依存的に増加した(**図 2A, B**)．anti-rmMPO によって活性化した mGEC は TNF-α やケモカインの発現も上昇した．これらのことから，MPO-ANCA は生体で腎糸球体血管内培養細胞に直接作用して，接着分子・サイトカイン・ケモカインの発現を誘導し，好中球等の白血球が接着・遊走に関与していると考えられる．これは，MPO-ANCA が直接腎糸球体組織の血管内皮細胞の接着分子の発現を誘導することで，MPO-ANCA 関連血管炎の臓器特異性を決定づける新たな要因になる可能性を示唆している．また，標的分子が mGEC 上に存在する moesin であることを突き止めた(**図 2C**)[12]．

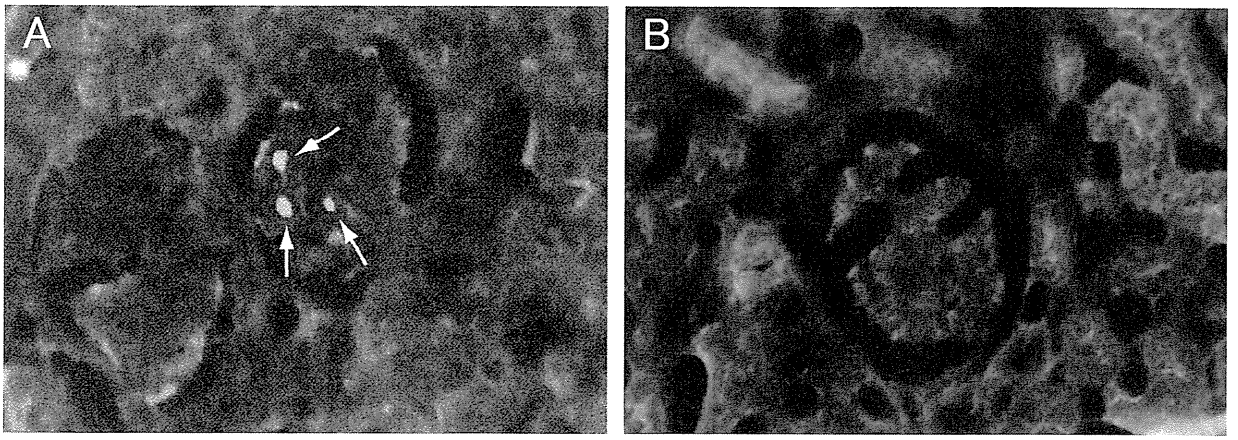

図 3. 量子ドットを結合した anti-rmMPO の腎糸球体への集積(Hoshino A *et al*, 2007[15]より改変引用)
A：anti-rmMPO IgG **B**：control rabbit IgG

3）MPO-ANCA のエピトープ部分と moesin との相同性

MPO-ANCA が抗原の MPO 以外の moesin と反応したことは，両者に交差性があることを示している．そこで両者の分子構造を解析したところ，MPO のいくつかの箇所で相同的なアミノ酸配列が認められた．そして，興味深いことに，病態と関連した MPO-ANCA のエピトープ部位である MPO の H 鎖の N 末端の一部に moesin と相同性を示すアミノ酸配列が認められた[12]．そこで，筆者らが，MPO-ANCA の患者の血清が抗 moesin 抗体を有しているかを調べたところ，抗 moesin 抗体と MPO-ANCA 値が必ずしも相関せず，好中球表面に反応したことから，MPO-ANCA と連携した新たな ANCA 抗体の存在の可能性が示唆されている(投稿準備中)．

4．血管炎 *in vivo* イメージング解析

近年，血管炎の *in vivo* イメージング解析が報告されはじめてきている．Little ら[13]は，好中球走化性を有する CXCL1 ケモカインを局所投与したとき，human MPO 免疫群で白血球数が増加し，さらに細静脈に血管内皮傷害による出血がみられることを報告している．また，anti-mouseMPO IgG とリポ多糖(lipopolysaccharide：LPS)を C57BL/6 マウスに投与し，糸球体における微小循環のイメージングから接着した白血球数の増加を認め[14]，同モデルで高投与量(200 μg/mouse)の場合は，*in vitro* の報告と異なり，β_2-インテグリンではなく α_4-インテグリンが好中球の接着に関与していることを報告している[15]．これらの解析は，血管内皮細胞に対する anti-MPO 抗体の影響を検討していないため，血管内皮細胞を観察対象として接着分子，ケモカイン，サイトカインの発現を含めた *in vivo* イメージング解析が必要である．筆者ら[16]は，より高感度に MPO-ANCA の生体内動態をモニタリングするために，強い蛍光性と退色の少ない量子ドットを anti-MPO IgG に結合したものを作製し，これを C57BL/6 マウスに投与したときに腎糸球体に結合することを示した(**図 3**)．これまで，腎糸球体を *in vivo* イメージング解析した報告はほとんどなく，今後は，このような，*in vivo* と *in vitro* をつなぐ新たな観察法を用いた腎糸球体における ANCA，白血球の動態を解析していくことが重要である．

