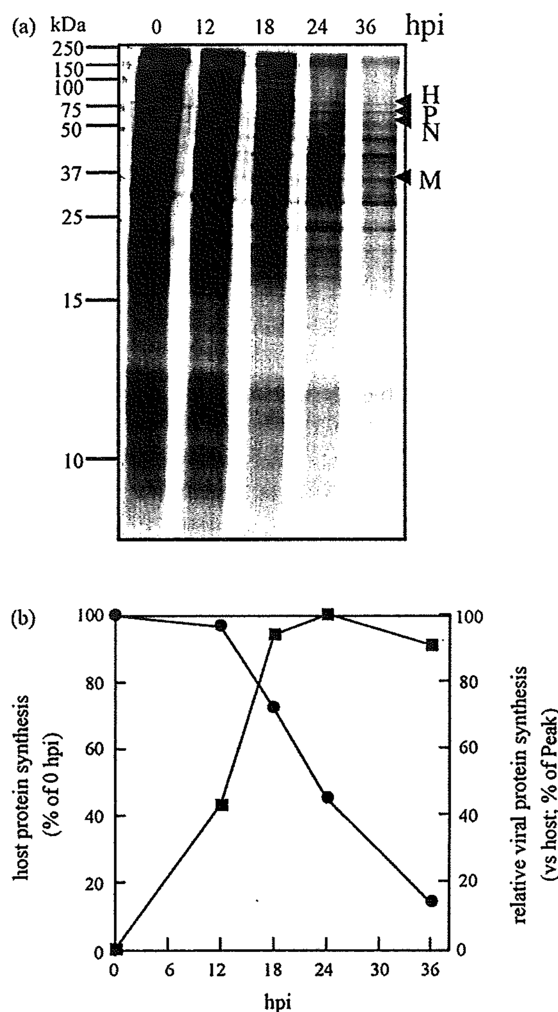

Fig. 1. Protein synthesis in B95a cells infected with MeV-HL. (a) B95a cells infected with MeV-HL were labeled with a mixture of [^{35}S] methionine/cysteine for 1 h and collected at the indicated time. Labeled proteins were separated by 12% SDS-PAGE gel. The proteins derived from MeV-HL are indicated to the right of the image. (b) Quantitation of host protein synthesis (closed circle) and relative viral protein synthesis to host protein synthesis (closed square). The rates of protein synthesis were determined from images as described in the text.

indicate that field isolated MeVs, which maintain their virulence, have an ability to induce shut-off of host protein synthesis, whereas only the MeV-Ed does not.

3.2. *Effect of MeV-HL infection on GAPDH mRNA*

The expression level of the GAPDH gene, one of house keeping genes, was measured as a representative to determine whether MeV-HL-induced inhibition of host protein synthesis occurs at the transcription stage (Fig. 3). The relative expression level of GAPDH

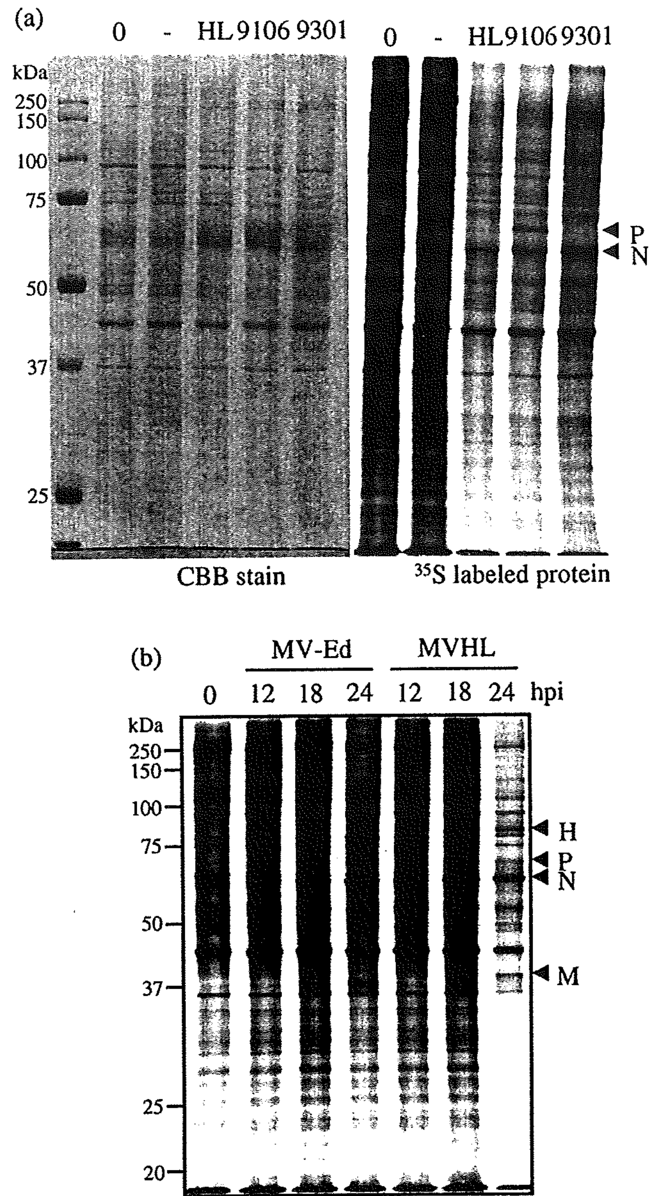

Fig. 2. The shut-off effect of other strain of MeV (a) Protein synthesis at 24 hpi in B95a cells infected with field isolates of MeV was examined similar to Fig. 1a (right panel) (0: 0 hpi; - :mock-infected B95a cells). To confirm the equivalence of protein, the autoradiograph gel was stained with Coomassie brilliant blue (left panel). (b) The protein synthesis in MeV-Ed- or MeV-HL-infected B95a cells was examined similar to Fig. 1a.

