研究成果の刊行に関する一覧表

書籍

該当なし

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
今川彰久、花房俊昭	劇症1型糖尿病	臨床病理	58(3)	216-224	2010
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Ⅴ．研究成果の刊行物・別刷

トピックス

▷トピックス：注目される新しい病態・疾患概念と臨床検査－内分泌・代謝疾患編－(1)◁

劇症1型糖尿病

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Fulminant Type 1 Diabetes Mellitus

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Fulminant type 1 diabetes is a novel subtype characterized by a markedly rapid progression and almost complete destruction of pancreatic beta cells. The number of patients in Japan has been speculated to be 5,000–7,000. A marked decrease of beta cells in addition to alpha cells and mononuclear cell infiltration both in the endocrine and exocrine pancreas are characteristic pathological findings in recent-onset fulminant type 1 diabetes. Laboratory examinations have revealed a high blood glucose level, near normal hemoglobin A1c, ketosis or ketoacidosis, elevation of serum pancreatic exocrine enzymes, and absence of anti-islet autoantibodies such as anti-glutamic acid decarboxylase (GAD) antibody or anti-insulinoma-associated antigen-2 (IA-2) antibody at disease onset. Genetic factors of *HLA-DR-DQ*, *CTLA-4*, and *HLA-B* are associated with this subtype. Both diagnostic criteria for screening and establishing have been announced by the Japan Diabetes Society. In approximately half of fulminant type 1 diabetes, HbA1c was lower than 6.2% at disease onset, indicating that newly proposed diagnostic criteria of diabetes (HbA1c $\geq$ 6.5%) from the joint committee of the American Diabetes Society, the European Association for the Study of Diabetes, and the International Diabetes Federation are not applicable to fulminant type 1 diabetes. In conclusion, all medical practitioners must remember that fulminant type 1 diabetes, an extremely rapidly progressing type of diabetes, does exist, and must pay special attention to avoid overlooking this disease.

[Rinsho Byori **58** : 216～224, 2010]

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【Key Words】 fulminant (劇症), diabetes (糖尿病), idiopathic (特発性), insulitis (膵島炎), diagnostic criteria (診断基準)

現在糖尿病は，1型糖尿病と2型糖尿病，その他特定の機序・疾患によるもの，妊娠糖尿病の4つに分類されている。日本において大多数をしめるのは生活習慣を背景として発症する2型糖尿病であり，インスリンの分泌低下とインスリン作用不全がその成因である。1型糖尿病はこれとは異なり，インスリンを分泌する膵β細胞の破壊により生じ，通常は絶対的インスリン欠乏に至る糖尿病と定義されてい

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る。したがって，インスリン注射による治療はほぼ必須である。1型糖尿病の多くは自己免疫により膵β細胞が破壊されると考えられており，患者血中に存在する自己抗体はそのマーカーと考えられてきた[1]。

劇症1型糖尿病は2000年に報告された1型糖尿病に属する新しい臨床病型である[2)~5)]。すなわち，膵β細胞が破壊されて発症する糖尿病であるが，発症は他の1型糖尿病と比べても，非常に急速であり，一般の1型糖尿病では発症時には約20%の膵β細胞が残存しているのに対し，劇症1型糖尿病では発症時にほぼ完全に破壊されている。従来1型糖尿病の診断マーカーと考えられてきた自己抗体が陰性で，ケトアシドーシスを伴って発症し，発症時に著明な高血糖を認めるにも拘らず，過去1～2ヵ月の平均血糖を示すHbA1c値は正常または軽度上昇にとどまり，発症時に既に内因性インスリン分泌の指標（血中/尿中Cペプチド）は著しく低下しているといった特徴を有する疾患である。また，発症時に血中膵外分泌酵素の上昇を認めることも特徴の1つである。

I. 疫　　学

日本糖尿病学会劇症型糖尿病調査委員会が2004年に発表した全国調査の結果では，劇症1型糖尿病の頻度は急性発症1型糖尿病のうち19.4%であった[2]。ごく最近，同委員会が新規に行った調査では，インスリン自己注射施行患者における劇症1型糖尿病患者の割合は，平均0.61%であった。日本人においてインスリン自己注射施行患者数は110～120万人と推計されていることから，日本における劇症1型糖尿病患者数は6,710～7,310人と推計できる。これとは別に，地域糖尿病患者登録レジストリである「愛媛スタディ」において，全糖尿病患者における劇症1型糖尿病患者割合は0.2%であった[6]。継続的な治療を受けていると推測される患者数246万9,000人の0.2%が劇症1型糖尿病患者であるとすれば，日本における劇症1型糖尿病患者数4,938人であると推計できる。以上の事実を総合的に検討すると，日本における劇症1型糖尿病患者数は5,000～7,000人であると推測するのが妥当と思われる。

海外では，韓国において小児を含めた新規発症1型糖尿病患者99名中7名（7.1%）が劇症1型糖尿病であったことが報告されている[7]。また，中国長沙市の病院において，2001～2008年に入院した全糖尿病患者9,493名中11名が劇症1型糖尿病であったことが報告されている[8]。他のアジア諸国，およびヨーロッパにおいても症例が報告されており[9]，劇症1型糖尿病が人種を越えた臨床病型であることが明らかにされつつある。

なお，本邦の全国調査における，劇症1型糖尿病患者の男女比は，ほぼ1：1であった。また，平均発症年齢は男性43歳，女性35歳，20歳未満の患者は8.7%で，他の患者はすべて成人であった[3)4)]。劇症1型糖尿病は発症に妊娠が関与するという報告があり，全国調査において，妊娠に関連して発症した1型糖尿病患者15名のうち，14名が劇症1型糖尿病であった。また，妊娠合併劇症1型糖尿病においては児の予後が非常に悪いことも明らかになっている[10]。

II. 病　　理

劇症1型糖尿病における代表的な膵病理所見は，マクロファージやT細胞といった免疫担当細胞の膵島および膵外分泌領域への浸潤である（**Fig. 1**）。この所見は発症直後に死亡した劇症1型糖尿病患者の剖検膵組織において認められるが，発症1～5ヵ月後の膵組織では認めないことから，発症後は時間経過とともに急速に消失することが推測される。発症直後に死亡した劇症1型糖尿病患者3名を対象とした著者らの検討では，観察した膵島のうち68.8%にT細胞の浸潤を92.6%にマクロファージの浸潤を認めた[11]。Tanakaらも同様に3名の発症直後に死亡した劇症1型糖尿病患者膵組織を検討しており，マクロファージ/T細胞の浸潤を報告している[12]。著者らの検討では，このマクロファージ/T細胞は，ウイルスの構成成分を認識するToll-like receptor（TLR）であるTLR3，TLR7，TR9を発現していた。特に，ウイルスが複製される際に生じる二本鎖RNAを認識するTLR3はT細胞の62.7%，マクロファージの84.7%に発現していた。この所見は，膵島局所においてウイルス感染の結果，免疫機構が活性化されていることを示唆するもので，これらの浸潤細胞がβ細胞傷害に直接関与していることが強く示唆される。実際，一部の症例においては，*in situ* hybridizationの手法を用いて，エンテロウイルスRNAの存在も報告されている（**Fig. 2**）[11]。