5．病態解明や治療法開発に有用なモデルマウス

血管炎を自然発症するマウスには，NZB/WF1，MRL/lpr，*Candida albicans* 由来分子(CADS や

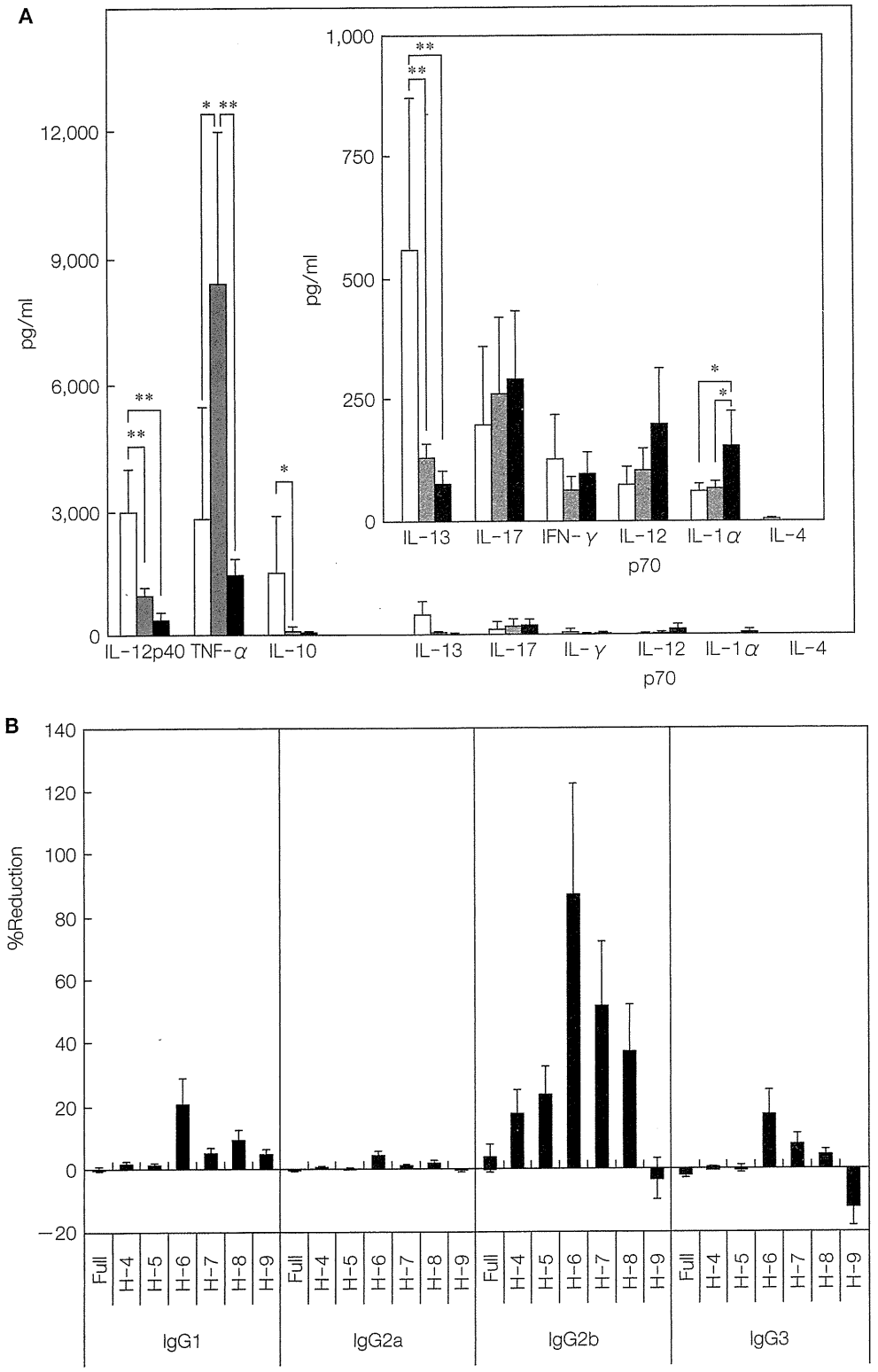

図 4. MPA モデルの SCG/Kj マウスでの DSG 治療によるサイトカインの産生と IgG サブクラスの変動(Tomizawa K *et al*, 2010[20] より引用)

A. 血清中のサイトカインレベル

B. 血清中の IgG サブクラス

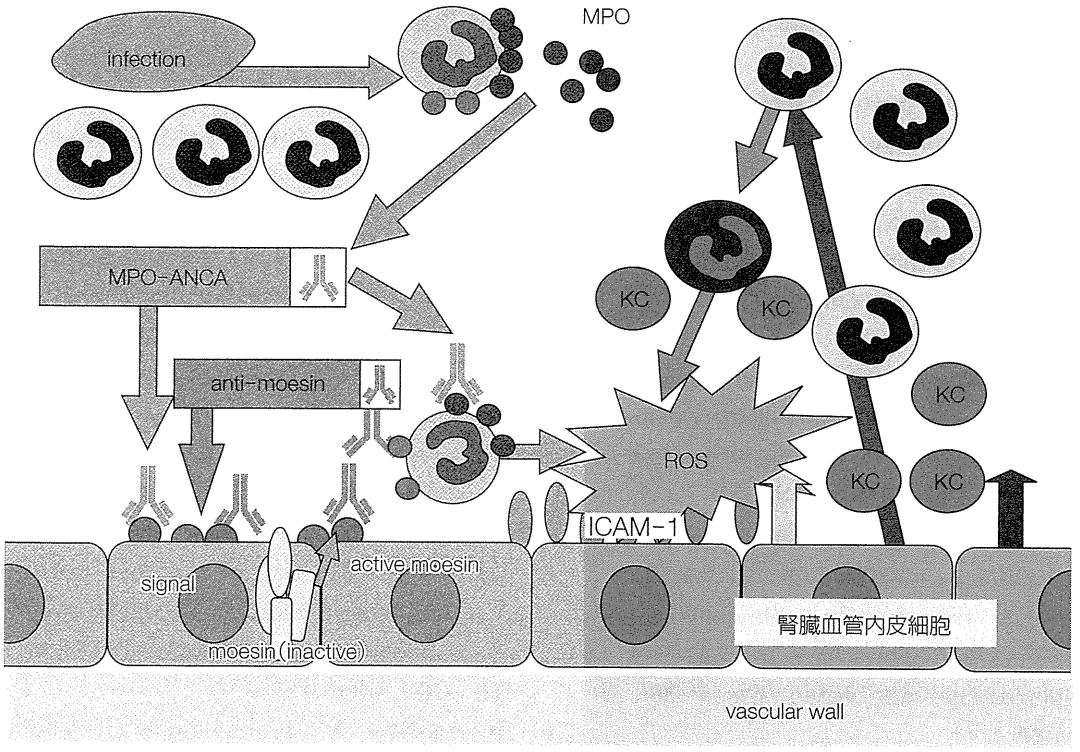

図 5. MPO-ANCA 関連血管炎の発症機序

CAWS)によって誘導される冠状動脈血管炎マウスもある[17)18)]．また，CAWS，MPO や補体 C5a によって，好中球が IL-17 の産生に関与していることもわかってきている[16)]．また，MPA のモデルとして SCG/Kj マウスは，半月体形成性糸球体腎炎を急速に自然発症し，発症・進行に好中球機能亢進が関与し[3)19)]，血清 MPO-ANCA 値が半月体形成に先立ち上昇する．このことから，MPO-ANCA が血管炎誘導に関与していることが示されている[3)]．さらに筆者ら[20)]は，SCG/Kj マウスでの治療法を検討した．15-deoxyspergualin(DSG)を腹腔投与し，半月体形成性腎炎が病理組織学的軽減化されることと比例する MPO-ANCA のエピトープを解析し，H-6 領域の関与を確認した．また，MPO-ANCA 関連性疾患では，ヒト血清のみならず，本モデル動物での治療において，IgG サブクラスおよびサイトカインの発現レベルに違いがあることが判明している(**図 4**)．

おわりに

これまでの血管炎の発症機序解析の研究から，MPO-ANCA による好中球の活性化が血管内皮細胞傷害に重要な役割を果たしていることがわかっている．しかしながら，血管内皮細胞側の傷害される機構の解明が進んでいないのが現状である．血管内皮細胞の傷害を間接的な生化学的手法で解析したことに加え，誘導系のモデルマウスが作製され，ノックアウトマウスや *in vivo* バイオイメージング技術の発展が有効になると思われる．さらに，血管内皮細胞に MPO-ANCA の標的分子が登場し，MPO-ANCA 関連血管炎発症における臓器特異的な好中球・血管内皮細胞の役割が明らかになるものと考えられる．現状で筆者が提案する血管炎発症機序のモデルを**図 5** に示す．

