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研究課題名（和文）腸管出血性大腸菌感染症に対する再生医療を応用した治療法とワクチンの開発

研究課題名（英文）Effects of Multilineage-differentiating stress-enduring (Muse) cells on brain damages induced by Shiga toxin 2 in vivo and in vitro

研究代表者

藤井　潤（Fujii, Jun）

鳥取大学・医学部・教授

研究者番号：６０２７１４４１

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研究成果の概要（和文）：腸管出血性大腸菌(EHEC)感染症は、急性脳症を引き起こす。本研究費で、世界で初めて、再生医療に用いられているMuse細胞を静注することで、EHEC O111経口感染マウスモデルを救命することができた。そのメカニズムを解析するため、マウスアストロサイト初代培養を用いて、培養上清にベロ毒素2型(Stx2)とLPSを添加することで、アストロサイト(AST)が活性するin vitro系を確立した。AST培養後に、Stx2(10ng/mg)とLPS(1 microg/ml)を加え、GFPでラベルしたMuse細胞とnon-Muse細胞を加えた。その結果Muse細胞は活性化されたASTを有意に抑制した。

研究成果の概要（英文）：Non-Muse cells are normal bone marrow mesenchymal stem cells minus Muse cells. NOD-SCID mice were infected with an injection directly into the stomach, with a bacterial suspension of STEC O111 1010 CFU (100% mortality). On day 2 after inoculation of STEC, bone marrow-derived Muse cells or non-Muse cells (bone marrow-MSCs other than Muse cells) were administered intravenously from the tail vein of the mouse model. An intravenous injection of human Muse cells had a strong effect in reducing the mortality rate ($p<0.01$) in the oral infection mouse model with STEC O111. In vitro experiment, reactivity of GFAP was maximized when 10 ng/ml of Stx2 and 1 µg/ml were exposed for a 12 h period. On greater observation many GFP-Muse cells were had attached to reactive astrocytes and some Muse cells surrounded astrocytes, and it seems that they may have killed the reactive astrocytes. In this microarray analysis, we discovered the factor X that may be involved in the reactive astrocytes.

研究分野：　実験動物学

キーワード：　EHEC　Muse cell　Stx2　CNS

１．研究開始当初の背景

Previously, we developed a mouse model of impaired blood-brain barrier (BBB) function caused by oral inoculation of Shiga toxin 2c (Stx2c)-producing E. coli O157:H− (Fujii J et al. 62. 3447-3453. Infect Immun 1994). In this model, we detected apoptosis associated with activated caspase-3 in motor neurons in the anterior horn of the spinal cord and the reticular formation in the medulla oblongata (Fujii J at al. 8(3):e58959. PLoS One 2013). I was already reported that a combination of Stx1 and lipopolysaccharide (LPS) activated rat astrocytes as reactivate astrocyte (rAST). Multilineage - differentiating stress enduring (Muse) cells are collectable as cells positive for the pluripotent surface marker stage specific embryonic antigen-3 (SSEA-3) (Dezawa M et al, 8(7):1391-415 Nat Protoc 2013). Muse cells are stress-tolerant and secrete pro-survival factors that play a key role in regulating the cell response to DNA damage following internal or external injury and reduce the inflammatory response and subsequent apoptosis. Non-Muse cells are normal bone marrow mesenchymal stem cells minus Muse cells. Muse cells can differentiate into multiple cell types, Recently, Sphingosine-monophosphate (S1P) was reported to release from the damaged tissue and it was recognized as an S1P receptor (S1P-R2) in Muse cells. Phase I clinical tests of myocardial infarction have presently been undertaken in Mitsubishi's Life Science Institute at Gifu University (Dezawa M et al. 122(8):1069-1083 Circ Res 2018).

２．研究の目的

The purpose of this study is that one intravenous injection of Muse cells have a strong impact in reducing the mortality rate and weight loss in the oral infection mouse model with STEC O111. And also, in the vitro study, human Muse cells suppress mouse astrocytes induced by the combination of Stx2 and LPS. Using microarray, immunomodulation of Muse cells is thought to be a mediate factor that is well known as anti- inflammatory and anti-apoptotic effects.