これとは別に著者らは本疾患において標的となっている膵β細胞について検討している。形態計測法

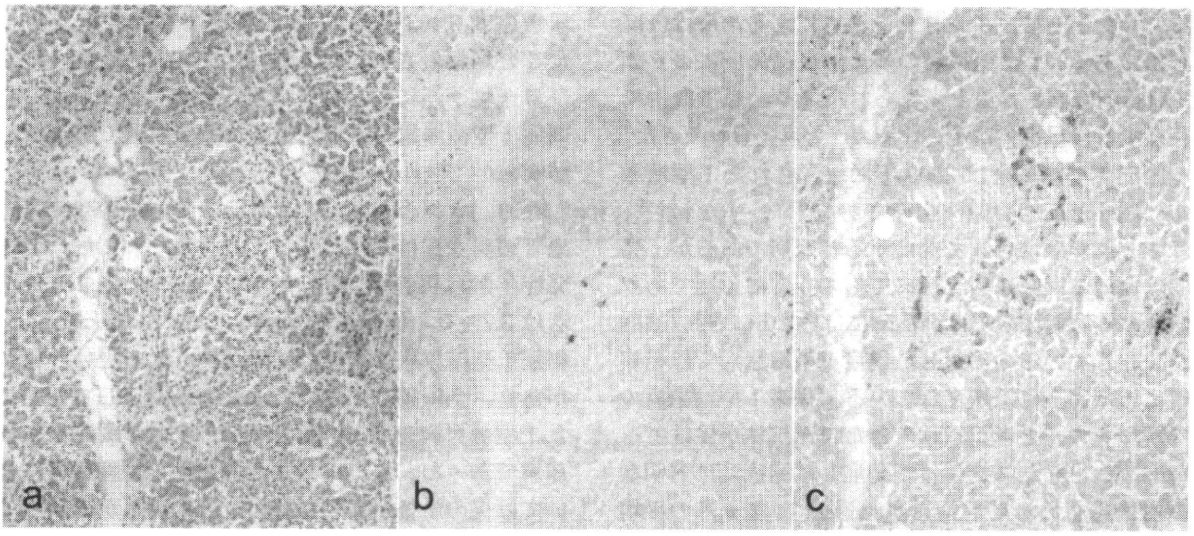

Figure 1　Histological findings of fulminant type 1 diabetes soon after the disease onset.
In HE staining (**a**) and immune staining for insulin (**b**) and glucagon (**c**), massive infiltration of mononuclear cells to islet and exocrine pancreas was observed (referred from 11 with minor modification).

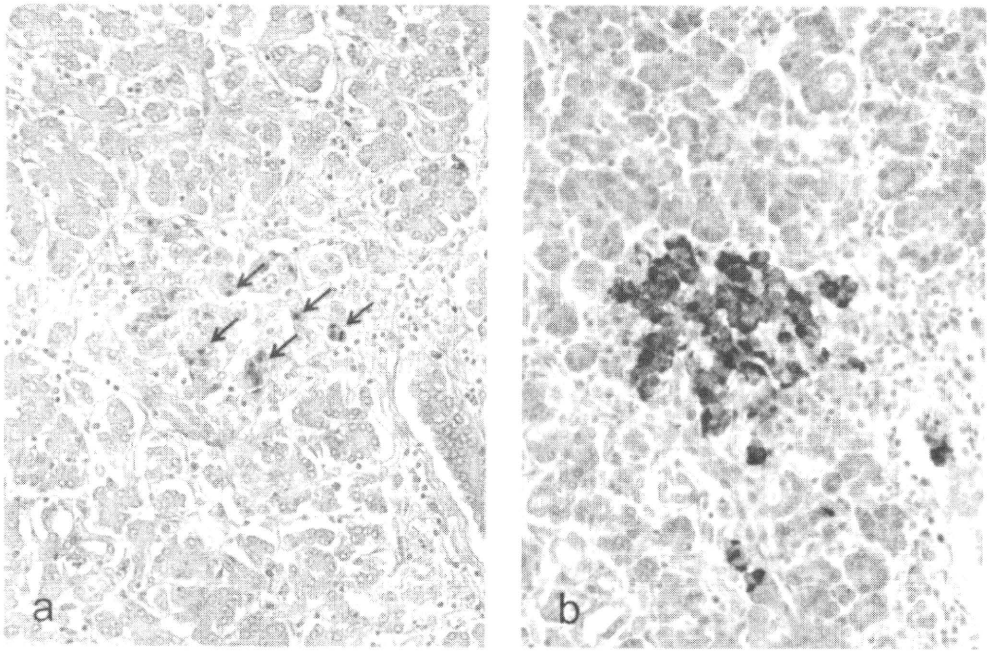

Figure 2　Expression of enterovirus RNA in the pancreas of fulminant type 1 diabetes soon after the disease onset.
Enterovirus RNA was visualized in an islet by *in situ* hybridization (**a**). Glucagon positive cells were observed in the serial section (**b**) (referred from 11 with minor modification).

により測定したβ細胞面積は，正常対照の0.4%に著減しており，糖尿病発症後同じ期間を経過した自己免疫性1型糖尿病患者膵(14.5%)に比べても，ほぼ完全なβ細胞傷害といえる[13]。また，β細胞の消失は発症直後の組織においても同様に認められ，β細胞面積は正常対照の0.1%であった。病理組織で認められるβ細胞の消失は，著しく低値を示す患者血中/尿中Cペプチドと一致する所見である。

次に，α細胞面積の減少は自己免疫性1型糖尿病では認められないが，劇症1型糖尿病では正常の33.1%に減少していた。これは劇症1型糖尿病においては，膵β細胞の破壊が，β細胞特異的なメカニズムではなく。α細胞も巻き込んだものであることを示唆している。

また，膵外分泌組織においては，単核球浸潤を認めるが，壊死，出血，浮腫，脂肪変成など膵炎で特徴的な所見は認められない[2)13)]。

III. 病　　因

劇症1型糖尿病の病因は，ウイルス感染とそれに伴う免疫現象が関与していると推測される。

ウイルス感染が関与していると考える根拠は，1)劇症1型糖尿病における急速な発症経過，2)前述の全国調査において劇症1型糖尿病の72%の症例に先行感染症状を認めたこと[3)]，3)ヒトヘルペスウイルス(HHV)6型，コクサッキーB3ウイルス，同B4ウイルス，インフルエンザBウイルスなどウイルス感染に伴って劇症1型糖尿病を発症したという症例の存在[14)15)]，4)膵組織においてウイルスRNAが存在するという症例の存在(前述)，5)発症早期劇症1型糖尿病患者におけるエンテロウイルス抗体価の上昇[16)]，などである。最後に述べたエンテロウイルス抗体の検討に用いた測定系は，特定のウイルスではなく，エコーウイルス，コクサッキーA群ウイルス，コクサッキーB群ウイルスなど広範囲のエンテロウイルスに反応する抗体を検出し得る測定系である。したがって，このウイルス抗体価が上昇していたことは，劇症1型糖尿病患者では繰り返しエンテロウイルスに感染していたこと，すなわち劇症1型糖尿病患者はエンテロウイルスに易感染性であることを示すと解釈できる。

最近，劇症1型糖尿病調査委員会で行われた発症時の患者血清におけるウイルス抗体価の研究結果が報告された[17)]。この研究では，55名の劇症1型糖尿病患者の発症時の血清を検討したところ，合計13項目に及ぶ様々な既知のウイルス抗体価の有意な上昇を認めた。しかし，特定のウイルス抗体価の上昇は認められなかった。したがって，劇症1型糖尿病では，特定のウイルスにより直接的な膵β細胞傷害が生じるのではなく，ウイルス感染に伴って抗ウイルス免疫反応が惹起され，その反応にβ細胞が巻き込まれて傷害されるという仮説が考えられる。もちろん，未知のウイルスによって直接β細胞傷害が生じる可能性もある。

一方，免疫反応の関与が示唆される所見として，病理の項で述べたように発症直後に死亡した劇症1型糖尿病患者の剖検膵組織において，マクロファージやT細胞といった免疫担当細胞が膵島および膵外分泌領域に浸潤しているという所見があげられる。また，発症後の劇症1型糖尿病患者末梢血にGAD反応性のTh1細胞が増加していることが明らかになっている[18)]。

劇症1型糖尿病では，従来の1型糖尿病のマーカーであり，自己免疫の指標であった膵島関連自己抗体は認めないが，これらの所見から，劇症1型糖尿病における膵β細胞傷害に免疫反応が関与していることは確実と思われる。しかし，数々のウイルス感染の証左から，この免疫反応のprimary antigenは，ウイルス抗原であり，したがって「自己免疫」ではなく，「抗ウイルス免疫」ではないかというのが著者らの現在の考えである。患者膵でのエンテロウイルスRNAの存在やマクロファージ/T細胞におけるTLR3発現は，膵島局所においてウイルス感染の結果，免疫機構が活性化されている事実を示唆する所見と思われる。

次に，発症に関連する遺伝素因として，免疫反応に関係する3つの遺伝子が報告されている。

第1は，HLA(human leukocyte antigen)遺伝子のうちclass IIとよばれる領域のDR-DQ遺伝子である[19)]。全国調査の一環として，115名の劇症1型糖尿病患者においてclass II HLA血清型を検討し，190名の健常者と比較した結果，HLA-DR4アレルは，劇症1型糖尿病患者の44.7%に認められたが，健常コントロール(21.8%)に比べ，有意に高頻度(オッズ比2.90)であった。