文　献

1) Radice A *et al*：Antineutrophil cytoplasmic

antibodies (ANCA). *Autoimmunity* **38** : 93-103, 2005
2) Jennette JC *et al* : Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood* **75** : 2263-2264, 1990
3) Ishida-Okawara A *et al* : Contribution of myeloperoxidase to coronary artery vasculitis associated with MPO-ANCA production. *Inflammation* **25** : 381-387, 2001
4) Falk RJ *et al* : Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A* **87** : 4115-4119, 1990
5) Reumaux D *et al* : Effect of tumor necrosis factor-induced integrin activation on Fcγ receptor II-mediated signal transduction : relevance for activation of neutrophils by anti-proteinase 3 or anti-myeloperoxidase antibodies. *Blood* **86** : 3189-3195, 1995
6) Rarok AA *et al* : Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. *J Leukoc Biol* **74** : 3-15, 2003
7) Hess C *et al* : Induction of neutrophil responsiveness to myeloperoxidase antibodies by their exposure to supernatant of degranulated autologous neutrophils. *Blood* **96** : 2822-2827, 2000
8) Xiao H *et al* : Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* **110** : 955-963, 2002
9) Fujii A *et al* : Epitope analysis of myeloperoxidase-specific anti-neutrophil Cytoplasmic antibody (MPO-ANCA) associated glomerulonephritis. *Clin Nephrology* **53** : 242-252, 2000
10) Suzuki K *et al* : Analysis of risk epitopes of anti-neutrophil antibody MPO-ANCA in vasculitis in Japanese population. *Microbiol Immunol* **51** : 1215-1220, 2007
11) Nagao T *et al* : Up-regulation of adhesion molecule expression in glomerular endothelial cells by anti-myeloperoxidase antibody. *Nephrol Dial Transplant* **22** : 77-87, 2007
12) Nagao T *et al* : Direct activation of glomerular endothelial cells by anti-moesin activity of anti-myeloperoxidase antibody. *Nephrology Dialysis Transplant* **26** : 2752-2760, 2011
13) Little MA *et al* : Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood* **106** : 2050-2058, 2005
14) Ruth AJ *et al* : Anti-neutrophil cytoplasmic antibodies and effector CD4+cells play non-redundant roles in anti-myeloperoxidase crescentic glomerulonephritis. *J Am Soc Nephrol* **17** : 1940-1949, 2006
15) Kuligowski MP *et al* : Antimyeloperoxidase antibodies rapidly induce alpha-4-integrin-dependent glomerular neutrophil adhesion. *Blood* **113** : 6485-6494, 2009
16) Hoshino A *et al* : Trafficking of QD-conjugated MPO-ANCA in murine systemic vasculitis and glomerulonephritis model mice. *Microbiol Immunol* **51** : 551-566, 2007
17) Nagi-Miura N *et al* : Lethal and severe coronary arteritis in DBA/2 mice induced by fungal Pathogen, CAWS, Candida albicans water-soluble fraction. *Atherosclerosis* **186** : 310-320, 2006
18) Kobayashi S *et al* : Anti-neutrophill cytoplasmic antibody-associated vasculitis, large vessel vasculitis and Kawasaki disease in Japan. *Kidney Blood Press Res* **33** : 442-455, 2010
19) Kinjoh K *et al* : Genetic selection for crescent formation yields mouse strain with rapidly progressive glomerulonephritis and small vessel vasculitis. *Proc Natl Acad Sci U S A* **90** : 3413-3417, 1993
20) Tomizawa K *et al* : Reduction of MPO-ANCA epitopes in SCG/Kj mice by 15-Deoxyspergualin treatment restricted by IgG2b associated with crescentic glomerulonephritis. *Rheumatology(Oxford)* **49** : 1245-1256, 2010

Mod Rheumatol (2010) 20:54–62
DOI 10.1007/s10165-009-0239-0

ORIGINAL ARTICLE

Clinicoepidemiological manifestations of RPGN and ANCA-associated vasculitides: an 11-year retrospective hospital-based study in Japan

Yusuke Suzuki · Yukihiko Takeda · Daisuke Sato · Yasuhiko Kanaguchi · Yuichi Tanaka · Shigeto Kobayashi · Kazuo Suzuki · Hiroshi Hashimoto · Shoichi Ozaki · Satoshi Horikoshi · Yasuhiko Tomino

Received: 2 October 2008 / Accepted: 7 September 2009 / Published online: 10 October 2009

Abstract Antineutrophil cytoplasmic antibody (ANCA)-associated small-vessel vasculitides are major causes of rapidly progressive glomerulonephritis (RPGN). Although recent papers suggest differences in clinicoepidemiological manifestations of ANCA-associated vasculitis between Japan [microscopic polyangiitis (MPA) ≫ Wegener's granulomatosis (WG)] and Europe (WG ≫MPA), little is known about the prevalence and serological pattern. We retrospectively analyzed 27 RPGN patients who were admitted in our hospital over the past 11 years and who could be basically followed for more than 1 year, concerning the incidence of ANCA-related vasculitis, the presence of (MPO)/proteinase 3 (PR3)-ANCA and their clinical outcomes. As there were no PR3-ANCA single positive and/or WG patients, all patients were serologically divided into four groups; Groups I: MPO-ANCA single-positive patients ($N = 11$), II: MPO-ANCA and PR3-ANCA double-positive patients ($N = 3$), III: anti-glomerular basement membrane antibody (anti-GBM Ab)-positive patients ($N = 6$), and IV: all negative patients ($N = 7$). Patients in Groups II/III showed more severe manifestation at admission. However, in Group I, only 36.3% patients avoided death and/or dialysis-dependent end-stage renal disease. Most patients in Group IV were women (85.7%), and 50% of these patients was diagnosed as having rheumatic diseases. Every patient in Groups I–III was treated with oral corticosteroid and/or methylprednisolone pulse therapy. Most patients treated with immunosuppressants showed severe prognosis because of frequent recurrences of vasculitis and infectious episodes after repeated and prolonged treatments with immunosuppressants. Present analysis further confirms the epidemiological and serological differences in ANCA-related RPGN between Japan and Europe, and reinforced the fact that ANCA-associated vasculitis is the most serious causal disease for RPGN.

Keywords MPO-ANCA · PR3-ANCA · Microscopic polyangiitis (MPA) · Wegener's granulomatosis

Y. Suzuki · Y. Takeda · D. Sato · Y. Kanaguchi · Y. Tanaka · S. Horikoshi · Y. Tomino (✉)
Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan
e-mail: yasu@juntendo.ac.jp

S. Kobayashi · H. Hashimoto
Department of Internal Medicine, Juntendo Koshigaya Hospital, Saitama, Japan

K. Suzuki
Department of Immunology, Chiba University Graduate School of Medicine, Inflammation Program, Chiba, Japan

S. Ozaki
Division of Rheumatology and Allergy, Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan

Introduction

More than 80% of patients with active, untreated, necrotizing small-vessel vasculitis associated with an absence or paucity of immunoglobulin (Ig) deposition in vessel walls have circulating antineutrophil cytoplasmic antibody (ANCA) [1]. The major clinicopathological expressions of ANCA-associated small-vessel vasculitis are Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg–Strauss syndrome, and renal-limited vasculitis

(RLV). The incidence of WG among the ANCA-associated small-vessel vasculitides is higher than that of MPA and/or RLV in northern Europe [2–6], whereas conversely, the incidence of MPA/RLV is higher than that of WG in Japan [7]. Two nationwide Japanese surveys demonstrated that the number of patients with MPA and/or RLV is sixfold higher than those with WG in Japan [7–11]. In addition, it is also known that the presence of myeloperoxidase (MPO)-ANCA/proteinase 3 (PR3)-ANCA among Japanese patients with ANCA-associated vasculitides differs from that in European countries [4, 5, 12, 13]. Therefore, recent clinical studies indicate that ANCA-associated systemic vasculitides differ epidemiologically and serologically between Japanese and European countries [7, 11]. However, underlying mechanisms to explain the clinicoepidemiological difference remain unclear.

Vasculitis is a pathological process characterized by inflammation and necrosis of blood-vessel walls. In the kidney, vasculitis preferentially affects the small vessels (arterioles, capillaries, and venules). Therefore, microscopic hematuria with or without proteinuria is consistent with renal vasculitis. Over the past decade, clinical and experimental studies have provided compelling evidence that ANCA is a primary pathogenic factor in renal vasculitis, mainly by augmenting leukocyte–endothelial interactions [14–17]. The renal features of ANCA-associated systemic vasculitis include oliguria, microscopic hematuria, and proteinuria. Its central pathological feature is a pauci-immune focal segmental fibrinoid necrosis with extracapillary proliferation that may become crescentic glomerulonephritis with the accumulation of macrophages and epithelial cells in Bowman's space. This histopathological hallmark is frequently associated with rapid deterioration of renal function, clinically diagnosed as rapidly progressive glomerulonephritis (RPGN). Recent studies revealed an increasing incidence of ANCA-associated vasculitides in the older population [3, 18]. Therefore, RPGN patients with ANCA-associated vasculitides may show poor prognosis. In fact, although the prognosis of patients with RPGN is regarded as having improved over the past 20 years [14–17, 19], the prognosis of older patients with RPGN and their long-term renal survival is still a serious concern [11, 15, 20].