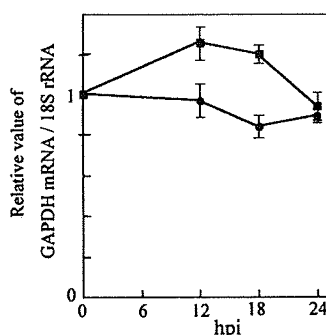

Fig. 3. The mRNA levels of GAPDH in B95a cells infected with MeV-HL. The expression levels of GAPDH mRNA in mock- (closed circle) or MeV-HL- (closed square) infected B95a cells were determined using one-step real-time RT-PCR and shown as means of three experiments.

mRNA to 18S rRNA was not suppressed by MeV-HL infection. Since the transcription level of GAPDH did not decrease during the period of shut-off, the inhibition of host protein synthesis in MeV-HL-infected B95a cells is suggested to occur at a post-transcription stage.

3.3. Modification of eIF4G, eIF4E and 4E-BP1 by MeV-HL infection

Previous reports on other viruses indicated that cap-binding proteins such as eIF4G, eIF4E, and 4E-BP1 are major targets for the virus-induced shut-off of host protein synthesis [6]. Picornavirus (except for cardiovirus) cleaved the eIF4G, which is one of the subunits of cap-binding complex eIF4F, by viral protease, $2A^{pro}$ [15–17]. This cleavage results in inhibition of binding of eIF4F to cap of host mRNA. In adenovirus- or influenza virus-infected cells dephosphorylation of eIF4E, which is a cap-binding protein, is observed [18–20]. Phosphorylation of eIF4E increases its affinity for the cap of mRNA [21]. Therefore, dephosphorylation of eIF4E by viral infection results in decrease of the affinity for the cap and may inhibit cap-dependent translation. The eIF4E is also regulated by eIF4E-binding protein-1 (4E-BP1). Encephalomyocarditis virus (EMCV), poliovirus and VSV dephosphorylate 4E-BP1 [22,23]. Dephosphorylated 4E-BP1 binds to eIF4E strongly, resulting in the suppression of cap-dependent translation. Considering these functions of cap-binding proteins, we first examined the characteristics of these three proteins in B95a cells at intervals after inoculation with MeV-HL. As shown in Fig. 4a, eIF4G was not cleaved throughout the course of MeV-HL-infection. Moreover, dephosphorylation was not observed for eIF4E and 4E-BP1 until 36 hpi (Fig. 4b and c). These results indicate that eIF4G and eIF4E are not involved in MeV-HL-induced shut-off of host protein synthesis as their function appear to be intact.

3.4. Accumulation of phosphorylated eIF2α in MeV-HL-infected B95a cells

Given that the modification of eIF4F was not detected in MeV-HL-infected B95a cells, we then focused on phosphorylation of eIF2α. It was reported that the interferon-inducible

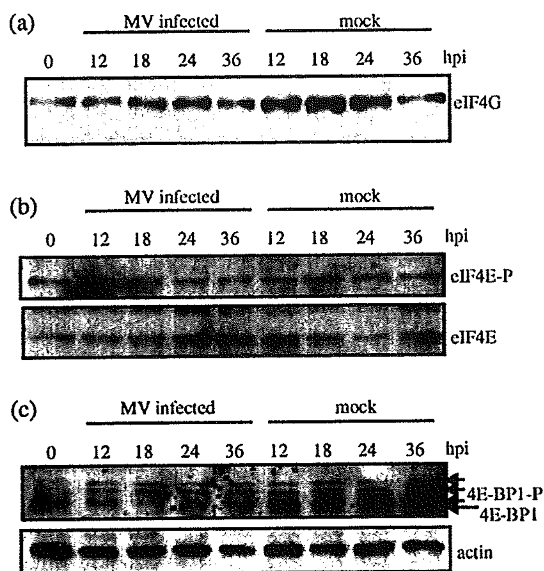

Fig. 4. Modification of the components of the eIF4F complex in MeV-HL-infected B95a cells. Expression levels of eIF4G and the phosphorylated states of eIF4E and 4E-BP1 in mock- or MeV-HL-infected B95a cells were determined by Western blotting assay. (a) Cell lysates were subjected to 6% SDS-PAGE and the proteins were transferred onto nitrocellulose membrane. The eIF4G was detected by Western blotting assay using rabbit antibody against eIF4G. (b) Detection of phosphorylated eIF4E by Western blotting assay using antibodies against phospho-eIF4E at serine 209 (upper panel) or eIF4E (lower panel) antibody. (c) The phosphorylation state of 4E-BP1 in the mock- or MeV-HL-infected B95a cells was examined by Western blotting assay using goat antibody against 4E-BP1. The quantity of protein was normalized to that of β-actin determined by goat antibody against β-actin.

PKR, known as a kinase that phosphorylate eIF2α at serine 51 [24], is activated by dsRNA during the infection with RNA viruses and involved as a host defense in preventing the translation of viral transcripts, concomitantly with the inhibition of host mRNA translation [25]. Considering such function of eIF2α, we analyzed the phosphorylation state of eIF2α by Western blotting assay with an antibody against phospho-eIF2α or eIF2α (Fig. 5). The ratio of phospho-eIF2α in MeV-HL-infected B95a cells increased after 12 hpi and reached a maximum (3.9-fold increase) at 18 hpi, although the effect was lower than that observed in the control with thapsigargin that induces eIF2α phosphorylation through ER-stress [26]. Thereafter, the ratio was sustained until 36 hpi. Phosphorylation of eIF2α occurred at a relatively early stage of infection, prior to the clear inhibition of host protein synthesis. The acceleration of host shut-off was accompanied by an increase in phosphorylation of eIF2α.