2番目の遺伝因子として，免疫反応の調節に関係する分子であるCTLA-4(cytotoxic T lymphocyte antigen-4)が報告されている。CTLA-4は抗原提示細胞(マクロファージや樹状細胞)上に発現する分子で，T細胞のB7-1(CD80)およびB7-2(CD86)と結合し，免疫系の反応調節に関係するシグナル，中でも免疫機構を不活化させる刺激(副刺激)を伝達すると考えられている。Kawasakiらの報告によると，劇症1型糖尿病55名，自己免疫性1型糖尿病91名，健常者369名において，*CTLA-4*の2つのSNP，＋49GGおよびCT60について検討したところ，劇症1型糖

尿病はCT60AA多型との関連を認めた(p<0.05，オッズ比2.68)が，＋49G>A多型との関連を認めなかった。これに対し，自己免疫性1型糖尿病では＋49GGおよびCT60GG多型との関連を認めた(p<0.001)[20]。

ごく最近，3番目の遺伝因子として，Kawabataらにより，class I HLAである*HLA-B*と劇症1型糖尿病との関連が報告された。すなわち*HLA-B*4002*アレルを有する頻度は劇症1型糖尿病92名では17.4%であり，健常対照者434名における頻度(6.7%)に比べて，有意に高値で，オッズ比2.9であった[21]。この*HLA-B*4002*はHLA-DR4の有無とは無関係に高頻度であることも明らかにされた。また，急性発症自己免疫性1型糖尿病454名では9.3%，緩徐進行1型糖尿病174名では6.3%であり，これらと比べても劇症1型糖尿病では*HLA-B*4002*アレルが高頻度であった。Class I HLAはウイルス感染とそれに伴う免疫反応に関係する分子であり，遺伝学的にも，劇症1型糖尿病の成因には，ウイルス感染と免疫応答が関与していることを示唆するものと考えられる。

現在劇症1型糖尿病を対象とした全ゲノム関連解析が進行中であり，劇症1型糖尿病の新しい遺伝因子が明らかにされることが期待される。

IV. 診　　断

口渇，多飲，多尿といった高血糖症状が急激に出現し，増悪することが特徴である。また，発症時にケトーシス，ケトアシドーシスが必発であるので，全身倦怠感，嘔気，嘔吐，などの症状が前面に出ることも多い。こういった症状は往々にして不定愁訴としてとらえられ，誤診につながる可能性があるので十分な注意が必要である。

日本糖尿病学会劇症型糖尿病調査委員会では診断に関して2つの基準を作成している。1つは，劇症1型糖尿病を見落とさないための「スクリーニング基準」であり，もう1つは，劇症1型糖尿病を確実に診断するための「診断基準」である[4]。

スクリーニング基準は，1)糖尿病症状発現後1週間前後以内でケトーシスあるいはケトアシドーシスに陥る，2)初診時の(随時)血糖値が288mg/dl(16.0mmol/l)以上である，という2項目のみからなるが，臨床現場において直ちにしかも容易に聴取ないし測定できる項目に絞り，劇症1型糖尿病を強く疑う所見を示したものである。この基準に当てはまる患者については，劇症1型糖尿病を強く疑い，直ちに入院可能な病院に紹介して精査・加療する必要がある。

これに対し，診断基準(**Table 1**)は，今後の病態や成因の解明のために作成されたより厳密な基準である。スクリーニング基準に「HbA1c<8.5%」という項目を加えたが，HbA1c値が血糖値に比較して不釣り合いに低いことは，急速な血糖の上昇を意味しており，劇症1型糖尿病の大きな特徴である。スクリーニング基準にこの項目を入れなかったのは，HbA1cの院内測定が不可能な施設において，診断と

Table 1　Diagnostic criteria for fulminant type 1 diabetes (2004)

下記1～3のすべての項目を満たすものを劇症1型糖尿病と診断する。

1. 糖尿病症状発現後1週間前後以内でケトーシスあるいはケトアシドーシスに陥る(初診時尿ケトン体陽性，血中ケトン体上昇のいずれかを認める)。
2. 初診時の(随時)血糖値が288mg/dl(16.0mmol/l)以上であり，かつHbA1c値<8.5%である。
3. 発症時の尿中Cペプチド<10μg/day，または，空腹時血清Cペプチド<0.3ng/mlかつグルカゴン負荷後(または食後2時間)血清Cペプチド<0.5ng/mlである。

<参考所見>

A)原則としてGAD抗体などの膵島関連自己抗体は陰性である。
B)ケトーシスと診断されるまで原則として1週間以内であるが，1～2週間の症例も存在する。
C)約98%の症例で発症時に何らかの血中膵外分泌酵素(アミラーゼ，リパーゼ，エラスターゼ1など)が上昇している。
D)約70%の症例で前駆症状として上気道炎症状(発熱，咽頭痛など)，消化器症状(上腹部痛，悪心・嘔吐など)を認める。
E)妊娠に関連して発症することがある。

(referred from 4)

Table 2 Clinical and laboratory findings of fulminant and type 1A diabetes at disease onset

	劇症型	自己免疫性
N	161	137
有症状期間(日)	4.4±3.1	36.4±25.1
随伴症状		
口渇(%)	93.7	93.3
感冒様症状(%)	71.7	26.9
腹部症状(%)	72.5	7.5
意識レベル低下(%)	45.2	5.3
妊娠合併*(%)	21.0	1.5
HbA1c(%)	6.4±0.9	12.2±2.2
尿中Cペプチド(μg/day)	4.3±4.0	21.0±14.8
初診時血糖値(mg/dL)	799±360	434±212
動脈血pH	7.13±0.13	7.31±0.12
下記のいずれかの膵外分泌酵素上昇(%)	98.0	39.5
血清アミラーゼ上昇(あり/なし)	74/54	11/81
血清エラスターゼ上昇(あり/なし)	54/9	1/37
血清リパーゼ上昇(あり/なし)	50/9	5/38
抗GAD抗体(陽性／陰性)	7/138	114/14
IA-2抗体(陽性／陰性)	0/43	31/24

(文献4を改変して引用)

数字は平均値±SD。*妊娠可能年齢(13〜49歳)の女性患者(劇症1型糖尿病62名，自己免疫性1型糖尿病68名)における頻度。

治療開始が遅れる可能性があることを危惧してのことである。この他に，インスリン分泌が枯渇していることの証明として，Cペプチドの低値および低反応が加えられている。

一方，劇症1型糖尿病のみにみられる所見ではないため診断基準には盛り込まなかったが，高頻度に認められる所見を「参考所見」として診断基準に付記された。

診断基準に記載されている項目のうち，検査所見をまとめると，1)高血糖，2)それに不釣り合いな(＝正常あるいは軽度上昇にとどまる)HbA1c，3)尿中あるいは血中ケトン体の上昇，4)動脈血液ガス検査におけるアシドーシス，5)血中膵外分泌酵素の上昇，6)1型糖尿病のマーカーである抗GAD(glutamic acid decarboxylase)抗体，抗IA-2(insulinoma-associated antigen 2)抗体，膵島細胞抗体(islet cell antibodies: ICA)，インスリン自己抗体(insulin autoantibody: IAA)などの膵島関連自己抗体陰性，となる。ただし，劇症1型糖尿病患者のうち約5%において低抗体価ながらGAD抗体を認めると報告されており，これらの抗体が陽性であるからと言って劇症1型糖尿病の診断を除外するものではない。全国調査における初診時の臨床所見・検査所見を**Table 2**に示す。

最近，HbA1c測定は慢性高血糖状態を正確に反映し，糖尿病合併症のリスクとよく相関すること，採血時間を問わないなど検査法として血糖測定に比べ優れることなどの理由から，米国糖尿病学会(ADA)，欧州糖尿病学会連合(EASD)，国際糖尿病会議(IDF)の合同委員会は，「A1C ≥6.5%」を新しい糖尿病診断基準とする案を発表した。しかし，前述のように，劇症1型糖尿病は発症時に非常に急激に血糖が上昇するため，HbA1cがあまり上昇しないことがある。そこで，新糖尿病診断基準(案)の劇症1型糖尿病診断における妥当性を検証する目的で，全国から日本糖尿病学会劇症1型糖尿病調査委員会に登録された劇症1型糖尿病患者99名(HbA1cの標準物質がJDS Lot2に切り替わった2001年3月以降[〜2006年6月]発症)について，発症時のHbA1cについて検討した。なお，米国における「A1C 6.5%」は，日本における標準物質JDS Lot2を用いた場合，6.2%に相当するため，6.2%をカットオフ値とした。その結果，劇症1型糖尿病患者99名の随時血糖値はすべての