In this study, we retrospectively evaluated RPGN patients over the past 11 years who could be followed for more than 1 year or died within 1 year after onset, and we analyzed the incidence of ANCA-related vasculitis in RPGN, presence of MPO/PR3-ANCA, and their clinical outcomes. This hospital-based analysis may aid the understanding of clinicoepidemiological differences and serve as a baseline for future therapeutic approaches to ANCA-associated systemic vasculitides.

Materials and methods

Patients and assessment of disease manifestation

Twenty-seven patients with RPGN who were admitted to the Division of Nephrology of Juntendo University Hospital from April 1996 to December 2006 and could be followed for at least for 1 year after first admission ($N = 20$) or died within 1 year after onset ($N = 7$) were enrolled in this study. Renal involvement with features of glomerulonephritis, including erythrocyturia, erythrocyte cylindria, and glomerular proteinuria was seen in all patients. Patients with rapid aggravation of renal dysfunction with >30% rise in serum creatinine (Cr) levels over several days to a few months were defined as having RPGN [9, 10]. The hospital Ethical Committee approved the study design.

Age, gender, blood pressure, complete blood count (CBC), and serum markers such as C-reactive protein (CRP), Cr, MPO/PR3-ANCA and antiglomerular basement membrane (anti-GBM) antibody, and urinalysis were assessed at onset and admission and followed for >1 year. For evaluation of lung lesions, chest/abdominal X-rays and/or computed tomography (CT) scans were also examined and followed. Renal biopsies were performed in some patients (three men, four women; age 52.57 ± 12.34 years) who were in relatively good condition at admission. Correlation between clinical outcomes with or without each treatment, such as hemodialysis, plasmapheresis, steroid (oral corticosteroid and/or methylprednisolone pulse therapy), and immunosuppressants (mainly cyclophosphamide), and these clinical markers was evaluated. As most patients treated with immunosuppressants were followed not only by nephrologists but also by rheumatologists, indication of immunosuppressants was typically based on the clinical manual or guidelines of the Committee for Intractable Vasculitides in Japan, Ministry of Health, Labor, and Welfare, Japan. In addition, clinical severity of RPGN in each case was graded by the grading score of the Committee for Guidelines on Diagnosis and Therapy of Rapidly Progressive Glomerulonephritis in Japan, Special Study Group on Progressive Glomerular Disease, Ministry of Health, Labor and Welfare, Japan [9] (Tables 1 and 2). Patients were graded as follows: grade 1: 29.7% ($N = 8$), grade 2: 48.1% ($N = 13$), grade 3: 18.5% ($N = 5$) and grade 4: 3.7% ($N = 1$).

Serological tests for MPO-ANCA, PR3-ANCA, and anti-GBM antibody (anti-GBM Ab) were conducted using enzyme-linked immunosorbent assay (ELISA). MPO or PR3 were immobilized on microplates as antigens for each ELISA. Normal ranges in each MPO-, PR3-ANCA and anti-GBM Ab titers were settled under 10, 10, and 10 EU, respectively. All RPGN patients were serologically divided

into four groups, as follows: Group I: MPO-ANCA single-positive patients ($N = 11$, male:female = 6:5), Group II: MPO-ANCA and PR3-ANCA double-positive patients ($N = 3$, M:F = 3:0), Group III: anti-GBM -Ab-positive patients ($N = 6$, M:F = 2:4) (including MPO-ANCA-positive patients: $N = 4$, M:F = 2:2), and Group IV: all MPO-/PR3-ANCA and anti-GBM-Ab-negative patients ($N = 7$, M:F = 1:6, systemic lupus erythematosus: 3, rheumatoid arthritis: 1, MPA: 1, IgA nephropathy: 1, Henoch-Schonlein purpura: 1).

Statistical analyses

The significance of differences in age between each group (Table 1) was assessed by paired Student's t test using StatView statistical software (Hulinks, Tokyo, Japan).

Results

The number of RPGN patients treated in our hospital has been increasing since 2003 (Fig. 1). Although there was no clear difference in incidence between male and female patients (M:F 12:15), onset age of male RPGN patients ($N = 12$, 67.5 ± 13.55) was older than that of female patients ($N = 15$, 57.6 ± 10.91) ($P < 0.05$).

Serological analyses revealed no PR3-ANCA single-positive patients with RPGN (Table 1). Consistent with this result, WG patients were not included in this study. The age at first medical examination or admission (years old), serum Cr (mg/dl), CRP (mg/dl) and hemoglobin (Hb) (g/dl) were examined. The average age of patients in Group IV (54.3 ± 11.9) was significantly lower than those in other groups ($p < 0.05$) (Table 1). Serum Cr at admission in Group III was higher than that of the other groups (p value; Group III vs. Groups I, II and IV; 0.31, 0.06, and 0.15) (Table 2). There was no clear difference in anemia at admission among the groups (Table 2). CRP at admission in Group IV tended to be low, whereas in other groups, it varied widely (Table 2). Serological changes of MPO- and PR3-ANCA and a-GBM Ab at admission or just before admission/final data after treatments (EU) in each patient is summarized in Table 1.

The severity of RPGN was evaluated by a grading score [9], as shown in Tables 1 and 2. Average grades in each group are shown in Fig. 2 (Groups I vs. II vs. III vs. IV; 1.73 ± 0.65, 2.67 ± 1.53, 2.50 ± 0.55, and 1.57 ± 0.53). Higher grades at admission were observed in Groups II and III (Fig. 2).

Nine of 27 patients (six men: 66.67 ± 16.06 years old; grade 1: 0, grade 2: 4, grade 3: 4, grade 4: 1) showed abnormal shadows suggesting interstitial pneumonitis on X-ray and CT scan analyses summarized in Table 1. Fifty-six percent of those patients showed abnormal shadows indicating alveolar hemorrhage. Only one Group IV (serologically negative) patient had interstitial pneumonitis with alveolar hemorrhage. Average Birmingham Vasculitis Activity Score (BVAS) for patients with lung lesions was 21.33 ± 6.0 at admission. Their BVAS were correlated with grading scores [9] (grade 2: 19.75 ± 6.95, grade 3: 21.5 ± 5.80, grade 4: 27). In particular, three MPO-ANCA/anti-GBM Ab double-positive cases in Group III showed high BVAS (25, 27, and 30) at admission, with severe lung lesions (Table 1). In addition to interstitial pneumonitis, these patients showed vasculitis-related severe eye lesions and cardiovascular complications or stroke with visual disturbance.

We performed renal biopsies in seven patients (grade 1: 4, grade 2: 3) (Table 1). Patients with IgA nephropathy, lupus nephritis, and Goodpasture syndrome were included. All patients showed cellular and fibrous cellular crescents in >50% of glomeruli. Pauci-immune patterns in immunofluorescence analysis were observed in three patients (Group I/grade 1: 1, Group I/grade 2: 1, Group II/grade 1: 1), whereas one patient in Group III showed cellular crescents with linear IgG deposition in glomeruli. One patient in Group II/grade 2 showed 75% crescent formation (15/20 glomeruli) with not only perinuclear ANCA (P-ANCA) but also anti-hepatitis-C-virus (HCV) antibody and cryoglobulin. The systemic lupus erythematosus (SLE) patient (Group IV/grade I) showed cellular crescent formation with glomerular C1q deposition.