3.5. Suppression of MeV-HL-induced phosphorylation of eIF2α in B95a cells stably expressing S51A mutated human eIF2α

Involvement of phosphorylation of eIF2α in shut-off of host protein synthesis in MeV-HL-infected B95a cells was examined using B95a cells that stably express

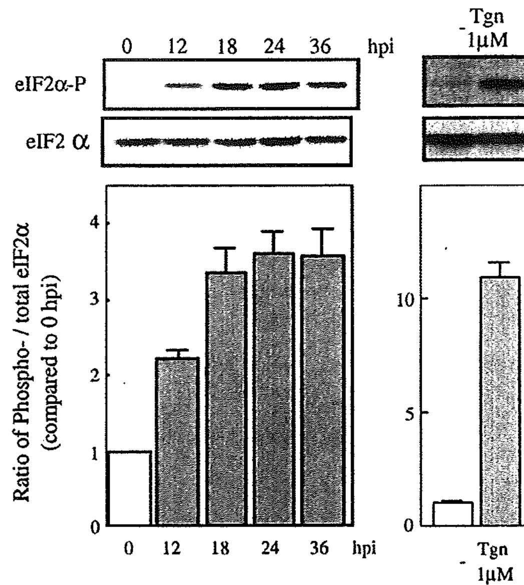

Fig. 5. Phosphorylation of eIF2α in MeV-HL-infected B95a cells. Lysates of mock- or MeV-HL-infected B95a cells were analyzed by Western blotting assay using antibodies against eIF2α-P (phosphorylated form at serine 51) or eIF2α on the same membrane (left, top). Quantitation of the relative amounts of phospho-eIF2α and the total eIF2α was measured and the ratio of phosphorylated eIF2α to total eIF2α (vs. 0 hpi) is shown as a bar graph (left, bottom). As a control experiment, B95a cells were treated with 1 μM thapsigargin for 1 h and shown as the same way as left column (right). The values are means ± standard errors of triplicate determinations.

eIF2α mutant, of which phosphorylation site serine 51, was replaced to alanine (B95a-2αS51A) and is able to inhibit the phosphorylation of endogenous eIF2α [27]. As a control experiment, the B95a cells that stably express wild type of eIF2α (B95a-2αWT) were used. The phosphorylation rate of total eIF2α in B95a-2αWT cells apparently increased at 18 hpi (Fig. 6a), whereas that in B95a-2αS51A cells was significantly inhibited. Shut-off of host protein synthesis was noted from 12 hpi in B95a-2αWT cells similar to the parental B95a cells. In B95a-2αS51A cells, shut-off of host protein synthesis was suppressed until 18 hpi (Fig. 6b and c) and the rate of host protein synthesis was higher than that of B95a-2αWT cells throughout the test period. These results indicate that the phosphorylation of eIF2α involved in shut-off of host protein synthesis in MeV-HL-infected B95a cells.

4. Discussion

In the present study, we showed that MeV-HL induces the shut-off of host protein synthesis in B95a cells. This shut-off is not specific feature of MeV-HL because other field isolates, 9106 and 9301 strain, also induce the shut-off in B95a cells. On the other hand, MeV-Ed that has been reported not to induce the shut-off in epithelial or epithelial-like cells did not induce the shut-off of host protein synthesis in B95a cells as well. Therefore, the inability of MeV-Ed to induce shut-off is suggested to be a characteristic of this strain

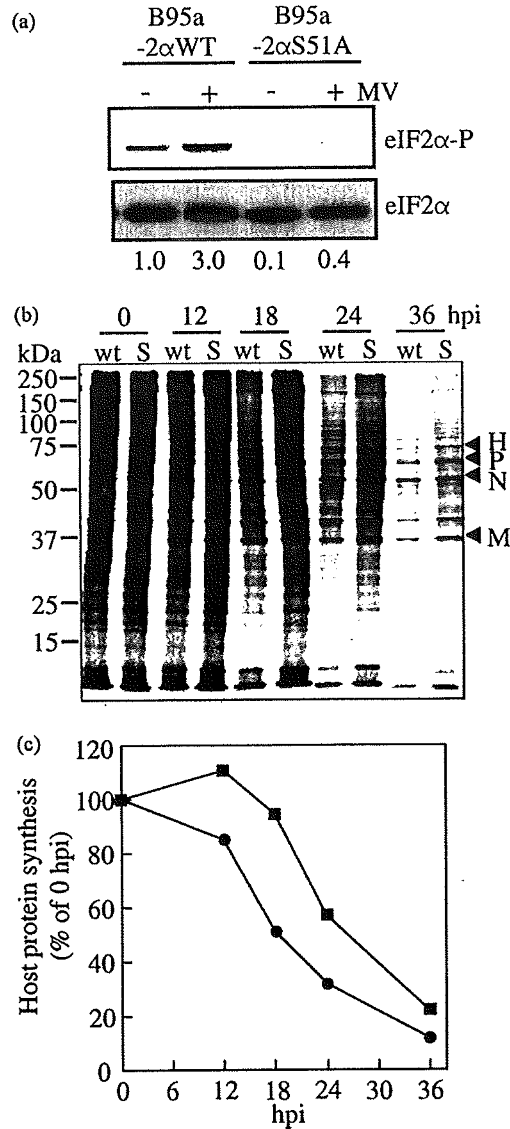

Fig. 6. MeV-HL-induced shut-off in eIF2αWT and S51A expressing cells. (a) eIF2α was detected by Western blotting assay using antibodies against phospho-eIF2α (upper panel), or eIF2α (lower panel) on the same membrane. The ratio of phosphorylated eIF2α (vs. mock infected B95a-2αWT) is shown under each lane. (b) Protein synthesis in MeV-HL-infected B95a-2αWT (wt) and B95a-2αS51A (S) cells was examined similar to Fig. 1a. Viral proteins are indicated to the right of the image. (c) The rates of host protein synthesis in B95a-2αWT cells (closed circle) or B95a-2αS51A (closed square) were determined from Fig. 6b by quantitation similar to Fig. 1b.

and independent of cell type. Similarly to MeV, Smith et al. reported that ability of reovirus to induce the shut-off of host protein synthesis is dependent of the viral strain [28].

The shut-off of host protein synthesis by virus infection was reported to be caused by a number of mechanisms such as inhibition of transcription, degradation of host mRNA and inhibition of translation. As the level of GAPDH mRNA was unaltered in MeV-HL-infected B95a cells, the shut-off by MeV-HL is suggested to be caused by inhibition of translation.