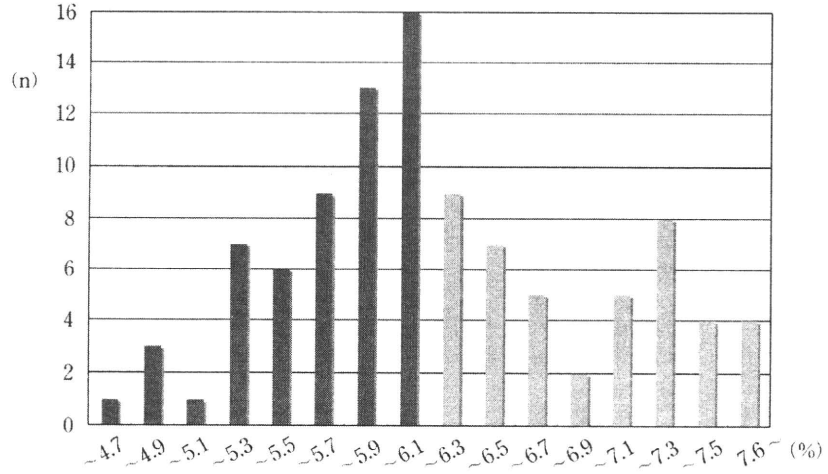

Figure 3　HbA1c levels of fulminant type 1 diabetes at disease onset.
HbA1c levels were below 6.2% in approximately half of the patients.

Table 3　Clinical findings of fulminant type 1 diabetes according to HbA1c levels at disease onset

発症時の HbA1c	＜6.2%	≧6.2%
n(M/F)	55(22/33)	44(22/22)
年齢	41±16*	48±15
罹病期間(日)	4±6	5±4
口渇なし	5	6
血糖値＜200mg/dl	0	0
血糖値(mg/dl)	747±353	851±386
pH	7.15±0.14	7.16±0.23
血中Cペプチド(ng/dl)	0.15±0.11*	0.25±0.20

*p＜0.05

患者で200mg/dl以上であったが，HbA1cの新糖尿病診断基準(案)を満たさない患者は55名(55.6%)存在した(**Fig. 3**)。すなわち，血糖が急激に上昇する劇症1型糖尿病では，HbA1cのみを用いた新診断基準(案)をみたさない患者を多数含むことが明らかになった。**Table 3**には新糖尿病診断基準(案)を満たす患者と満たさない患者の検査所見を示す。5名の患者では典型的な高血糖症状である口渇を認めず，かつHbA1c＜6.2%であった。当然ではあるが，症状とHbA1c値のみではなく，血糖値を診断基準に組み入れる必要がある[22]。

また最近，自己免疫性膵炎において患者血中に認められるアミラーゼ α2Aやheat shock protein 10に対する抗体が，劇症1型糖尿病患者においても認められるということも報告されており，多数例での検討が待たれる[23][24]

V. 治　　療

A. 急性期(発症時)の治療

劇症1型糖尿病は非常に急激に進行するため，発症時に診断が遅れると糖尿病ケトアシドーシスのために死の転帰をとることがある。心肺停止を来たした症例とそれ以外の症例の差が何に由来するものであるのかは，現在のところわかっていないが，急性期(発症時)の治療で大切なことは，できるかぎり早期に発見(診断)し，早期に治療を開始することである。

そのためには，まず医療従事者がこのような糖尿病の存在を知っていることが大切である。次に，救急受診患者といえどもルーチンで尿検査を行うことが大切で，典型的な高血糖症状を呈さない患者においても，尿糖と尿ケトンがともに強陽性であれば，劇症1型糖尿病を含め，1型糖尿病を強く疑う。次

に，直ちに簡易血糖測定器で血糖を測定し，糖尿病の診断を確定する。劇症1型糖尿病であれば，診断時の血糖値は90%の症例で400mg/dl以上である。

糖尿病ケトーシス(ケトアシドーシス)の診断がつけば，即入院の上，治療を開始する。この際の治療目標は，合併症が生じることなくケトアシドーシスの状態から脱出することである。具体的には，一般のケトアシドーシスの治療と同じく，脱水の補正，血糖値・電解質の管理，アシドーシスの是正を行う。

B. 慢性期の治療－血糖コントロール

糖尿病ケトアシドーシスが改善すれば，一般の1型糖尿病と同じように強化インスリン療法を行う。内因性インスリン分泌能が病初期から枯渇しているので，血糖コントロールの難しい症例が多い。インスリン治療を中断すると短期間でケトーシスに陥るので，外来での管理において，医療従事者と患者の双方がこのことをよく認識しておく必要がある。具体的には，sick dayの自己管理について，患者さんに十分説明し，理解してもらうことが大切である。また，現在までに，劇症1型糖尿病でインスリン治療が不要になる時期(いわゆるハネムーン期)が報告された例はない。

実際には，(超)速効型インスリン＋持効型インスリンの投与あるいは持続皮下インスリン注入療法(CSII)が標準的な治療である。

VI. 予　　後

急性期の予後に関しては，前述した通りで，1日の診断の遅れが不幸な転帰につながる可能性があり，来院時に心肺停止に陥った症例も報告されている。

急性期を無事経過した症例の長期予後に関しての報告はないが，早期に合併症が進行しやすいという報告がある。これは劇症1型糖尿病患者41名とコントロールである自己免疫性1型糖尿病患者76名について，発症5年後までの細小血管障害の進行を調査したものである。その結果，発症5年後には，劇症1型糖尿病患者のうち10名(24.4%)に何らかの合併症を認めた。これに対し，自己免疫性1型糖尿病で発症5年後に合併症の発症を認めた患者は2名(2.6%)に過ぎず，劇症1型糖尿病患者では有意に合併症が進行しやすいことが明らかになった。今回の調査では，網膜症は毛細血管瘤や網膜小出血などいわゆる単純網膜症(SDR: simple diabetic retinopathy)の出現，腎症はmicroalbuminuriaの出現(UAE＞30mg/g Cr)，神経障害はアキレス腱反射の減弱をもって判定しているが，劇症1型糖尿病患者のうち，発症5年後には網膜症は9.8%，腎症は12.2%，神経障害も12.2%の症例で進行を認め，どの細小血管合併症においても有意に高率に進行していた。その理由として，高い平均血糖値の影響が第一に考えられるが，内因性インスリン分泌能低下による血糖の不安定性，あるいはCペプチドの低下が関与していることも考えられる[25]。

VII. おわりに

近年確立された臨床病型である劇症1型糖尿病の概念，疫学，病因，病理組織，診断，治療，予後について概説した。劇症1型糖尿病は発症時の診断が遅れると生命予後に影響する糖尿病の最重症型であることを強調して稿を終えたい。

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Exendin-4, a glucagon-like peptide-1 receptor agonist, suppresses pancreatic β-cell destruction induced by encephalomyocarditis virus

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ABSTRACT

Viral infection is one of the important factors for the pathogenesis of type 1 diabetes. Particularly, in fulminant type 1 diabetes, rapid β-cell destruction is suggested to be triggered by viral infection. Recently, glucagon-like peptide 1 (GLP-1) receptor agonists have been reported to have direct beneficial effects on β-cells, such as anti-apoptotic effect, increasing β-cell mass, and improvement of β-cell function. However, their effects on β-cell destruction induced by viral infections have not been elucidated. In this study, we used an encephalomyocarditis virus (EMCV)-induced diabetic model mouse to show that a GLP-1 receptor agonist, exendin-4, prevents β-cell destruction. Nine-week-old male DBA/2 mice were intraperitoneally injected with EMCV (200 plaque forming units (PFU) mouse^{-1}). Low (20 nmol kg^{-1} d^{-1}) or high (40 nmol kg^{-1} d^{-1}) doses of exendin-4 were administered for 10 d, starting from 2 d before the infection, and the rate of diabetic onset was evaluated. In addition, the number of infiltrating macrophage per islet and the ratio of β-cell area to islet area were determined. The effects of exendin-4 on infected β-cells and macrophages were investigated by using MIN6 and RAW264 mouse macrophages. The incidence of diabetes was significantly lower in the high-dose exendin-4-treated group than in the control group. Furthermore, the β-cell area was significantly more preserved in the high-dose exendin-4-treated group than in the control. In addition, the number of macrophages infiltrating into the islets was significantly less in the high-dose exendin-4-treated group than in the control group. In vitro, exendin-4 reduced β-cell apoptosis, and tumor necrosis factor α (TNFα), interleukin β (IL-β), and inducible nitric oxide synthase (iNOS) production of infected or lipopolysaccharide (LPS)-stimulated macrophages. These results suggested that exendin-4 limits β-cell destruction by protecting β cells and reducing the inflammatory response of macrophages.