Patient and renal prognoses were divided into four groups: survival, dialysis-dependent end-stage renal disease (ESRD) alone, patient death alone, and ESRD/patient death. The prognosis for each grade of patients is shown in Fig. 3a, whereas the prognosis of each group of patients is summarized in Fig. 3b and Table 2. In MPO-ANCA single-positive Group I, only four patients did die or enter ESRD (4/11; 36.4%). Three patients in Group I who died had sepsis or lethal gastrointestinal bleeding with or without colonic penetration (Table 2). In the other groups, all patient deaths occurred within 1 year after onset. The major causes of patient death were severe infections and subsequent disseminated intravascular coagulation (DIC). Although all cases of death in Group III were due to pneumonia, one death, in a patient 93 years old, was based on aspiration pneumonia (Table 2). One patient in Group IV died because of cerebral hemorrhage.

All patients in Groups I, II, and III were treated with oral corticosteroid and/or methylprednisolone pulse therapy. Hemodialysis therapy was introduced directly without therapy by steroid or immunosuppressants in two of seven patients in Group IV. Methylprednisolone pulse therapy was used for most patients in Groups II and III (Group I: 46%, Group II: 100%, Group III: 83.3%, and Group IV:

Table 1 Diagnosis and disease activity

	Age	Sex	Year of admission	Serological analysis						Diagnosis	Grade	BVAS	Lung lesions	Lung XP/CT	Renal bx	Lesions
				MPO/ ANCA	(EU)	PR3/ ANCA	(EU)	a-GBM	(EU)							
Group I																
1	59	M	1996	+	100/<10	–		–		RLV/HCV	1		–		+	C/F Cres, Pim
3	58	M	1999	+	24/<10	–		–		MPA	2	15	+	RS		
4	84	M	1999	+	660/12	–		–		MPA	3	20	+	RS/EP		
9	65	F	1996	+	831/12	–		–		MPA/HCV/LC	2		–		+	C/F Cres, Pim
12	42	F	1998	+	142/<10	–		–		MPA	2		–			
17	76	F	2003	+	510/<10	–		–		RLV	2		–			
21	74	M	2005	+	640/<10	–		–		RLV	1		–			
22	68	M	2005	+	138/<10	–		–		RLV	1		–			
23	56	F	2006	+	142/<10	–		–		MPA	2	16	+	PH/RS/NO/CV		
24	55	M	2006	+	144/<10	–		–		RLV	1		–			
25	72	F	2006	+	88/<10	–		–		RLV	2		–			
Ave	64.45															
SD	11.9															
Group II																
2	75	M	1997	+	24/<10	+	24/<10	–		PN/RA	3	14	+	RS/PL/GC		
5	69	M	2000	+	175/<10	+	12/<10	–		MPA	4	27	+	RS		
6	63	M	2004	+	920/69	+	24/<10	–		MPA	1		–		+	C Cres, Pim
Ave	69															
SD	6															
Group III																
8	70	M	2002	+	208/<10	–		+	660/<10	RA	2		–			
14	42	F	2003	–		–		+	20/<10	GP s/o	2		–		+	C Cres, linear IgG
18	53	F	2003	+	91/<10	–		+	300/<10	MPA	2	30	+	PH/RS/PL/NO		
20	70	F	2004	+	42/<10	–		+	132/<10	MPA	3	25	+	PH/RS		
21	63	F	2005	–		–		+	117/<10	RLV	3		–			
26	93	M	2006	+	232/<10	–		+	24/<10	MPA	3	27	+	RS/EP/PH		
Ave	65.17															
SD	17.4															
Group IV																
7	42	M	2003	–		–		–		MPA	2	18	+	PH/RS/PL	+	C Cres, IgG±
10	49	F	1998	–		–		–		HSP	2		–			
11	57	F	1998	–		–		–		SLE	2		–			
13	38	F	2001	–		–		–		IgAN	1		–		+	C Cres, IgA3+

Table 1 continued

	Age	Sex	Year of admission	Serological analysis						BVAS	Lung lesions	Lung XP/CT	Renal bx	Lesions
				MPO/ ANCA	(EU)	PR3/ ANCA	(EU)	a-GBM	(EU)	Diagnosis	Grade					
15	70	F	2002	–		–		–		RA/Cryogl	1		–			
16	58	F	2003	–		–		–		SLE	2		–			
19	66	F	2004	–		–		–		SLE	1		–		+	C Cres, C1q 1+
Ave	54.3	(*$P < 0.05$, vs. Groups I–III)														
SD	11.9															

MPO/ANCA myeloperoxidase antineutrophil cytoplasmic antibody, *EU* equivalent unit, *PR3/ANCA* proteinase 3 antineutrophil cytoplasmic antibody, *a-GBM* antiglomerular basement membrane, *BVAS* Birmingham Vasculitis Activity Score, *XP/CT* X-ray/computed tomography, *bx* biopsy, *MPA* microscopic polyangiitis, *RLV* renal limited vasculitis, *HCV* hepatitis C virus, *RA* rheumatoid arthritis, *LC* liver cirrhosis, *PN* polyarteritis nodosa, *GP* Goodpasture syndrome, *HSP* Henoch-Schönlein purpura nephritis, *SLE* systemic lupus erythematosus, *IgAN* immunoglobulin A nephropathy, *Cryogl* cryoglobulin, *RS* reticular shadow, *EP* emphysema, *PH* pulmonary hemorrhage, *NO* nodular opacity, *CV* cavitation, *PL* pleuritis, *GC* granulomatous change, *C Cres* cellular crescent, *F Cres* fibrous crescent, *Pim* pauci-immune, *Ave* average, *SD* standard deviation, + positive, – negative

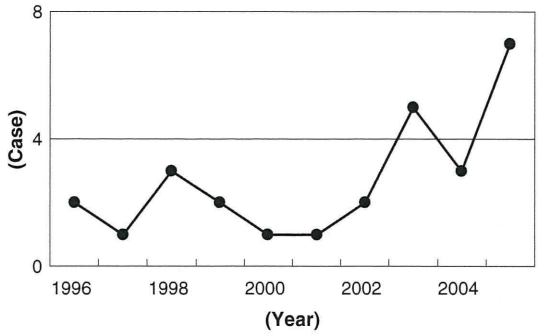

Fig. 1 Annual numbers of rapidly progressive glomerulonephritis (RPGN) patients who were admitted to Division of Nephrology, Juntendo University Hospital, from 1996 to 2006 and could be followed for more than 1 year

57%). Four of six patients in Group III received plasmapheresis therapy. The use of methylprednisolone pulse therapy increased with severity (grade 1: 50%, grade 2: 61.5%, grade 3: 80%, and grade 4: 100%). Only patients in grades 2 (30.7%) and 4 (100%) were treated with immunosuppressants, mainly with cyclophosphamide (Table 2). Eighty percent of patients treated with immunosuppressants died. These patients had strong disease activity and thus showed frequent recurrences of vasculitis and episodes of opportunistic infections, including *Candida albicans* and cytomegalovirus after treatments, even with prophylaxis treatments (Table 2).

Discussion

This study evaluated serological and prognostic outcomes in 27 patients with RPGN at the Division of Nephrology, Juntendo University Hospital, in the past 11 years. Although the average age of all RPGN patients was around 60 years (62.4 ± 13.3), that of male patients was >5 years older than that of female patients. However, this was partly due to the fact that serologically negative patients (M:F = 1:6) included relatively young women with rheumatic diseases and primary glomerulonephritis. In fact, the average age of ANCA-positive cases (66.6 ± 12.2) was older than that of the serologically negative group (54.3 ± 11.9).