The shut-off of host translation is caused mainly by inhibition of the cap-dependent mechanism [6]. Contrary to many other virus-infected cells in which the components of the eIF4F complex including eIF4G, eIF4E and 4E-BP1 are involved in cap-dependent translation, they were not modified by MeV-HL infection. Therefore, the cap-binding activity of eIF4F complex appears to be intact. Instead, phosphorylation of eIF2α in MeV-HL-infected B95a cells was noted (Fig. 5). The phosphorylation rate of eIF2α correlated with the inhibition of host protein synthesis after MV infection. In addition, in B95a-2αS51A cells that stably expressed the eIF2α-S51A mutant, the shut-off phenomenon appeared to be suppressed compared with those in B95a and B95a-2αWT cells (Fig. 6). Therefore, phosphorylation of eIF2α is suggested as one of the mechanisms particularly at the early stage for the induction of host shut-off by MeV-HL infection.

Conner and Lyles reported that phosphorylation of eIF2α in VSV-infected cells suppressed viral translation rather than host translation [22]. In the case of MeV-HL infection, the suppression effect on host proteins was obviously much greater than that on viral proteins (Figs. 1a and 6b). MeV-HL mRNA may be more resistant to the effect of phosphorylated eIF2α than cellular mRNA. The mechanisms of the selective synthesis of viral protein in the shutoff stage of MeV-HL-infected cells are currently under investigation.

Recently, we also reported that the N protein of MeV-HL inhibits host translation by the binding to eIF3-p40 [13]. In our report, the inhibitory effect of the N protein is partial and inhibitory rate reaches a plateau at approximately 50–60%. On the other hand, MeV-HL-infection suppressed about 90% of the host translation (Fig. 1b). Experiment using eIF2αS51A mutant in this study, in which the inhibition of eIF2α phosphorylation observed in18 hpi lasted 24 hpi (data not shown) showed that the shut-off was inhibited at 18 hpi but became partial after 24 hpi (Fig. 6c). The expression level of the N protein increases rapidly after 18 hpi and reaches a peak at 24 hpi (data not shown). Taken together, we hypothesize that in MeV-HL-infected B95a cells the accumulation of phosphorylated eIF2α probably resulting from the replication of viral genome occurs at a relatively early stage of infection initiating the shut-off and then binding of increased N protein binds to eIF3-p40 and enhance the shut-off of host translation at later stage of infection.

Acknowledgement

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変貌する感染症

エボラ出血熱

Ebola hemorrhagic fever

特集

髙田 礼人
TAKADA Ayato

変貌する感染症－人類の備えは十分か？

Key words　フィロウイルス　病原性　自然宿主　伝播経路

エボラウイルスとマールブルグウイルス

エボラ出血熱は，フィロウイルス科に属するエボラウイルスの感染によって引き起こされる(図1)．フィロウイルス科には他にマールブルグウイルスが属しており，これら2つのウイルスはいずれも急性で致死率の高い感染症の病原体である．最初に発見されたのはマールブルグウイルスであり，1967年に実験用にアフリカからドイツ(マールブルグ大学)に輸入されたサルが感染源となり，感染患者からウイルスが分離された．一方，エボラウイルスは1976年にアフリカのコンゴ民主共和国(旧ザイール)で最初に見つかった．現在のところ，マールブルグウイルスは一属一種のみが知られているのに対し，エボラウイルス属は系統学的に4種(ザイール，スーダン，アイボリーコーストおよびレストン)に分類されている[1]．

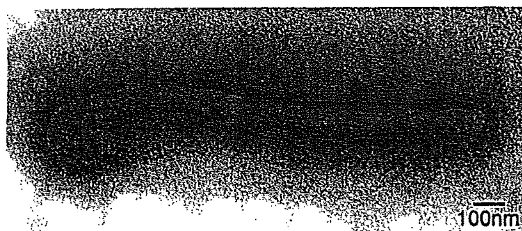

図1　エボラウイルスの電子顕微鏡像
ウイルスは直径約80nmの糸状粒子で，粒子内にマイナス鎖RNAをウイルスゲノムとしてもつ．粒子表面は宿主細胞膜由来の脂質2重膜(エンベロープ)に覆われている．

北海道大学人獣共通感染症リサーチセンター　教授

フィロウイルスは，ヒトを含む霊長類に重篤な出血熱を引き起こし，その致死率はきわめて高い．感染初期には，発熱，悪寒，倦怠，食欲不振，吐き気，下痢，呼吸速拍，喉痛あるいは筋肉痛等の風邪に似た症状を起こす．その後，血液凝固不全に伴う出血傾向に陥り，最終的に痙攣を伴うショック症状を起こし発症後6～9日で死亡する．病原性が強いこと，そして効果的な予防・治療法が実用化されていないことから，エボラウイルスおよびマールブルグウイルスは，Biosafty Level 4(BSL-4)と呼ばれる高度生物学的封じ込め施設で取り扱わなければならない病原体である．

フィロウイルスによる感染症の発生頻度は近年増加している(表1，図2)．例えば，2001～2007年にかけてガボン，コンゴ共和国およびコンゴ民主共和国でザイールエボラウイルス，2005および2007年にはアンゴラおよびウガンダでマールブルグウイルスの感染による出血熱が流行し，多数の死者を出した．さらに，2008年にはウガンダで，恐らく新種のエボラウイルスによる感染症が流行した．一方，表1に示したもののうちいくつかは，