1. Introduction

The onset of type 1 diabetes is strongly associated with genetic factors (such as HLA type) and environmental factors. Particularly, viral infection is regarded as the most important environmental factor [2,3]. In Japan, fulminant type 1 diabetes accounts for about 20% of the cases of acute-onset type 1 diabetes and is characterized by extremely rapid β-cell destruction. Fulminant type 1 diabetes appears to be triggered by viral infection based on the following findings: (1) it occurs after flu-like symptoms; (2) anti-enterovirus antibody titers in these patients are higher than those in healthy individuals [4–6]; (3) macrophages infiltration into islets was detected in autopsy pancreatic tissue from patients who died soon after the onset of fulminant type 1 diabetes; and (4) enteroviral antigen or RNA was detected in some patient's pancreas [7,8].

Encephalomyocarditis virus (EMCV) belongs to the Picornaviridae family and causes diabetes, myocarditis, and paralysis in susceptible mice. After EMCV infection, diabetes occurs within a week as a result of almost complete destruction of β-cells. The clinical course of this model resembles that of fulminant type 1 diabetes in humans [9].

Glucagon-like peptide-1 (GLP-1), a hormone that is secreted from the L-cells of the small intestine during digestion, enhances the glucose-dependent insulin secretion and decreases glucagon secretion [10–12]. GLP-1 receptors are abundantly expressed not only in β-cells but also in many other cells [1]. Recently, it was reported that much higher blood GLP-1 levels than physiological level brought by GLP-1 receptor agonist injection exhibit many effects, including anti-apoptotic and proliferative effects on β-cells [13–16], decreased food intake [22], and protection against heart injuries [23–25].

Here, we show that a high-dose exendin-4, a GLP-1 receptor agonist, suppresses EMCV-induced β-cell destruction through not only anti-apoptotic effect on β-cells but also anti-inflammatory effects against macrophages.

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2. Materials and methods

2.1. Animals

All animal experimentation was conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The study protocol was approved by the Animal Care and Use Committee of Osaka Medical College. Male DBA/2 mice 9 weeks old were purchased from CLEA Japan Inc. and housed in bio-safety facilities at Osaka Medical College. Mice were maintained at a constant room temperature of 25 °C with a 12 h photoperiod. They were fed a standard rodent chow and given access to water ad libitum.

2.2. Virus

Encephalomyocarditis virus (EMCV) was kindly provided by Katsuaki Dan (Keio University, Tokyo, Japan). The virus was grown in DBA/2 mice or L929 cells. The viral titer was determined by a plaque assay on L929 cells as described previously [17,18].

2.3. Viral infection and measurement of blood glucose and pancreatic insulin content

Nine-week-old male DBA/2 mice, which are the most susceptible strain to EMCV, were intraperitoneally injected with 200 plaque forming units (PFU) EMCV in 0.1 mL phosphate-buffered saline (PBS) pH 7.4. Low (20 nmol kg^{-1} d^{-1}) ($n = 7$) or high (40 nmol kg^{-1} d^{-1}) ($n = 7$) doses of exendin-4, a GLP-1 receptor agonist, were intraperitoneally injected for 10 d, starting from 2 d before the infection. Control mice ($n = 14$) were intraperitoneally injected with corresponding dose of PBS for same period. Blood samples were obtained from the tail vein and glucose levels were measured by using Glutest Sensor (Sanwa, Japan) for 14 d after infection. Mice with blood glucose levels greater than 250 mg dL^{-1} were considered to be diabetic. Fifteen days after infection, intraperitoneal glucose tolerance test (IPGTT) was performed with 1 g glucose kg^{-1}. Afterwards, the mice were sacrificed to measure the pancreatic insulin content.

Pancreatic insulin content was measured by using the acid ethanol method [28]. Briefly, fresh pancreas was homogenized in acid ethanol (0.18 N hydrochloric acid, 75% ethanol), and then incubated overnight at 4 °C. After centrifugation, the supernatant was neutralized. The insulin concentration of the extract was measured by using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Sibayagi, Japan). The protein concentration was measured by using the bicinchoninic acid (BCA) method. The pancreatic insulin content was calculated by dividing the insulin concentration by the protein concentration.

2.4. Histopathology of the pancreas

Sixteen control mice infected with EMCV and 16 mice treated with high-dose exendin-4 were sacrificed at 48, 72, 96, and 120 h after infection, and then their pancreas was excised, fixed with 4% paraformaldehyde, and embedded in paraffin. Four-micrometer-thick paraffin sections were deparaffinized in xylene and rehydrated in a graded ethanol series (100% to 50%). After washing in PBS, the target retrieval was performed with proteinase K for 6 min. Peroxidase activity was inhibited by immersing the sections in 3% methanolic hydrogen peroxide for 15 min, and non-specific binding of the antibody was blocked by pre-incubation with Non-Specific Staining Blocking Regent (X0909 Dako, Japan). The sections were then incubated at room temperature for 30 min with either rat anti-Mac-2 antibody (CL8942AP Cedarlane Laboratory, 1:500 dilution) or guinea pig polyclonal anti-swine insulin antibody (A0564 Dako, Japan, 1:800 dilution). After washing in PBS 3 times, the slides were incubated with secondary antibodies for 30 min at room temperature; Envision + (K4003 Dako, Japan) was used for the insulin slides and Vectastain Elite ABC Rat Kit (Vector Laboratories, Burlingame, CA) was used for Mac-2 slides. After washing in PBS, the antibody binding was detected by using diaminobenizidine (DAB). Finally, the slides were counterstained with hematoxylin. The β-cell area was measured as the percentage of the insulin-positive area per islet area by using a Scanscope XT Digital Slide Scanner (Aperio). The number of macrophages was counted by using a Scanscope XT Digital Slide Scanner and expressed as the number of Mac-2 positive cells per islet area. At least 5 islets were chosen at random from each section for statistical analysis.

2.5. Flow cytometry apoptosis assay of a β-cell line

MIN6, a β-cell line, was used for the apoptosis assay. MIN6 cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) with 15% fetal calf serum (FCS). Confluent dishes were incubated with EMCV (about 1 PFU $cell^{-1}$) for 48 h or 2 μM staurosporine (Sigma–Aldrich) for 18 h with or without 12 nM exendin-4. In the apoptosis assay with staurosporine, the control wells were incubated with the corresponding concentration of dimethyl sulfoxide (DMSO). After incubation, cell dissociation buffer (Gibco) was added to each well, and then the cell samples were washed with ice-cold medium and centrifuged for 5 min at 500g. Then, the supernatant was thrown away. For the latter process, Annexin V-FITC Kit System for Detection of Apoptosis (Beckman Coulter, Inc., Fullerton, CA) was used. Briefly, samples were diluted with binding buffer to 5×10^5–5×10^6 cells mL^{-1}. Afterwards, the Annexin V-FITC and propidium iodide (PI) solutions were added, and the samples were incubated on ice in the dark for 10 min. Immediately afterwards, the apoptosis rate was measured by flow cytometer, BD FACSAria™ (Becton, Dickinson and Company).

2.6. Exendin-4 pretreatment of LPS activated or EMCV infected macrophages

After washing with PBS, RAW264 macrophage cells were incubated with or without 12 nM exendin-4 for 1 h. Then, EMCV (1 PFU $cell^{-1}$) was added and incubated for 12 h, or 1 μg mL^{-1} LPS (Sigma–Aldrich) was added and incubated for 1 h. To inhibit the exendin-4 signal, RAW264 cells were incubated with 5 μM MDL-12330A (Sigma–Aldrich), an adenylate cyclase inhibitor, at the same time that the exendin-4 was added.