Although we did not change the method for measuring ANCA and anti-GBM Ab in the study period, RPGN patients mainly with ANCA-associated vasculitis increased after 2003. In particular, in 2005, we had seven patients. This increment was consistent with the nationwide tendency [9, 10], suggesting that the increasing incidence may be partly due to an aging society, increased opportunity for serological measurement of MPO-/PR3-ANCA, and an increase in referral rates from home doctor to tertiary center hospitals such as university hospitals, based on an

Table 2 Treatment profiles and prognosis

	Diagnosis	Grade	s-Cr (mg/dl)	Hb (g/dl)	CRP (mg/dl)	Urinalysis prot (g/day, g/gCr)	Treatment				Prognosis				Causative bacteria/virus	Prophylaxis treatment
							Hema	Steroid/ pulse	ImSup		HD	ESRD	Death	Cause of death		
Group I																
1	RLV/HCV	1	4.69	10.4	0.3	2	+	+					+	Col Penet./GIB		ST/AMPB
3	MPA	2	16.74	8.5	1.9	0.79	+	+			+	+				ST
4	MPA	3	5	7.6	1.3	2.3	+	+			+	+				ST
9	MPA/HCV/LC	2	3.19	6.2	0.3	4.8	+	+								–
12	MPA	2	3.16	8.3	33.5	ND	+	+	+	CY	+	+	+	Sepsis/DIC	*S. aureus*/ *P. aeruginosa*	ST/AMPB
17	RLV	2	4.85	9.4	1.2	3.5	+	+			+	+	+	Col Penet/Pnm	*P. aeruginosa*	ST
21	RLV	1	8.84	8.5	1.8	6.1	+	+			+	+				ST
22	RLV	1	1.77	10.9	0.2	ND	+	+								–
23	MPA	2	5.54	7	4.3	2.1	+	+								ST/AMPB
24	RLV	1	11.02	7.3	0.9	3.4	+	+			+	+				ST
25	RLV	2	3.68	7.9	0.1	ND	+	+								ST
		Ave	6.23	8.36	4.16											
		SD	4.4	1.4	9.8											
Group II																
2	PN/RA	3	5.01	6.1	17.5	0.3	+	+			+	+				ST
5	MPA	4	4.18	8.9	16.7	1.3	+	+	+	CY	+	+	+	UTI/DIC/GIB	*P. fluorescans*/ *C. albicans*	ST/AMPB
6	MPA	1	4.18	10.8	2.3	0.83	+	+								–
		Ave	4.46	8.60	12.17											
		SD	0.48	2.36	8.55											
Group III																
8	RA	2	5.74	9.3	6.3	0.59	+	+	+	CY			+	Pnm/DIC	*P. carini*/ *C. albicans*	ST/AMPB
14	GP s/o	2	8.68	10	6.9	3.84	+	+								ST/AMPB
18	MPA	2	8.77	6.5	1.3	ND	ND	+	+	CY/CA/AZ	+	+	+	Pnm/DIC/GIB	CMV/ S epidermidis	ST/AMPB/GCV
20	MPA	3	8.95	10	0.6	4.64	+	+			+	+				ST
21	RLV	3	6.3	8.8	23	0.06	+	+			+	+				ST
26	MPA	3	3.25	8.7	5.7	1.5	+	+			+	+	+	Aspiration Pnm		ST/AMPB
		Ave	6.95	8.88	7.30											
		SD	2.3	1.3	8.1											

Table 2 continued

	Diagnosis	Grade	s-Cr (mg/dl)	Hb (g/dl)	CRP (mg/dl)	Urinalysis prot (g/day, g/gCr)	Treatment			Prognosis			Causative bacteria/virus	Prophylaxis treatment
							Hema	Steroid/ pulse	ImSup	HD	ESRD	Death	Cause of death	
Group IV														
7	MPA	2	4.29	10	1.2	2.2	+	+						ST/AMPB
10	HSP	2	13.29	6.6	0.3	4.35	+	+		+	+			–
11	SLE	2	6.29	8.6	16.9	0.6	+	+	+ CY	+				ST/AMPB
13	IgAN	1	2.01	10	0.1	3.3	+	+						–
15	RA/Cryogl	1	1.84	10.9	0.2	4.85	+			+	+			–
16	SLE	2	4.95	6.9	1.2	1.4	+	+		+	+	+	Stroke	ST
19	SLE	1	1.94	8.7	0.8	1.5	+	+						ST
		Ave	4.9	8.8	3.0									
		SD	4.1	1.6	6.2									

MPA microscopic polyangiitis, *RLV* renal limited vasculitis, *HCV* hepatitis C virus, *RA* rheumatoid arthritis, *LC* liver cirrhosis, *PN* polyarteritis nodosa, *GP* Goodpasture syndrome, *HSP* Henoch-Schönlein purpura nephritis, *SLE* systemic lupus erythematosus, *IgAN* immunoglobulin A nephropathy, *Cryogl* cryoglobulin, *s-Cr* serum creatinine, *Hb* hemoglobin, *CRP* C-reactive protein, *prot* protein, *Hema* hematuria, *ImSup* immunosuppressant, *HD* hemodialysis, *ESRD* end-stage renal disease, *ND* not done, *CY* cyclophosphamide, *AZ* azathioprine, *CA* cyclosporine, *Col Penet* colonic penetration, *GIB* gastrointestinal bleeding, *Pnm* pneumonia, *DIC* disseminated intravascular coagulation, *UTI* urinary tract infection, *CMV* cytomegalovirus, *ST* sulfamethoxazole trimethoprim, *AMPB* amphotericin B, *GCV* ganciclovir

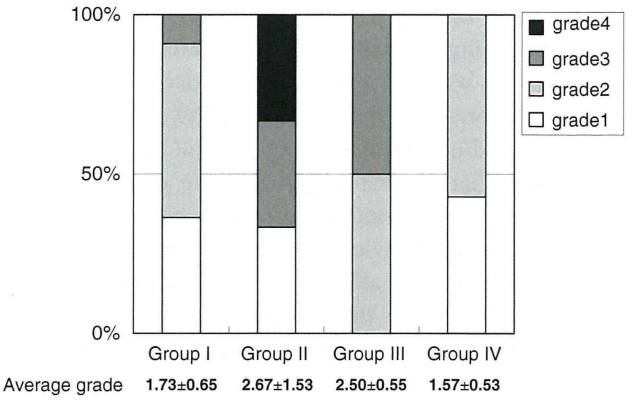

Fig. 2 Clinical grades in severity of each group at admission

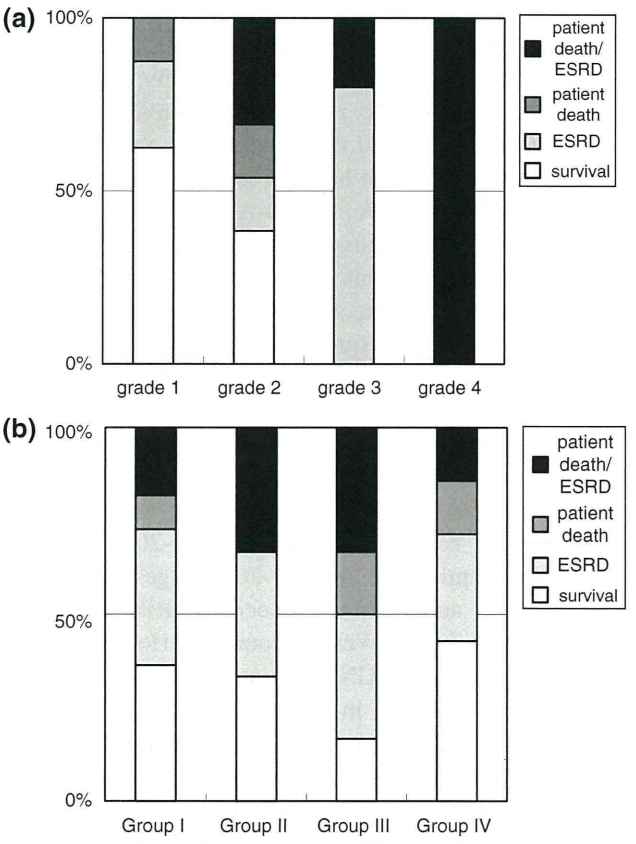

Fig. 3 Prognosis of each grade (**a**) or group (**b**). *ESRD* dialysis-dependent end-stage renal disease

improved recognition of RPGN and ANCA-associated vasculitis by general practitioners and family physicians [10]. Indeed, the Japan RPGN Registry Group first published Japanese guideline for RPGN in 2002 [9, 11].