0371-1900/08/¥50/頁/JCLS

表1 エボラおよびマールブルグウイルスによる感染症の流行

ウイルス種	発生年	発生国	感染患者数(%致死率)
マールブルグウイルス	1967	ドイツ，ユーゴスラビア	32(23)
	1975	南アフリカ，ジンバブエ	3(33)
	1980	ケニア	2(50)
	1987	ケニア	1(100)
	1998-2000	コンゴ民主共和国(旧ザイール)	149(83)
	2005	アンゴラ	374(88)
	2007	ウガンダ	4(25)
ザイールエボラウイルス	1976	コンゴ民主共和国	318(88)
	1977	コンゴ民主共和国	1(100)
	1994	ガボン	51(60)
	1995	コンゴ民主共和国	315(79)
	1996	ガボン	37(57)
	1996	ガボン，南アフリカ	61(74)
	2001-2002	ガボン，コンゴ共和国	124(79)
	2002-2003	ガボン，コンゴ共和国	143(90)
	2003	コンゴ共和国	35(83)
	2005	コンゴ共和国	12(75)
	2007	コンゴ民主共和国	264？(71？)[a]
スーダンエボラウイルス	1976	スーダン	284(53)
	1979	スーダン	34(65)
	2001-2002	ウガンダ	423(40)
	2004	スーダン	17(41)
アイボリーコーストエボラウイルス	1994	コートジボアール	1(0)
ウガンダエボラウイルス？	2007-2008	ウガンダ	200？(50？)[a]
レストンエボラウイルス	1989	アメリカ合衆国	4？(0)[b]
	1992	イタリア	0[c]
	1996	アメリカ合衆国，フィリピン	0[c]

[a]正確な数が不明.
[b]発症したのはサルのみ．感染したサルを取り扱った関係者数名の血中抗体の上昇が認められた.
[c]発症したのはサルのみ.

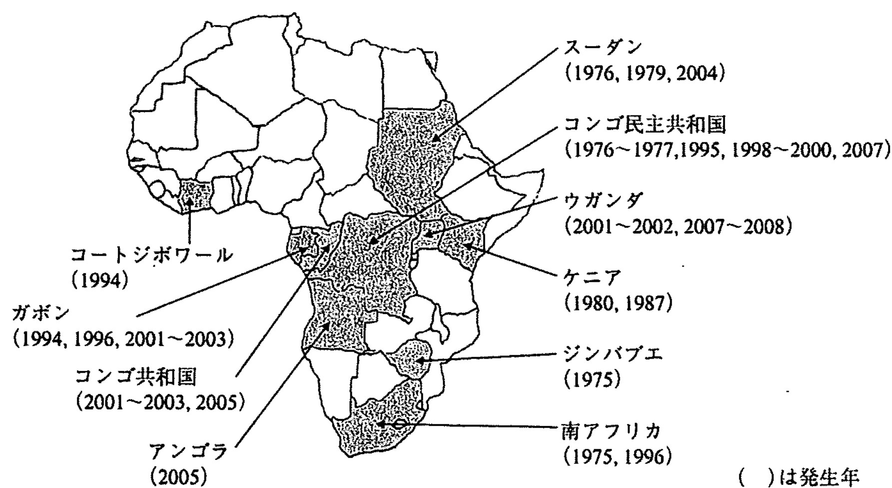

図2 アフリカにおけるエボラまたはマールブルグ出血熱の発生国

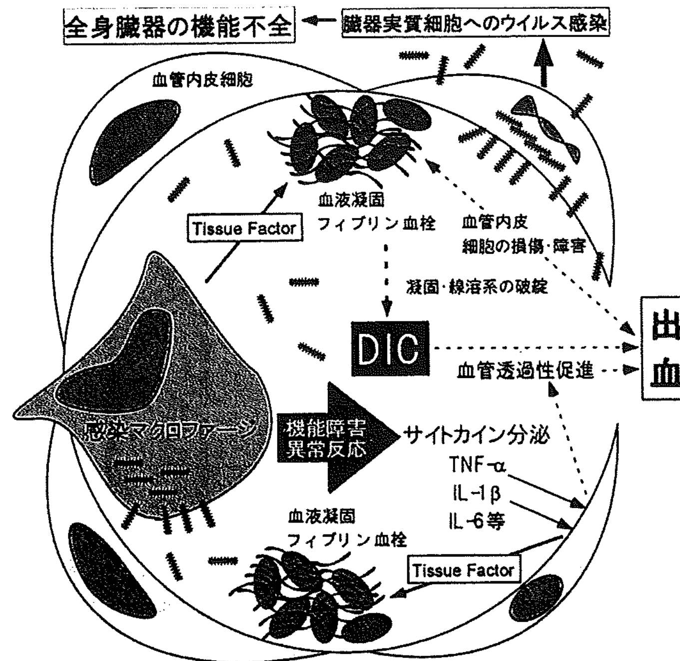

図3 エボラ出血熱の発症メカニズム
(高田礼人：蛋白質核酸酵素 52(10)：1242-1247, 2007, 図3に追加・改変)

フィロウイルス常在地域で感染し，自国に帰国してから発症した事例である．最近の例では，2008年7月にウガンダ旅行からオランダに帰国した女性が，自国でマールブルグ出血熱を発症するという例が報告された．交通機関の発達や国際交流の活発化によって，中央アフリカ諸国以外の国にとって，輸入感染症としてのエボラおよびマールブルグ出血熱の重要性が高まっていると言える．

エボラウイルスの増殖と感染病態

エボラウイルスの体内への侵入経路は，主に粘膜や傷口である．体内に侵入したエボラウイルスの最初の標的細胞は，マクロファージ，単球および樹状細胞等の抗原提示細胞と考えられている[2]．抗原提示細胞には自然免疫系の主要な担い手として，ウイルス感染に対する免疫応答を正常に誘導するための非常に重要な機能がある．しかし，エボラウイルスの感染により，これらの細胞が機能障害あるいは異常反応を起こし，効果的な免疫応答を誘導できなくなることがエボラウイルスの病原性に深く関与していると考えられている[2)~4)]．また，エボラ出血熱感染者において，正常な免疫応答が誘導されることなく死に至る理由の1つは，エボラウイルスの複数の蛋白質が宿主の免疫応答を阻害する機能を持っていることである[2)~4)]．最終的に感染後期には，ウイルスが全身臓器の血管内皮細胞や実質細胞に感染し，それらの機能を破壊するために，多臓器不全に陥っていると考えられる．

