Fig. 6. Lectin staining of two protein fractions extracted from the hearts of transgenic (TG) and wild-type (WT) mice ($n = 3$) at 10 weeks of age (A: fraction 1; B: fraction 2). After the confirmation of the presence of proteins on blot membranes by SyproRuby blot stain (one of three blots is shown for each fraction), the blots were stained with three lectins (ConA, MAA, PNA). Lectin binding was visualized with chemiluminescence. No evident difference was found on fraction 2 lectin blots containing proteins with lower solubility (*e.g.*, membrane proteins). However, some differentially stained bands (arrows) between TG and WT hearts were found in fraction 1 (soluble proteins): ~100 kDa by ConA, and ~250 kDa by MAA and PNA. ConA staining indicated that ~100 kDa proteins from TG hearts lost their high-mannose-type glycosylation, which was present in those of WT hearts. PNA staining, which recognizes Gal-GalNAc sugars, indicated that the sugar portion of the 250-kDa band in WT mice was highly sialylated, and less sialylated in TG mice. MAA staining, which recognizes α2,3-sialylation of sugars, such as Gal-GalNAc, confirmed higher sialylation of proteins of the same size in WT mice than TG mice. Thus, heart proteins in the TG mice were less sialylated than those of WT mice even though ST3Gal-II, one of the sialyltransferases, was overexpressed in the TG mice. M: Molecular weight markers. Western blots indicate that heart expression of calreticulin (C) and calnexin (D) were significantly higher in the TG hearts than that in WT hearts ($p < 0.05$).

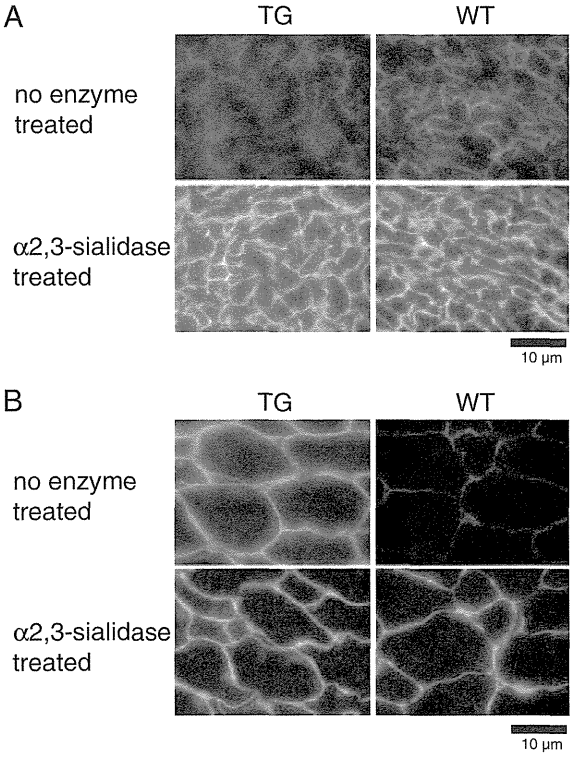

Fig. 7. Frozen sections of heart (A) and rectus femoris muscle (B) treated with or without α2,3-sialidase and stained with biotinylated PNA lectin, followed by FITC-avidin D. Bars = 10 μm. PNA binding was localized to pericellular regions without sialidase treatment, whereas the reactivity was essentially the same between TG and WG hearts. The reactivity in TG muscle was higher than in WT muscle. After sialidase treatment, PNA binding in both became evident equally in TG and WT heart and muscle.

fully understood. Some are known, such as inflammation of the myocardium in viral carditis and loss of or abnormalities in genes encoding structural proteins, particularly in hereditary cardiomyopathies.[19] However, other idiopathic cardiomyopathies occur, for which the causes remain unknown. Our TG mice had no apparent disruption of genes encoding cardiomuscular proteins and no inflammatory response in heart tissues. These features suggest that our TG mice may be useful for examining the mechanism of some idiopathic cardiomyopathies.

Ganglioside abnormalities are unlikely to cause heart symptoms in the TG mice because no apparent change in ganglioside composition was detected in TG hearts, contrary to our expectation that the overexpressed ST3Gal-II would increase the content of sialylated glycolipids, such as GD1a. The absence of ganglioside changes suggests that the substrate preference predicted by *in vitro* experiments[4] does not always coincide with the *in vivo* situation. The absence of changes may be because there is little GM1, a substrate for ST3Gal-II, in mouse hearts.[20] GM3 is a major ganglioside in both mouse and human hearts.[20),21] However, in human hearts, other gangliosides (GM1, GD1a, GD1b, and GT1b) are also detected by more sensitive assays with a GM1-specific choleragenoid.[21] Additionally, GM1 can act as a cardioprotectant against hypoxic damage in neonatal rat myocardial cells.[22] Thus, further analysis with more sensitive assays is needed to clarify the possible involvement of ganglioside abnormalities in cardiomyopathies.