The ratio of the number of patients with ANCA-associated vasculitis among all RPGN patients was 74% (20/27), slightly higher than results of nationwide Japanese RPGN surveys (MPO-ANCA 58.7%; PR3-ANCA 51.7%; MPO-ANCA 3.2%, and PR3-ANCA 3.8%) [9, 10]. Our

cases did not include patients with RPGN due to WG or PR3-ANCA single-positive RPGN. This finding appears to be consistent with the results of the survey and support previous reports that incidence of MPO-ANCA-positive vasculitis and/or glomerulonephritis in Japan is much higher than that of PR3-ANCA-positive patients [7–10]. This tendency is reversed in European countries [3, 4, 12, 13, 21]. Although previous papers discussed human leukocyte antigen (HLA) allele frequency and environmental factors in relation to the discrepancy [14–17, 22, 23], underlying mechanisms remain unclear. At least, MPO-ANCA ELISA commercially available in Japan, including the method used in our study, exhibited high sensitivity and specificity for diagnosing ANCA-associated vasculitides and provided similar diagnostic value to those in Europe [24]. The rate of MPO-/PR3-ANCA double-positive cases in our study was 11.1% (3/27). Although the data was for a small number of patients only, this rate was higher than that in the results of nationwide Japanese surveys (3.8%) [10]. All three patients in our study were elderly men with a high level of severity. Although false positivity of PR3-ANCA in MPO-ANCA-positive patients should be carefully discussed and further study with more patients is required, this is interesting concerning the background of Japanese RPGN patients showing PR3-ANCA. At least in our study, this aging factor may influence the high grading scores, although there were only three patients.

A Japanese nationwide RPGN survey revealed that the primary disease of RPGN was anti-GBM nephritis in 6.5% (median age 54) [9, 10], Whereas reports from the USA and European countries showed that 12–20% of RPGN patients had anti-GBM Ab [24–26], suggesting that the frequency of anti-GBM-Ab-associated RPGN may be lower in Japan than in Western countries. However, in our study, frequency of RPGN with anti-GBM Ab was 22.2% (6/27), similar to that in Western countries. It is well known that RPGN with anti-GBM Ab frequently shows a severe clinical course with poor prognosis [9–11]. In fact, patients in our analysis showed higher Cr, CRP, and severity at admission and high mortality (50%). Five of six patients suffered ESRD and/or death. On the other hand, both MPO-ANCA single-positive (Group I) and serologically negative (Group IV) groups showed lower severity at admission. In MPO-ANCA single-positive patients, 10 /11 patients showed grades 1 or 2 at admission. However, as 7/11 patients in this group died or entered ESRD, there was a discrepancy in the initial grade and prognosis. Analysis of correlation between grade and prognosis revealed that all patients with grade 3 suffered ESRD and/or death, whereas grade 2 patients showed a highly variable prognosis, suggesting that careful treatment and evaluation of prognosis are required in grade 2 patients. However, grade 2 patients included those who had already been treated with steroid pulse and immunosuppressants in another hospital and then transferred to our hospital in severe condition. This initial treatment may influence the discrepancy of actual severity and grading score. Therefore, the reason about 40% patients in grade 2 died may be partly due to this initial bias.

Major causes of patient death (5/8 patients) were infectious complications, including DIC. This lethal infection was mainly linked to pneumonia by opportunistic pathogens, including *Pneumocystis carinii*, *Candida albicans*, and cytomegalovirus. This result was consistent with that of the nationwide survey [9–11]. Eighty percent (4/5) of patients who died due to severe infection had received immunosuppressants. Moreover, 80% of patients who were treated with the immunosuppressants in addition to steroid therapy died by infection-related causes. However, we found that most of these patients on immunosuppressants showed high disease activity with frequent recurrence of vasculitides. Therefore, one major reason they died due to opportunistic infections despite prophylactic pretreatments may be partly due to repeated and prolonged treatments with immunosuppressants because of recurrences. Immunosuppressive treatments may improve patient/renal survival rates [27–29] but are still closely linked to immunocompromised status, leading to lethal infectious complications, particularly in older patients and patients with strong activity of vasculitis.

This analysis further confirms clinicoepidemiological differences between Japanese and European patients with ANCA-related RPGN. In addition, the findings emphasize that ANCA-associated vasculitis is the most important causal disease for RPGN and has an extremely serious prognosis.

Acknowledgments We are very grateful to Drs. David Jayne, David Scott, and Richard Watts for critically reading the manuscript and their helpful discussion. This study was supported in part by grants from the Japan Health Science Foundation and the Ministry of Health, Labour, and Welfare, Japan.

Conflict of interest statement None.

References

1. Jennette JC, Falk RJ. Small-vessel vasculitis. N Engl J Med. 1997;337:1512–23.
2. Haugeberg G, Bie R, Bendvold A, Larsen AS, Johnsen V. Primary vasculitis in a Norwegian community hospital: a retrospective study. Clin Rheumatol. 1998;17:364–8.
3. Watts RA, Lane SE, Bentham G, Scott DG. Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. Arthritis Rheum. 2000;43:414–9.
4. Watts RA, Scott DG, Jayne DRW, Ito-Ihara T, Muso E, Fujimoto S, et al. Renal vasculitis in Japan and UK—are there differences