エボラウイルスを実験的に接種したサルでは，感染したマクロファージが血液凝固系活性化の引き金であるTissue Factorという蛋白質を過剰に発現する[2)5)]．Tissue Factorが過剰に産生されることによって，さまざまな臓器において末梢血管内での血液凝固反応が起こるので，血液中に循環する血液凝固因子が不足する．その結果，播種性血管内凝固症候群(DIC)となり，出血傾向に陥る(図3)．また，感染したマクロファージから過剰分泌されるTNF-α，IL-1βあるいはIL-6等のサ

イトカインは，非感染マクロファージあるいは血管内皮細胞における Tissue Factor の発現量をさらに増加させうる．同時に，TNF-αは血管内皮細胞の透過性を促進する[6]．ウイルスの感染によって血管内皮細胞が直接障害されることで，血液凝固・線溶系の破綻を引き起こす可能性もある．エボラウイルス感染において，以上のような血液凝固制御系の異常および血管内皮細胞機能障害が「出血熱」という特徴的な症状を引き起こすのであるが，感染した個体の死因は明らかに出血そのものではなく，ウイルスの全身感染および出血を契機とした多臓器不全と考えられる．

エボラ出血熱の疫学とウイルスの自然宿主

エボラ出血熱の発生は散発的で，主にアフリカに限局している（表1）．しかし，フィリピンからアメリカおよびイタリアに輸入された実験用のサルが発症した事例（レストンエボラウイルスが分離された）があることから，アジアにおけるエボラウイルスの存在が示唆される．現在までに，ヒトのエボラ出血熱がアジアで発生したという報告はないが，未知のエボラウイルスが何らかの野生動物に潜んでいる可能性は否定できない．一般に，エボラウイルスのようにヒトに対して急性で致死率の高い感染症を引き起こすウイルスが自然界で存続するためには，感染しても致死的感染に至らずにウイルスを保有している生物（自然宿主）の存在が必要である．ウイルスは持続的に個々の自然宿主体内あるいは個体群内で維持され，他の動物への感染源となる．

エボラウイルスの自然宿主に関して，これまでにいくつかの報告がある．1999年にフランスのパスツール研究所のグループが，中央アフリカに生息する齧歯類からエボラウイルスの遺伝子が検出されたと発表した[7]．しかし，感染性のある実際のウイルスおよびウイルスに対する抗体の存在が証明されていないので，これらの動物が自然宿主であるという結論には至っていない．現在は，食果コウモリがエボラウイルスの自然宿主として有力視されている．ガボンで研究を行っているフランスの研究グループが，中央アフリカに生息する食果コウモリから，ザイールエボラウイルス遺伝子の一部とウイルス特異的な抗体を検出したと発表したからである[8]．しかし，この報告でも感染性ウイルスが分離されていないため，コウモリがエボラウイルスの自然宿主として他の動物への直接の感染源となっているのか否かは実際には明らかではない．コウモリが偶然に他の動物（真の自然宿主動物）から感染した可能性も否定できないからである．

自然宿主であることを証明するには，長期的・持続的に感染性のあるウイルスが個体あるいは個体群から分離され，他の動物への感染源になっていることを，疫学的に検証する必要がある．現在のところ，自然宿主が保有するエボラウイルスそのものが直接すべての霊長類に強い病原性を示すのか（図**4a**，**b**），霊長類の間で感染を繰り返すうちに変異して病原性を獲得するのか（図**4c**），または感染しても発症しないサルまたはヒトが存在し，キャリアーとなりうるのか（図**4d**），これらはすべて不明のままである．

過去に発生したエボラ出血熱の流行の多くは，感染したサルに接触したヒトが最初の感染源となったと考えられる[9]．例えば，1994年にコート・ディボアール（アイボリーコースト）で発生したエボラ出血熱は，感染・発症したサルを解剖したヒトが感染した．また，2001～2003年にかけてアフリカのガボンとコンゴ共和国でヒトに流行したエボラ出血熱では，ザイールエボラウイルスの感染によって死んだサルに接触し感染したハンターが他のヒトへの感染源になり，ウイルス感染が拡大したことが追跡調査で判明している．この流行の時，中央アフリカのゴリラやチンパンジーにもエボラウイルスが蔓延し，それらの生息数が急激に減少した[10]．野生霊長類におけるエボラウイルス感染の大流行と，近年のヒトでのエボラ出血熱の