2.7. Isolation of RNA and quantitative real-time polymerase chain reaction

Total RNA was extracted from treated RAW264 cells by using the RNeasy Mini Kit (Qiagen, Tokyo, Japan). First-strand cDNA was synthesized by using the Transcriptor First strand cDNA synthesis kit (Roche Diagnostics GMBH, Mannheim, Germany), with the included random hexamers as the priming strategy. The resulting cDNAs were mixed with the LightCycler FastStart DNA Master Hybridization Probes reagent (Roche), TaqMan™ probes, and primers in LightCycler capillaries. Then, the sample carousel with the capillaries was centrifuged in the LightCycler Carousel Centrifuge and placed into the LightCycler. After denaturing for 10 min at 95 °C, 55 polymerase chain reaction (PCR) cycles were run. Each cycle consisted of 10 s at 95 °C and 20 s at 60 °C. Fluorescence was measured at the end of every cycle. After the final cycle, the capillaries were cooled for 2 s at 40 °C. Fluorescence curves were analyzed with the LightCycler software, Ver. 3.5. Cycles 15–55 were used to calculate the crossing points, which were defined

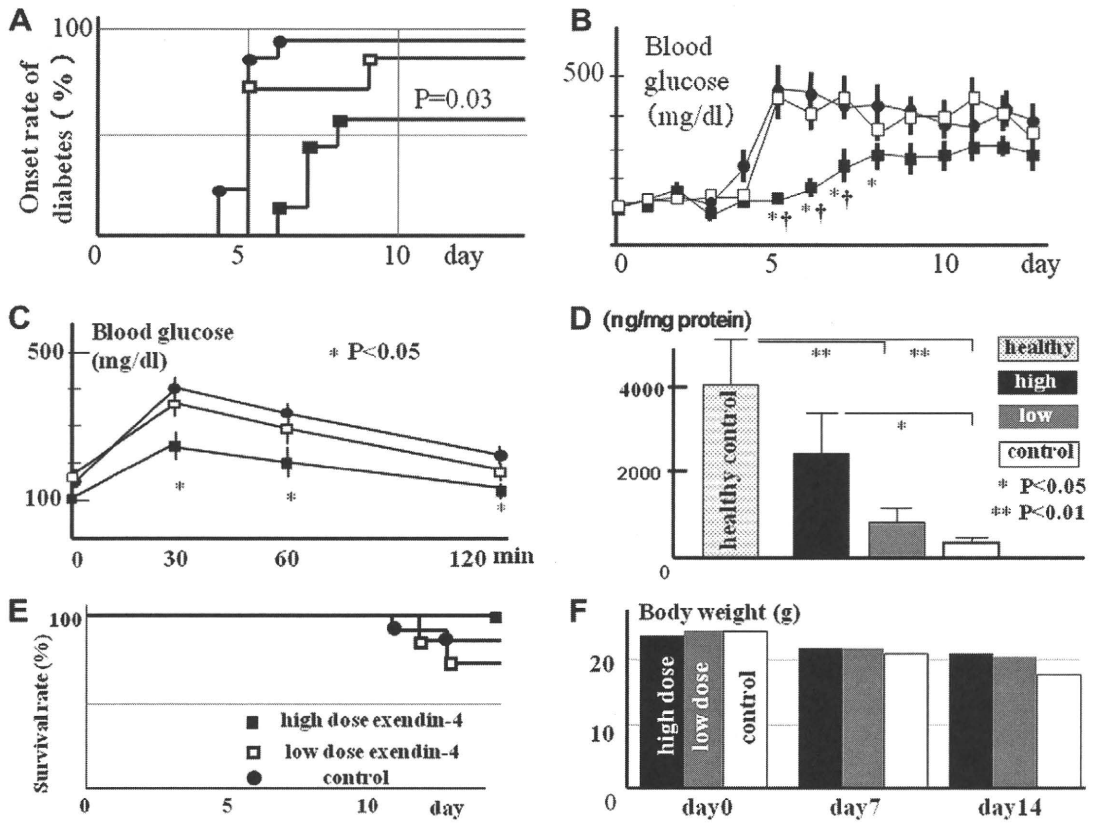

Fig. 1. Exendin-4 prevents the onset of diabetes in encephalomyocarditis virus (EMCV)-infected mice. (A) Changes in the incidence of diabetes during treatment with or without exendin-4 in EMCV-infected mice. ■, High-dose exendin-4; □, low-dose exendin-4; ●, control. $p = 0.03$ control vs. high-dose exendin-4. (B) The change in blood glucose levels after infection. ■, High-dose exendin-4; □, low-dose exendin-4; ●, control. $^{*}p < 0.05$ control vs. high-dose exendin-4, $^{\dagger}p < 0.05$ low-dose exendin-4 vs. high-dose exendin-4. (C) Intraperitoneal glucose tolerance test (IPGTT) 15 d after infection. ■, High-dose exendin-4; □, low-dose exendin-4; ●, control. $^{*}p < 0.05$ control vs. high-dose exendin-4. (D) Pancreatic insulin content 15 d after infection. Black bar, high-dose exendin-4; gray bar, low-dose exendin-4; white bar, control. The bar of healthy control ($n = 4$) was added. (E) Survival rate after infection. ■, High-dose exendin-4; □, low-dose exendin-4; ●, control. There was no significant difference between each group. (F) change in body weight after infection. Black bar, high-dose exendin-4; gray bar, low-dose exendin-4; white bar, control. There was no significant difference between each group.

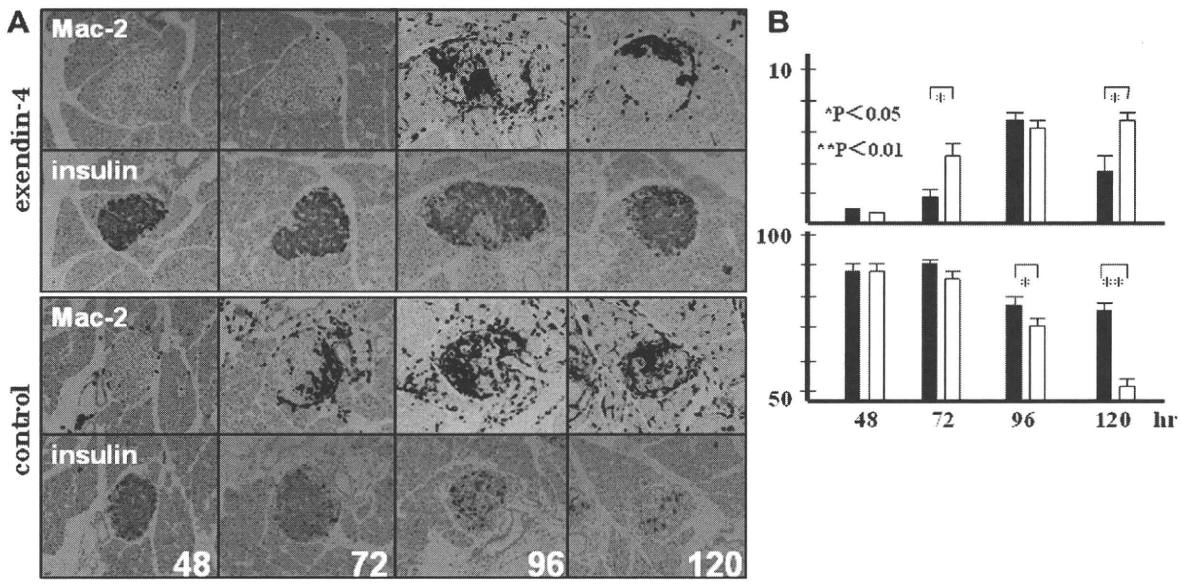

Fig. 2. Exendin-4 reduces macrophages infiltration into islets and β-cell destruction in EMCV-infected mice. (A) Histological changes in the high-dose exendin-4-treated group and control group at 48, 72, 96, and 120 h after infection. Mac-2 positive cells indicate macrophages, and insulin-positive cells indicate β-cells. Original magnification: 150×. (B) The upper panel shows number of macrophages per islet area ($\cdot 10^{-3}/\mu m^2$), and the lower panel shows the (β-cell area/islet area) ×100 in the high-dose exendin-4 group and the control group at 48, 72, 96 and 120 h after infection. Black bar, high-dose exendin-4; white bar, control. Statistical analysis: Student's *t*-test.

as the maximum of the second derivative from the fluorescence curves. Automated calculation was performed by the second derivative maximum method. The sequences of the primers and Roche universal probe number (#) are as follows: TNFα (GenBank

Accession No. M13049.1): left primer (TCTTCTCATTCCTGCTTGT GG), right primer (GGTCTGGGCCATAGAACTGA), and #49 probe; IL-1β (NM_008361.3): left primer (TGTAATGAAAGACGGCACACC), right primer (TCTTCTTTGGGTATTGCTTGG), and #78 probe; iNOS (NM_010927.3): left primer (GGGCTGTCACGGAGATCA), right primer (CCATGATGGTCACATTCTGC), and #76 probe; GAPDH (NM_008084): left primer (TGTCCGTCGTGGATCTGAC), right primer (CCTGCTTCACCACCTTCTTG), and #80 probe.