in epidemiology and clinical phenotype? Nephrol Dial Transplant. 2008,23(12):3743–5.
5. Lane SE, Scott DG, Heaton A, Watts RA. Primary renal vasculitis in Norfolk-increasing incidence or increasing recognition? Nephrol Dial Transplant. 2000;15:23–7.
6. Reinhold-Keller E, Herlyn K, Wagner-Bastmeyer R, Gross WL. Stable incidence of primary systemic vasculitides over five years: results from the German vasculitis register. Arthritis Rheum. 2005;53:93–9.
7. Fujimoto S, Uezono S, Hisanaga S, Fukudome K, Kobayashi S, Suzuki K, et al. Incidence of ANCA-associated primary renal vasculitis in Miyazaki prefecture: the first population-based, retrospective, epidemiologic survey in Japan. Clin J Am Soc Nephrol. 2006;1:1016–22.
8. Matsumoto Y, Inaba Y, Nakayama T, et al. Nationwide epidemiological survey of refractory vasculitis (anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, anti-phospholipid syndrome and temporal arteritis) in Japan. Annual report of the research committee on intractable vasculitides, Ministry of Health and Welfare of Japan [in Japanese], Tokyo. 1998. p. 15–23.
9. Sakai H, Kurokawa K, Koyama A, Arimura Y, Kida H, Shigematsu H, et al. Committee for the Guidelines on Diagnosis and Therapy of Rapidly Progressive Glomerulonephritis: guidelines for the management of rapidly progressive glomerulonephritis [in Japanese]. Jpn J Nephrol. 2002;44:55–82.
10. Yamagata K, Hirayama K, Mase K, Yamaguchi N, Kobayashi M, Takahashi H, et al. Apheresis for MPO-ANCA-associated RPGN-indications and efficacy: lessons learned from Japan Nationwide survey of RPGN. J Clin Apheresis. 2005;20:244–51.
11. Koyama A, Yamagata K, Makino H, Ariyama Y, Wada T, Nitta K, et al. A nationwide survey of rapidly progressive glomerulonephritis in Japan: etiology, prognosis and treatment diversity. Clin Exp Nephrol. 2009 (Epub ahead of pub).
12. Gonzalez-Gay MA, Garcia-Porrua C, Guerrero J, Rodriguez-Ledo P, Llorca J. The epidemiology of the primary systemic vasculitides in northwest Spain: implications of the Chapel Hill Consensus Conference definitions. Arthritis Rheum. 2003;49:388–93.
13. Booth AD, Almond MK, Burns A, Ellis P, Gaskin G, Neild GH, et al. Pan-Thames Renal Research Group: outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. Am J Kidney Dis. 2003;41:776–84.
14. Jennette JC, Xiao H, Falk RJ. Pathogenesis of vascular inflammation by anti-neutrophil cytoplasmic antibodies. J Am Soc Nephrol. 2006;17:1235–42.
15. Kallenberg CG, Heeringa P, Stegeman CA. Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides. Nat Clin Pract Rheumatol. 2006;2:661–70.
16. Morgan MD, Harper L, Williams J, Savage C. Anti-neutrophil cytoplasm-associated glomerulonephritis. J Am Soc Nephrol. 2006;17:1224–34.
17. van Paassen P, Cohen Tervaert JW, Heeringa P. Mechanisms of vasculitis: how pauci-immune is ANCA-associated renal vasculitis? Nephron Exp Nephrol. 2007;105:e10–6.
18. Satchell SC, Nicholls AJ, D'Souza RJ, Beaman M. Renal vasculitis: increasingly a disease of the elderly? Nephron Clin Pract. 2004;97:c142–6.
19. Levy JB, Winearls CG. Rapidly progressive glomerulonephritis: what should be first-line therapy? Nephron. 1994;67:402–7.
20. Haas M, Spargo BH, Wit EJ, Meehan MJ. Etiologies and outcome of acute renal insufficiency in older adults: a renal biopsy study of 259 cases. Am J Kidney Dis. 2000;35:433–47.
21. Hauer HA, Bajema IM, van Houwelingen HC, Ferrario F, Noel LH, Waldherr R, et al. European Vasculitis Study Group (EUVAS). Renal histology in ANCA-associated vasculitis: differences between diagnostic and serologic subgroups. Kidney Int. 2002;61:80–9.
22. Tsuchiya N, Kobayashi S, Kawasaki A, Kyogoku C, Arimura Y, Yoshida M, et al. Genetic background of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis: association of HLA-DRB1*0901 with microscopic polyangiitis. J Rheumatol. 2003;30:1534–40.
23. Yashiro M, Muso E, Itoh-Ihara T, Oyama A, Hashimoto K, Kawamura T, et al. Significantly high regional morbidity of MPO-ANCA-related angitis and/or nephritis with respiratory tract involvement after the 1995 great earthquake in Kobe (Japan). Am J Kidney Dis. 2000;35:889–95.
24. Ito-Ihara T, Muso E, Kobayashi S, Uno K, Tamura N, Yamashita Y, et al. A comparative study of the diagnostic accuracy of ELISA systems for the detection of anti-neutrophil cytoplasm antibodies available in Japan and Europe. Clin Exp Rheumatol. 2008;26:1027–33.
25. Couser WG. Rapidly progressive glomerulonephritis: classification, pathogenetic mechanisms, and therapy. Am J Kidney Dis. 1998;11:449–64.
26. 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.
27. Jennette JC. Rapidly progressive crescentic glomerulonephritis. Kidney Int. 2003;63:1164–77.
28. Jayne D, Rasmussen N, Andrassy K, Bacon C, Tervaert JW, Dadoniene D, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med. 2003;349:36–44.
29. Jayne DR, Gaskin G, Rasmussen N, Abramowicz D, Ferrario F, Guillevin L, et al. European Vasculitis Study Group: randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol. 2007;18:2180–8.

健常人におけるIFN-α産生能と加齢の影響
Ageing Effects On Human Type I IFN System In Healthy Subjects

[1]宇野賀津子、[1]八木克巳、[1]谷川真理、[1,2]吉川敏一、[1]藤田哲也

[1]Kazuko Uno, [1]Katsumi Yagi, [1]Mari Tanigawa, [1,2]Toshikazu Yoshikawa, [1]Setsuya Fujita

[1](財) ルイ・パストゥール医学研究センター

[2]京都府立医科大学

[1]Louis Pasteur Center for Medical Research, Kyoto, Japan

[2]Kyoto Prefectural University of Medicine, Kyoto, Japan

Abstract

In order to ascertain how critical type I IFN system is in the maintaince of human health, IFN-α production in response to Sendai virus stimulation was quantified by cohort analysis of subjects monitored for more than 10 years. Our previous cross-section studies of patients with various diseases (infectious disease, cancer, metabolic disease, and nephritis) revealed that in spite of the cause, many kinds of diseases impair IFN-α production. These results showed that IFN dysfunction despite the cause lead to higher risk of infectious diseases and cancer development. Data for long-term monitoring of IFN-α production were obtained retrospectively by examining over 24 years of records for 109 subjects; each satisfied the following conditions: 1) IFN-α production was monitored more than 6 times and for more than 10 years, 2) they had no history of cancer, autoimmune nor colonic infectious disease, 3) IFN-α production was quantified when they were 50 years old. Their periodical log transformed IFN-α values (y) were plotted vs. age (x) along with the fitting to a linear expression ($y=mx+n$) and quadratic formula ($y=ax^2+bx+c$) expression. The results of linear expression showed that IFN production in 5.5 % of the subjects followed a rising trend, 83.5 % were flat, and 11.0 % reflected a declining trend (no significant difference). On the other hand, the results of quadratic formula analysis showed that IFN production in the vast majority (77.1 %) were flat and in 6.4 % of subjects it followed a convex (Λ) shape. IFN production in 16.5 % of subjects had a concave (U) shape which means that their once declining IFN production recovered as they aged ($p<0.05$). These subjects experienced their lowest IFN-α production level at a mean age of 56.5±2.06 years. These results shows that 1) age effects on IFN-α production is mild, 2) IFN production does not decline with age. We therefore conclude that declining IFN production is more influenced by disease development rather than by ageing.

はじめに

自然免疫力とりわけウイルス感染の際に、重要な役割を果たすのが、Type Iインターフェロン（IFN）システムである。私たちは、人の末梢血によるIFN産生能を測定することにより、個々人のIFNシステムについて知ることが出来ると考え、センダイウイルスで刺激したときに産生されるIFN量を、バイオアッセイにて測定、IFN産生能として、測定してきた。この時に、産生されるIFNは主として、IFN-αであるが、ヒトによっては、IFN-βも産生されている。またこのIFN産生能は、遺伝的背景によって規定されてていて、高産生、低産生タイプが存在することを私たちは明らかにしている[1]。私たちはこれまでに、糖尿病、肺ガン、骨髄異形成症候群、HIV感染症、HCV感染症でIFN産生能が低下していることを報告している[2]。また、健常人とHCV感染症患者のIFN産生能の個人史の推移から、IFN産生能低下は発癌リスク増大に繋がることを明らかにしてきた[3]。