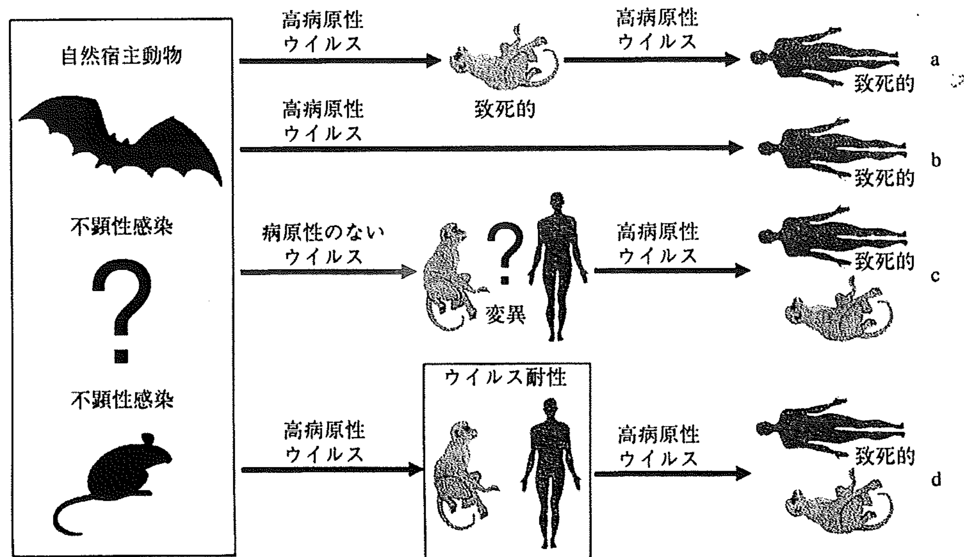

図4 推測されるエボラウイルスの伝播経路

発生頻度の増加は無縁ではないと思われる．

2000～2003年に流行したウイルスの遺伝子解析の結果，数種類のエボラウイルスが同時に流行していたことがわかった[10]．つまり，数種類のウイルスが自然宿主動物から別々にサルに伝播したようである．この事実は，自然宿主個体群内には，未知のエボラウイルスが維持されており，新種のエボラウイルスが今後ヒトに伝播し流行する可能性を示唆している．実際に，2008年にウガンダで流行したエボラウイルスは，これまでに知られていなかった種類である可能性が高い(米国CDCが解析中)．以上に述べたように，エボラウイルスの自然界における存続メカニズムは謎に包まれており，自然宿主となる野生動物を同定し，霊長類およびヒトへの伝播経路を解明することが重要な課題である．

予防・治療法の開発

現在までに，エボラウイルスに対するワクチン開発がさまざまな手法で実験的に試みられている．そのほとんどは，げっ歯類を感染動物モデルとした系では有効であるが，サルでの有効性が認められない．現在，有望なワクチン候補として期待されているのは，アデノウイルスまたはブタ水胞性口炎ウイルスにエボラウイルスの遺伝子の一部を人工的に組込んだ遺伝子組換えウイルスワクチンである[11][12]．これらは，サルを用いた感染防御実験で十分な効果が認められている．しかし，これらの遺伝子組換えウイルスは基本的に生ワクチンであり，ヒトにおける安全性は定かではない．さらに，それぞれのワクチンに一長一短があり，実際に使用できる体制が整うまでにはかなりの時間を要すると思われる．

ワクチン開発に関するもう一つの問題は，エボラ出血熱がいつ，どこで発生するのかを予測できない点である．中央アフリカのエボラウイルス常在地域の住民全員にワクチンを接種するのは現実的には困難である．また，自然宿主動物が確定されていないことから疫学的な発生予測も難しい．したがって，エボラウイルスのワクチンを必要とするのは，流行地域の医療関係者，野生動物管理関係者，エボラウイルス研究従事者，軍人あるいはバイオテロ対策従事者等に限られるだろう．

したがって，エボラ出血熱に対する対策としては，ワクチンを用いた予防的手段だけでなく，治

療法の開発が重要となる．これまでに，血液凝固系の破綻を制御する薬物に治療効果があることが，サルを用いた実験で示されている[13]．前述のように，エボラウイルスに感染したマクロファージは血液凝固を引き起こす細胞因子Tissue Factorを過剰産生する．致死量のエボラウイルスを感染させたサルに，Tissue Factorの活性を阻害する薬物であるnematode anticoagulant protein c2を投与すると約3割が生残した．また，敗血症の治療薬として欧米を始め多くの国で認可されている活性型プロテインCにも治療効果が認められている．致死量のエボラウイルスを接種したサルにこの薬剤を投与すると，一部が生残し，また死亡したサルでも生残時間が明らかに延長した[14]．これらの薬剤の発症抑制効果の詳細なメカニズムは明らかではないが，エボラ出血熱の症状を改善する薬剤発見の意義は今後の治療法の開発にとって大きい．

これまでに，ウイルスに対する抗血清を患者に投与する受動免疫による血清療法も試みられてきたが，その効果は不明である[15]．血清中にはウイルスの感染性を中和する抗体の他に，感染性を増強する抗体が存在するため[16][17]，このような血清で治療を行った場合には，病気を悪化させる可能性も考えられる．一方，ウイルスの感染性を中和するモノクローナル抗体を投与することによって予防・治療効果があることが，マウスおよびモルモットを使った実験で報告されている[15]．しかし，霊長類での感染防御効果は十分に検討されていない．

その他にも，インターフェロン，膜融合阻害ペプチド，ウイルスRNA合成阻害薬などが実験的に試みられているが[2][9]，いずれも単独では十分な効果が認められていない．今後は，数種類の方法を組み合わせて用いる治療法を検討する必要があるかもしれない．

おわりに

エボラウイルスの診断および研究には，BSL-4施設が必要であるが，わが国には稼動しているBSL-4施設が存在しない．先進国の中でBSL-4施設が稼動していないのは日本だけである．したがって，わが国ではエボラウイルスの病原性ならびに感染宿主応答の詳細な解析，さらには効果的なワクチンおよび治療法の開発のための基礎研究ができないだけではなく，エボラ出血熱が疑われる症例の確定診断すらも満足に行えない状態である．

これまでに，エボラ出血熱の発生は世界の限られた地域でしか認められていないが，近年の急激な国際化による人の移動および動植物の輸出入あるいは地球環境の変化によって，ウイルスが他の地域に拡散する可能性が高まっていると考えられる．また，エボラウイルスがバイオテロの手段として用いられる危険性も指摘されている．これらの問題は国際的に認識されており，わが国でも早急に対策を講じる必要がある．

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Antibody therapy as a future treatment option for Ebola virus infection

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Ebola virus causes lethal hemorrhagic fever in human and nonhuman primates. Effective prophylaxis and treatment for this disease are not yet available. Antisera and monoclonal antibodies specific to Ebola virus proteins have been tested for passive immunization in experimental animal models and clinical cases, and shown to be effective in mice and guinea pigs, whereas the evidence of protective efficacy in primates, including humans, remains elusive. In this review, we focus on research relevant to prophylaxis and treatment by passive immunization, and discuss the potential use of antibody therapy for Ebola virus infection. Nevertheless, there is no doubt that a comprehensive understanding of Ebola virus pathogenesis will aid in the development of therapeutic strategies against Ebola hemorrhagic fever.