In contrast to gangliosides, the glycosylation of heart glycoproteins was perturbed in TG mice, demonstrated by the protein and histochemical analyses with lectins. The effects of exogenous ST3Gal-II enzymes induced by transgenesis altered sugar portions of glycoproteins beyond the reaction catalyzed directly by the ST3Gal-II enzyme. However, sialylation changes in an abnormal state might be difficult to interpret because both skeletal and heart muscle tissues contain cytosolic sialidases with high activity at neutral pH.[23] Elevated ST3Gal-II activity induced by transgenesis in TG mice might cause tissue damage in hearts and skeletal muscles by higher sialylation. In contrast, if the cytosol is exuded

from damaged (necrotic/degenerated) tissue into interstitial spaces of the hearts and muscles, various cytosolic enzymes, including sialidases, may act on glycoproteins on the surface of adjacent muscle fibers, resulting in removal of terminal sialic acids from glycoproteins, particularly membrane-bound proteins. This tendency was evident in skeletal muscles (Fig. 7B). Complicated effects of exogenous ST3Gal-II are also suggested by transfection experiments of human ST3Gal-II in cell cultures.[24] In the experiments, the level of ST3Gal-II mRNA are not necessary consistent with those of monosialosyl globopentaosylceramide (MSGb5), a product catalyzed by ST3Gal-II.[24] Production of MSGb5 is highly dependent on cell types used for transfection, which may be due to cell-type-dependent sialidase activities.[24] In addition, altered protein glycosylation might be attributed to mislocalization of ST3Gal-II itself and/or the other glycosyltransferases in the Golgi apparatus by overloading of exogenous ST3Gal-II because expression-dependent mislocalizations were reported for glycosylation-related enzymes like α2,6-sialyltransferase,[25] GA2/GM2/GD2 synthase,[26] and human galactosyltransferase.[27] Thus, the perturbation in the glycoprotein sugar portions might have been caused by a complex imbalance of glycosylation–deglycosylation reactions in TG mice.

Elevated calreticulin and calnexin levels in TG hearts suggest that cardiac dilatation in TG mice is a response to ER-stress *via* elevated ERQC, caused by disorganized protein glycosylation in the ER/SR. Calreticulin and calnexin participate in a molecular chaperone system that integrates the processes of *N*-glycosylation and ERQC.[10] Calreticulin is a multifunctional protein involved in many functions, such as regulation of calcium homeostasis,[28] ERQC,[29] and interactions with various nuclear hormone receptors.[30] In the heart, calreticulin is highly expressed in the developing heart, but is strongly downregulated after birth.[31),32] The low level of calreticulin expression in adult heart is important for normal heart function because overexpression of calreticulin in the mouse heart leads to severe cardiac pathology, such as cardiac chamber dilatation,[33] resulting in complete heart block and sudden death.[34] These harmful effects of calreticulin overexpression may be due to various forms of ER stress.[35] Myocardiac cells overexpressing calreticulin are highly susceptible to apoptosis under oxidative stress.[36] The presence of underglycosylated proteins generates a signal leading to increased GRP78 gene expression,[37] which is an ER stress-associated protein.[38] Thus, ST3Gal-II overexpression in our TG mice might have perturbed protein glycosylation, which evoked ER stress and stimulated calreticulin expression, and the elevated calreticulin may have caused cardiac dilatation. However, the mechanism is not so simple, because calreticulin is not always elevated in dilated hearts; the calreticulin level is not influenced in some cases of human dilated cardiomyopathy.[39] Further investigations are needed to evaluate the involvement of calreticulin in TG hearts.

In conclusion, our transgenic mice (4C30 line) may represent a new form of cardiac dilatation with abnormal glycosylation. Although we do not yet have evidence for a direct connection between abnormal ST3Gal II expression and cardiac dilatation, our findings suggest that the glycosylation status of heart proteins should be evaluated carefully as a possible cause of DCM, especially noninflammatory cases, in humans. Our TG mice have potential as a new animal model for cardiac dilatation diseases, such as dilated cardiomyopathy, because the mice can be maintained as a homozygous line with 100% incidence of heart symptoms and a clear zygosity check system for the transgene allele is available.[14] The 4C30 strain is available from JCRB Laboratory Animal Resource Bank at the National Institute of Biomedical Innovation (http://animal.nibio.go.jp/).