2.8. Data analysis

The data for the incidence of diabetes and the survival rate were analyzed by the log-rank test. Other data were presented as mean (SEM). Unless noted, statistically significant differences among multiple groups were analyzed by one-way analysis of variance (ANOVA). Sheffe's *F*-test was used for comparing each group. A *p*-value less than 0.05 were considered a statistically significant difference.

3. Results

3.1. The effect of exendin-4 on the prevention of diabetes in EMCV-infected mice

Five days after the infection, the blood glucose level and the incidence of diabetes was significantly higher in the control mice than those in the high-dose exendin-4 treated mice (Fig. 1A and B). IPGTT performed 15 d after infection showed that the glucose levels at 30, 60, and 120 min were significantly lower in the high-dose exendin-4 group than those in the control mice (Fig. 1C). The pancreatic insulin content 15 d after infection was significantly higher in the high-dose exendin-4 group than that in the control group (Fig. 1D). After viral infection, the survival rate was the highest in the high-dose exendin-4 group (Fig. 1E). In addition, after viral infection, the body weight of all of the mice decreased, but the degree of weight reduction was less in the high-dose group than in other groups (Fig. 1F).

3.2. Effects of exendin-4 on β-cell mass and macrophage infiltration of islets

The β-cell area was dramatically decreased in the control group compared with that in the high-dose exendin-4 group, and the degree of macrophage infiltration into the islets was severer in the control group than in the high-dose exendin-4 group (Fig. 2A). To evaluate these findings quantitatively, we measured the percentage of the β-cell area to the area of each islet and the number of macrophages per islet area. After 96 h, the β-cell area was significantly less in the control group than in the high-dose exendin-4 group, and at 72 and 120 h, the number of macrophages was significantly more in the control group than in the exendin-4 group (Fig. 2B).

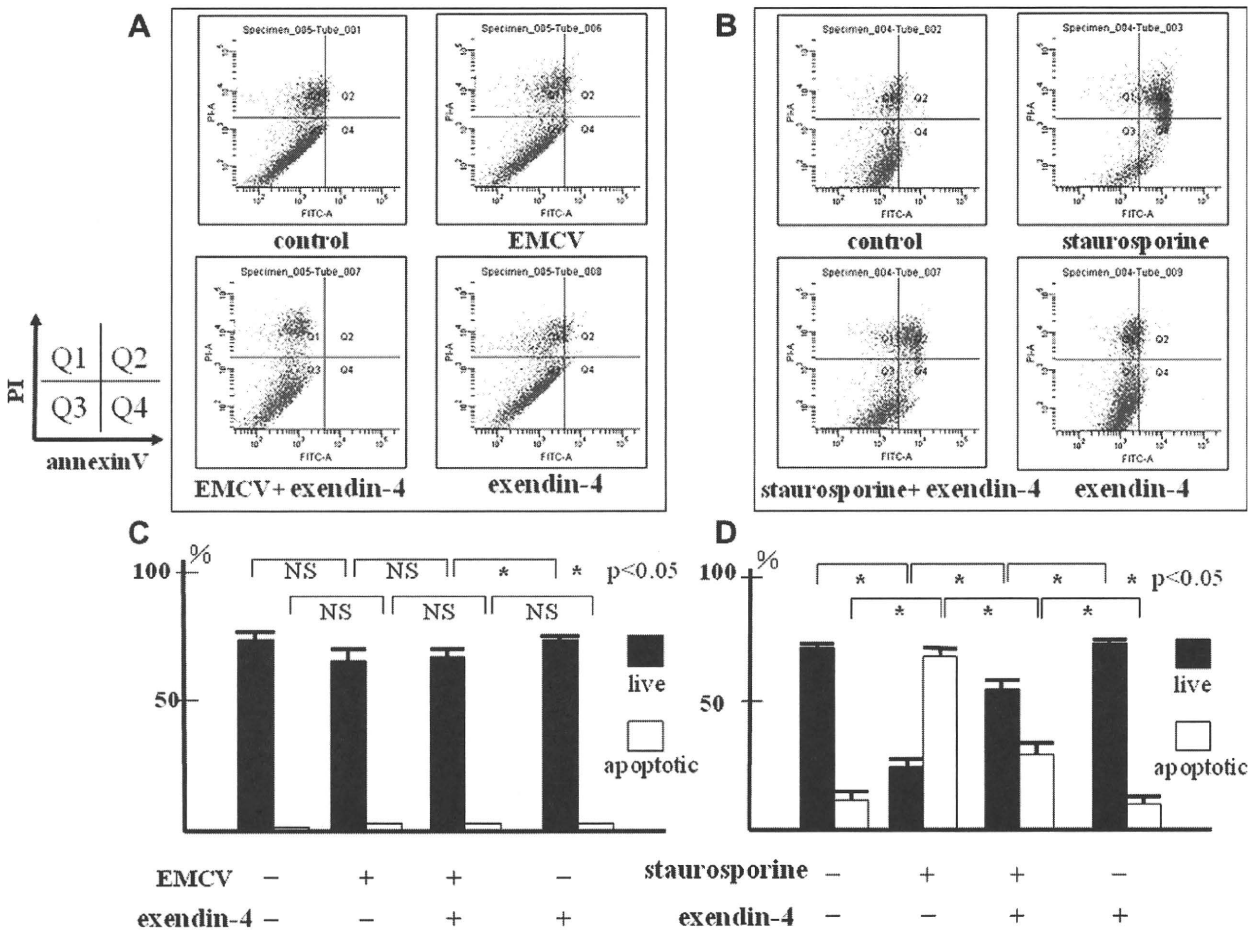

Fig. 3. Exendin-4 has an anti-apoptotic effect on β-cells, but EMCV does not directly induce apoptosis of β-cells. (A) MIN6 cells were incubated for 48 h with EMCV and exendin-4. (B) MIN6 cells were incubated for 18 h with staurosporine and exendin-4. Cells were co-stained with annexin V-FITC and propidium iodide (PI), and then analyzed by flow cytometry. A representative dot-plot is shown for each condition ($n = 3–5$). Q3, live cells; Q4, early apoptotic cells; Q2, late apoptotic cells; Q1, necrotic cells. (C and D) Comparison of the percentage of cells collected as live cells (Q3, black bar) or apoptotic cells (Q2 + 4, white bar) ($n \geq 3$ in each group). Statistical analysis: Student's *t*-test.