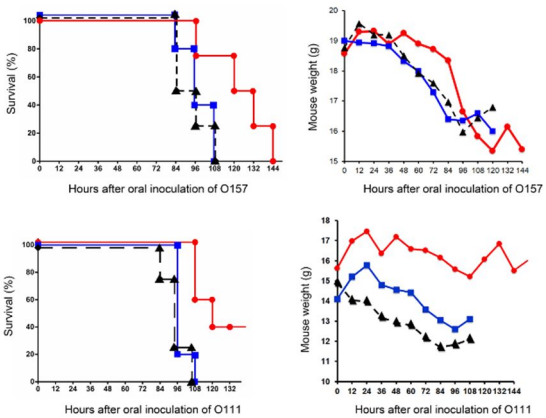
３．研究の方法

Seven week-old male NOD-SCID mice were infected with an injection directly into the stomach, with a bacterial suspension of STEC O157 10^{10} CFU 100% mortality). Also seven week-old male NOD-SCID mice were given an injection with STEC O111 10^{10} CFU (100% mortality) of bacterial suspension. On day 2 after inoculation of STEC, human bone marrow-derived Muse cells or non-Muse cells (bone marrow-MSCs other than Muse cells) were administered intravenously from the tail vein of the mouse model. Immunohistochemistry was performed after inoculation of STEC. An in vitro study was carried out, primary cultured CD57/B6 mouse AST was used by the co-culture of Muse cells dyed with green fluorescent protein (GFP). Dose-and time-dependent experiments, were then performed. GFP-Muse or non-Muse cells ware observed with the reactivated astrocytes for 6 or 12h. Finally, a microarray was performed.

４．研究成果

An intravenous injection of human Muse cells (50,000) had a strong effect in reducing the mortality rate ($p<0.01$) and weight loss ($p<0.001$) in the oral infection mouse model with STEC O111. In the O111 infected model, only astrocytes reacted with pons of the brain and there was no apoptosis, which may suggest that Muse treatment is more effective against O111 encephalopathy.

Fig. 1) Effectiveness of a single dose of human Muse or non-Muse cells in the EHEC O157 or O111 model (Survival curve and body weight loss)



In vitro experiment, reactivity of GFAP was maximized when 10 ng/ml of Stx2 and 1 μg/ml were exposed for a 12 h period. On greater observation many GFP-Muse cells were had attached to reactive astrocytes and some Muse cells surrounded astrocytes, and it seems that they may have killed the reactive astrocytes.

Fig. 2) Effects of human GFP-Muse or non-Muse cells on activated mASTs combined with Stx2 10ng/mg and LPS 1μg/ml in vitro

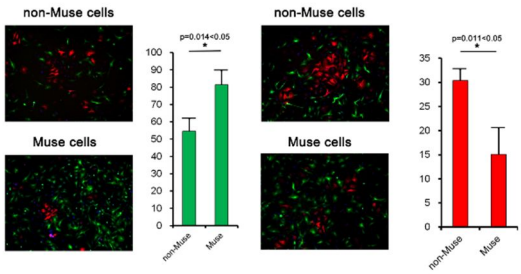
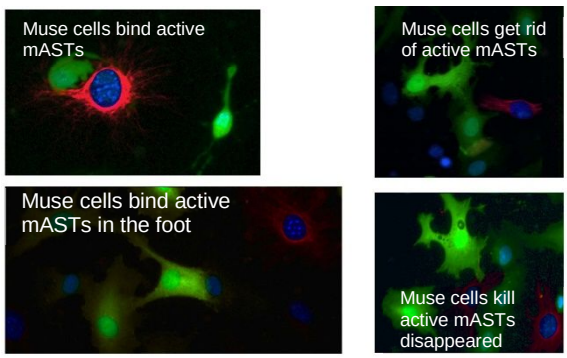
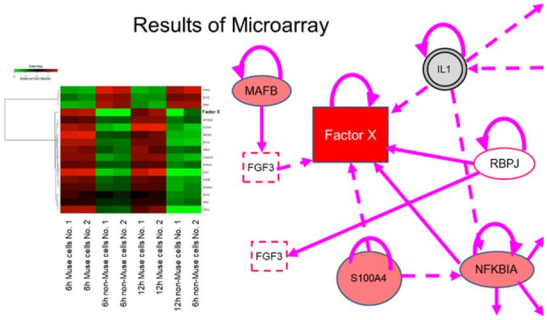


Fig. 3) Effects of human Muse or non-Muse cells on activated mASTs combined with Stx2 10ng/mg and LPS 1μg/ml in vitro



We added Muse or non-Muse cells at the same time as Stx2 and LPS to the culture. After 6 or 12 h, Muse or non-Muse cells and astrocytes were separated with human CD105 microbeads. And then we calculated the ratio of messenger RNA of Muse and non-Muse at each hour using a microarray. In this microarray analysis, we discovered the factor X that may be involved in the control of reactive astrocytes.

Fig. 4) Microarray
Ratio = human Muse / non-Muse cells



５．主な発表論文等（研究代表者、研究分担者及び連携研究者には下線）

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〔学会発表〕（計 4 件）

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〔図書〕（計 1 件）

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〔産業財産権〕

○出願状況（計　0　件）

○取得状況（計　0　件）

〔その他〕
ホームページ等
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６．研究組織
(1)研究代表者
藤井　潤(FUJII Jun)
鳥取大学・医学部・教授
研究者番号 ： 60191441
(2)研究分担者
森元　聡(MORIMOTO Satoshi)
九州大学・薬学研究院・教授
研究者番号：60191045

研究分担者
大原　直也(OHARA Naoya)
岡山大学・大学院医歯薬学総合研究科・教授
研究者番号：70223930

(3)連携研究者
出澤　真理(DEZAWA Mari)
東北大学・大学院医学系研究科細胞組織学分野・教授
研究者番号：50272323

(4)研究協力者
なし