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第3項　ネフローゼ症候群モデル(ICGNマウス)

ネフローゼ症候群は，重度のタンパク尿，低タンパク血症，高脂血症，浮腫を主たる症状とする疾病で，糸球体疾患を原因とする一次性と他の全身性疾患(紫斑病性腎炎，糖尿病，ループス腎炎など)に起因する二次性とに分類される。本稿で紹介するICR-derived glomerulonephritis(ICGN)マウスは一次性ネフローゼ症候群としての特性を備えた貴重なモデル動物である。

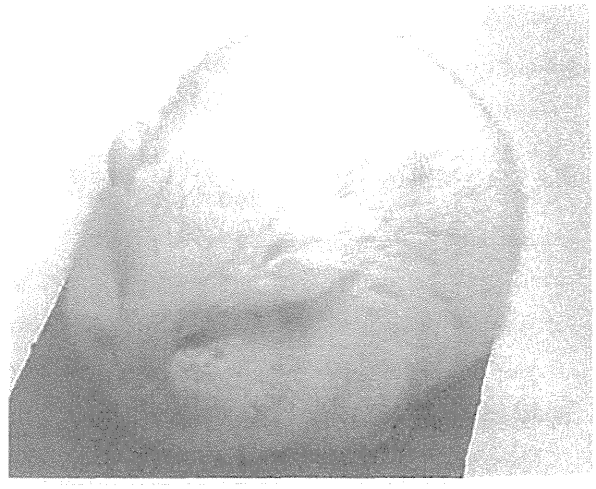

図1　全身性浮腫を呈した雄性ICGNマウス

1. ICGNマウスの由来および原因遺伝子

リンパ節腫大および脾腫を呈する雄性ICRマウスが，1986年に国立感染所研究所(旧国立予防衛生研究所)にて発見された。その個体と正常雌性マウス間の兄妹交配から得られた産仔の中で，浮腫を呈する個体をICGNマウスのF_1として育種が開始され[1]，兄妹交配による近交化を経て病態進行に個体差はあるものの全個体がネフローゼを発症する系統として確立された[2]。ネフローゼ症候群の発症機序は未解明であるが，2006年に細胞接着に関わるtensin2のnt1477-1484(exon18)8塩基の欠失が報告された[3]。Tensin2は正常な腎の糸球体上皮細胞および尿細管に発現していることが分かっているが，腎における機能は不明であり，今後の解析が待たれる。

2. 病　態

生後間もなくタンパク尿を呈し，続いて低タンパク血症，高脂血症，腎性貧血が認められ，末期には腎不全に陥るという臨床像はすべての個体に共通の特性である。しかし全身性浮腫は雄個体に多くに認められるが，個体差が大きく，病態進行により軽減することもあり，注意を要する(図1)。病理組織学的には，生後間もなく形態学的に正常な糸球体基底膜(glomerular basement membrane：GBM)が完成した直後にGBMの肥厚および上皮細胞足突起の消失が認められ，この時点で糸球体の選択的透過性が破綻していることが分かっている[4]。続いてメサンギウム領域の拡張，糸球体基底膜へのIgG，IgM，IgAの沈着が認められ，急速に糸球体硬化が進行する。病態末期には尿細管の拡張，リンパ球の浸潤も認められ(図2)，最後は腎不全で死亡するがこの時期についても個体差が大きく，多くは5ヵ月齢以降に死亡する。

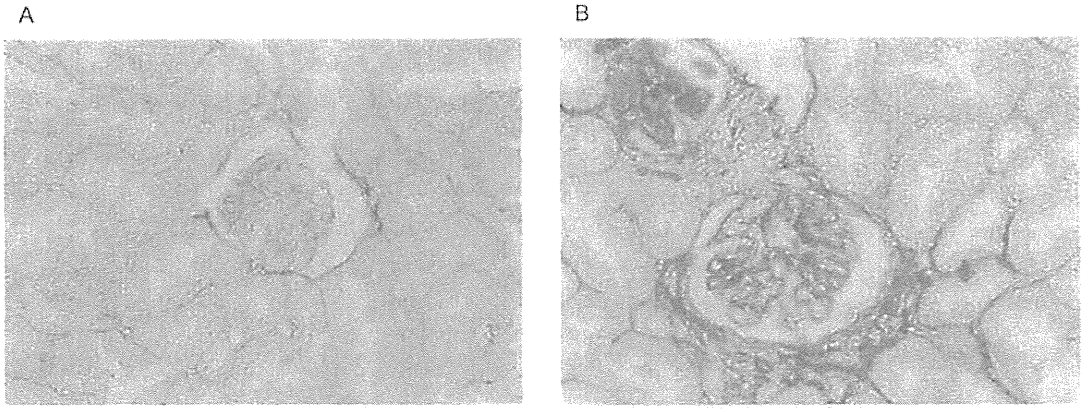

図2　発症初期(A)には認められない糸球体硬化，尿細管拡張，リンパ球浸潤が認められる病態末期の腎(B)