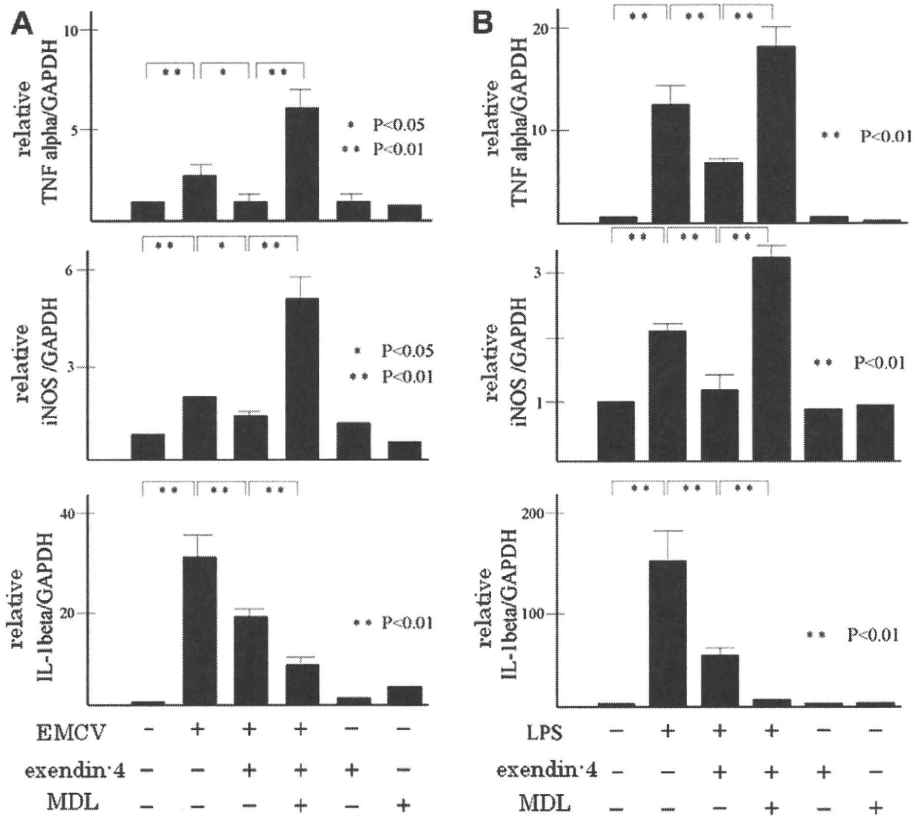

Fig. 4. Exendin-4 reduces the inflammatory response of macrophages. RAW264 cells were incubated with 12 nM exendin-4 for 1 h or with exendin-4 and 5 μM MDL-12330A for 1 h followed by treatment with EMCV (1 plaque forming unit (PFU) $cell^{-1}$) for 12 h (A) or lipopolysaccharide (LPS) (1 μg mL^{-1}) for 1 h (B). Then, the mRNA expression levels of tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), and interleukin β (IL-β) were determined by real-time quantitative polymerase chain reaction (RT-PCR). The gene expression level in RAW264 cells without the addition of anything was defined as 1.0 ($n = 3–7$). Statistical analysis: Student's *t*-test.

3.3. Exendin-4 protects β-cells from apoptosis, but EMCV does not directly destroy β-cells

To investigate whether exendin-4 has a preventive effect on EMCV-induced β-cell apoptosis, MIN6 cells were incubated with or without EMCV. Forty-eight hours after incubation, no significant difference was observed between the control cells and the EMCV-infected cells (Fig. 3A and C). Staurosporine treatment induced apoptosis in MIN6 cells, but pre-incubation with exendin-4 suppressed it (Fig. 3B and D).

3.4. Exendin-4 modulates macrophage function

Incubation of RAW264 cells with EMCV for 12 h induced a 2–30-fold increases in the RNA expression levels of TNFα, IL-β, and iNOS. Exendin-4 significantly suppressed EMCV-induced RNA expression (Fig. 4A). Likewise, LPS enhanced the RNA expression of TNFα, IL-β, and iNOS, but exendin-4 significantly suppressed them (Fig. 4B). As for TNFα and iNOS, MDL-12330A blocked the effects of exendin-4.

4. Discussion

This study has clearly shown that high-dose exendin-4 injection suppresses the onset of EMCV-induced diabetes. To the best of our knowledge, this is the first report showing that exendin-4 is useful for preventing diabetes induced by EMCV. By inhibiting the infiltration of macrophages into the islets and the expression of macrophage-derived chemical mediators, exendin-4 markedly reduced β-cell destruction. These results suggested that the anti-diabetic effect of exendin-4 occurs through mechanisms that are independent of the enhancement of glucose-dependent insulin secretion or glucagon suppression.

To investigate the mechanism underlying the preservation of β-cells, we first examined whether exendin-4 protects β-cells from EMCV-induced apoptosis. Surprisingly, the in vitro apoptosis assay showed that EMCV did not directly destroy β-cells. On the other hand, exendin-4 suppressed β-cell apoptosis induced by staurosporine, an apoptosis inducer. In mice infected with EMCV, the depletion of macrophages resulted in a much greater decrease in the incidence of diabetes [26,27], which suggested that EMCV did not directly destroy β-cells but macrophage played important role in β-cell destruction.

The infection of macrophages with EMCV induces the expression of chemical mediators [19] and that induce β-cell death [21]. Since macrophages express GLP-1 receptors [20], we hypothesized that chemical mediators, such as TNFα, IL-1β, and iNOS, from infected macrophages play a pivotal role in the destruction of β-cells and that exendin-4 modulates the expression of these mediators. Our results showed that exendin-4 suppressed their expression in infected macrophages. It has previously been reported that EMCV-infected mice treated with an antibody against IL-1β or TNFα or with an iNOS inhibitor exhibited a significant decrease in the incidence of diabetes [30]. Our results are in agreement with this report, which suggested the importance of macrophage-derived mediators in the pathogenesis of diabetes in this EMCV model.

The effect of GLP-1 is mainly mediated by the activation of adenylate cyclase and the elevation of intracellular cyclic AMP levels [29]. By using MDL-12330A, we demonstrated that the

elevation of cyclic AMP levels is essential for the attenuation of TNFα and iNOS mRNA expression in EMCV-infected cells. However, MDL-12330A did not inhibit the suppressive effect of exendin-4 on IL-1β mRNA expression, which indicated that this effect is not mediated by cyclic AMP.

In conclusion, our data suggested that exendin-4 prevents the onset of EMCV-induced diabetes in mice by suppressing the expression of TNF-α, IL-1β, and iNOS in activated macrophages and reducing the β-cell death. Because the macrophages infiltration into islets is observed predominantly in fulminant type 1 diabetes in humans, it has clinical significance to have clarified the effect that exendin-4 suppresses the inflammatory response of activated macrophages. These findings might provide new therapeutic strategies for type 1 diabetes including fulminant type 1 diabetes.

Acknowledgments

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Expression of chemokines, CXC chemokine ligand 10 (CXCL10) and CXCR3 in the inflamed islets of patients with recent-onset autoimmune type 1 diabetes

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Abstract. The aim of this study is to present direct evidence for the involvement of CXC chemokine ligand 10 (CXCL10) and CXCR3 in human autoimmune type 1 diabetes. We examined five patients with recent-onset type 1 diabetes and five control subjects without diabetes. Islet cell antibodies or GAD antibodies or both were detected in all five patients. We used double-immunofluorescence to detect the expression of CXCL10 and CXCR3 (the receptor of CXCL10). CXCL10 was detected in the islets of all five patients. Almost all (84.2 ± 10.3 %, mean ± SD) CXCL10-positive cells were insulin-positive in the islet area. CXCL10-positive cells with glucagons, somatostatins or pancreatic polypeptides were not detected at all. CXCL10 expression was not seen in any islet without beta cells. CXCR3 was detected in the islet areas of all five patients. Almost all (80.3 ± 13.4 %, mean ± SD) CXCR3-positive cells were CD3-positive T cells. Our study showed that CXCL10 was expressed in the remaining beta cells, and the infiltrating T cells expressed CXCR3, in pancreatic islets of patients with recent-onset type 1 diabetes. The interaction of CXCL10 and CXCR3 would contribute to the selective destruction of beta cells in the development of type 1 diabetes.

Key words: Biopsy, Chemokine, IP-10, CXCL10, CXCR3

TYPE 1A DIABETES is an autoimmune disease resulting from the destruction of pancreatic beta cells [1]. T cell dominant insulitis is a hallmark of this disease both in human and in rodent models. T cells are supposed to play a central role in beta cell destruction because they infiltrate beta cell-positive islets but disappear from the islets when beta cells are completely destroyed. In insulitis lesions, several cytokines induced by Th1 subtypes, including interferon gamma, tumor necrosis factor alpha and interleukin 1 beta, contribute to the final phase of beta cell damage.

Chemokines, a subgroup of cytokines, are defined as a family of small proinflammatory peptides that mediate the recruitment of different subsets of peripheral blood leukocytes [2]. Among them, CXC chemokine ligand 10 (CXCL10/ IP-10), an interferon-inducible chemokine, is known to be expressed stromal cell type of various lymphoid tissues and intestinal epithelial cells, and has activities related to the mechanism of immune response or inflammation including cell migration against monocytes and activated T cells [3], and inhibition of vascular neogenesis [4]. While its receptor, CXCR3 is expressed in various cell types such as activated T cells, Th0 cells, Th1 cells, and some of B lymphocytes and NK cells [5]. CXCL10 relates to the development of type 1 diabetes in rodent models. The expression of this chemokine in pancreatic beta cells increases with the severity of insulitis in NOD mice [6]. Islet-specific expression of CXCL10 accelerated diabetes development [7]. Elevated serum levels of CXCL10 are detected in the patients with recent-onset type 1 diabetes [8]. CXCR3, a receptor of CXCL10 in addition to CXCL9 and CXCL11, was also implicated in the development of type 1 diabetes. In CXCR3-deficient mice, the onset of type 1 diabetes is substantially delayed [9]. CXCR3 was expressed on the T

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