3. 腎線維症モデルとしての有用性

病態進行に伴って糸球体メサンギウム領域および尿細管間質にコラーゲン，ラミニン，フィブロネクチンなど細胞外マトリックス(ECM)の異常蓄積が認められることから，腎線維症モデルとして有用である[5](図3)。下述のように，ECM蓄積には産生亢進，分解抑制およびECM成分の修飾が原因となる。

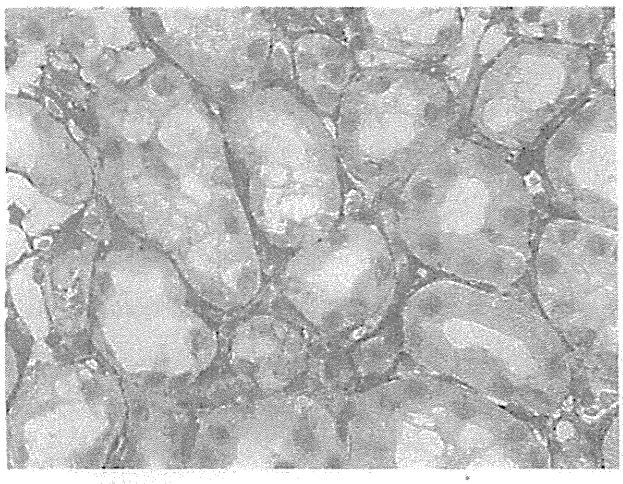

図3　尿細管間質部に細胞外マトリックス異常蓄積が認められる病態末期の腎

＜産生亢進＞

ICGNマウスの腎においては，I型コラーゲンやIII型コラーゲンなどの間質性成分の顕著な産生亢進が認められることが特徴である。一方，基底膜成分であるIV型コラーゲン，ラミニンなども糸球体に顕著に蓄積しているものの産生亢進は軽度である。

＜分解抑制＞

ECMの分解を担うmatrix metalloproteinase(MMP)は，コラゲナーゼ(MMP-1など)，ゼラチナーゼ(MMP-2，MMP-9)，ストロメライシン(MMP-3など)，膜型MMP(MMP-14など)などに大別される。ICGNマウス腎においては，MMP-1，MMP-2，MMP-9の活性低下が認められたが，MMP-3は正常腎での活性と同程度であった[6]。特にMMP-2，MMP-9といった基底膜成分の分解に関与する酵素活性が低下していることからIV型コラーゲンなどの蓄積には分解系の抑制が深く関与していることが示唆された。

＜ECM成分修飾＞

Lysyl oxidase(LOX)はコラーゲンやエラスチンなどを修飾する酵素として知られる。ICGNマウス腎においてLOXが増加しており，ECM成分が修飾されていることが示唆されている[7]。修飾されたECM成分が分解を受けにくくなっていることも線維化進行に関与している可能性が高い。

これらのすべての現象について，その制御に深く関与する因子がtransforming growth factor(TGF)-β_1であり，腎だけではなく多くの臓器の線維化進行に関与している。その機能はECM産生を亢進するだけではなく，ECM分解酵素のインヒビターやLOXのような修飾酵素の発現をも亢進することなどの多面的な作用が知られており，線維化現象のkey moleculeである。ICGNマウスの腎においてもTGF-β_1が増加しており，線維化に寄与している。興味深いことに，このマウスではTGF-β_1の細胞内シグナル伝達因子であるsmad4が細胞内に増加することで尿細管間質病変を増悪させていることが分かっている[8]。もちろんTGF-β independentな線維化の機序についてもさらなる精査が必要である。このようにICGNマウス腎においてECM産生・分解・修飾の異常が認められることから，線維化メカニズムの解析や治療法の開発などに利用可能である。これまでにICGNマウスを腎線維症モデルとして利用してhepatocyte growth factor[9]やangiotensin-converting enzyme inhibitor[10]による腎線維症抑制効果が報告されている。

4. 腎性貧血モデルとしての利用

赤血球の産生を促進するホルモンとして知られるエリスロポエチン(erythropoietin：EPO)は，主に腎の尿細管間質細胞で産生されるので腎疾患が進行すると産生量が低下し，貧血が起こる。ICGNマウスでは尿細管間質病変の進行とともに貧血が認められるので腎性貧血モデルとして適している[11]。ヒト組換EPO投与(5 U/mouse/day 5日間反復)により，ヘモグロビン濃度とヘマトクリット値が正常になる[12]。

以上紹介してきたようにICGNマウスは，我が国発の数少ないネフローゼ症候群の特性を備えた遺伝性のモデル動物であるが，病状の個体差が大きいことや繁殖が難しいことが原因となって利用例は多くない。現在は(独)医薬基盤研究所実験動物研究資源バンク(http://animal.nibio.go.jp/)からマウスや凍結胚が購入可能である。できるだけ多くの方が活用してくださることを期待している。

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