Keywords: antibody therapy, Ebola virus, monoclonal antibody, passive immunization

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Ebola virus (EBOV) is a nonsegmented, negative-strand RNA virus, and together with Marburg virus, it belongs to the family *Filoviridae* [1]. EBOV causes severe hemorrhagic fever (HF) in human and nonhuman primates, with the highest mortality rates of all the viral HFs, and is correspondingly classified as a biosafety level 4 agent. Ebola HF is characterized by generalized fluid distribution problems, hypotension, coagulation disorders, lymphopenia and hemorrhagic manifestations. The clinical symptoms in humans appear suddenly, several days after infection and death usually occurs 6–9 days after the onset of clinical symptoms [1,2]. Effective prophylaxis and treatment for the disease are currently unavailable.

The EBOV group has four distinct species, and the mortality rates of clinical cases caused by the two major species, Zaire and Sudan, are around 80 and 50%, respectively [1]. By contrast, Reston EBOV has been shown to be less pathogenic than Zaire EBOV in a nonhuman primate model and has not been associated with symptomatic infection in humans [1]. The molecular basis of the extraordinary pathogenicity of EBOV is largely unknown and still needs to be clarified.

Past outbreaks of Ebola HF were sporadic in Central Africa. However, since transmission routes of filoviruses to human and nonhuman primates remain unclear, the potential for the introduction of EBOV into nonendemic countries remains. Although several species of fruit bat are suspected as the natural reservoirs of filoviruses [3], no infectious virus has been isolated from these animals. In addition, EBOV is of public health concern because of its potential use in bioterrorism. Thus, there is an urgent need to develop effective vaccines and drugs against EBOV infection. Here we review the research relevant to prophylaxis and treatment for Ebola hemorrhagic fever, and discuss the potential use of antibody therapy for this disease.

Viral structure & functions of EBOV glycoproteins

EBOV has a nonsegmented, negative-strand RNA genome that is approximately 19 kb in length in its filamentous particle. The genome encodes at least seven structural proteins: nucleoprotein, virion structural protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24 and RNA-dependent RNA polymerase (L). Nucleoprotein, VP30, VP35 and L form the ribonucleoprotein complex with viral RNA. VP40, GP and VP24 are membrane-associated proteins. The GP forms spikes as a unique surface pattern on the virion [1].

The GP gene of EBOV has two overlapping reading frames. From transcriptionally edited GP mRNA, full length transmembrane GP is synthesized, while the unedited mRNA directs synthesis of the nonstructural soluble GP (sGP) that is secreted from EBOV-infected cells [1]. The GP is responsible for receptor binding and fusion of the virus with host cell membranes, and is the sole known target of neutralizing antibodies against EBOV.

Although it has been reported that EBOV GP interacts with several different cellular molecules, the β1 group of integrins, folate receptor α, C-type lectins, and Tyro3 receptor tyrosine kinase family [4–8], it remains elusive whether these molecules are required for both viral attachment and membrane fusion. It has also been demonstrated that EBOV utilizes some

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anti-GP antibodies for its entry into cells through interaction with the cellular Fc receptor or complement component C1q and its ligand, promoting viral attachment to cells [9]. The demonstration of this antibody-dependent enhancement (ADE) of EBOV infection raised fundamental questions regarding the development of GP-based EBOV vaccines and the use of anti-GP antibodies for passive immunization.

In contrast to GP, the functions of sGP are not well known, although potential roles of sGP in the pathogenesis of EBOV have been suggested [1,10]. Importantly, it has been hypothesized that sGP effectively absorbs anti-GP neutralizing antibodies because of its structural similarity to GP and abundant amounts present in the blood. Indeed, it was experimentally shown that sGP reduced the neutralizing activities of anti-GP serum [11]. A similar effect was also suggested for the products of GP ectodomain shedding [12].

Vaccines

Classical approaches to produce vaccines against filoviruses have been generally unsuccessful, especially in nonhuman primate models. For example, immunization with inactivated EBOV induced antibody responses and protected guinea pigs, but was inefficient in nonhuman primates [13], suggesting that B cells may play an important role in the protection of rodents. Recently, viral vector-based strategies were shown to be effective at protecting animals. Adenovirus, vesicular stomatitis virus and paramyxovirus vectors expressing EBOV proteins protected monkeys from lethal EBOV infection [14–16]. It should be noted that these vaccines induced only low, neutralizing antibody responses. Accordingly, the importance of $CD8^+$ T lymphocytes for the protective effects in mouse and monkey models was demonstrated, suggesting that activation of cytotoxic T cells is one of the key mechanisms underlying the protective response [13,17–19]. Since cellular immunity, including a cytotoxic T-cell response, cannot be fully induced by immunization with nonreplicative protein antigens such as inactivated virus vaccines, viral vector-based or DNA vaccines are promising candidates for an Ebola HF vaccine.

Passive immunization with polyclonal antibodies

The presence of neutralizing antibodies against EBOV was demonstrated in the sera of convalescent patients and experimentally infected nonhuman primates [20–22]. In some clinical cases, convalescent human sera were used for treatment protocols together with several drugs (e.g., recombinant human interferon) [23–25]. Even though most of the patients who received convalescent whole blood were treated very late in the course of the disease (7–15 days after onset of symptoms), the treatments seemed to moderate the symptoms and reduce case mortality rates. However, reliable conclusions could not be drawn from these studies owing to the possible effects of the concomitant drugs and to the inevitable lack of a control study. Under experimental conditions, protective efficacies of hyperimmune sera or purified polyclonal antibodies were evaluated in mice [26,27], guinea pigs [27,28], hamadryas baboons [28–30], and cynomolgus monkeys [27,31] (Table 1).

It was shown that the serum from mice subcutaneously infected with live EBOV protected recipient mice from challenge with lethal doses of the virus, although it remains unclear whether virus-induced immune factors other than antibodies (e.g. cytokines) affect the efficacy of such a treatment [26]. A commercially available IgG from hyperimmune horse serum to EBOV antigens was also shown to protect hamadryas baboons in earlier studies [28–30]. However, it did not consistently protect mice, guinea pigs or cynomolgus monkeys, although beneficial effects (reduction of the viral burden) were observed [27,31].

Our previous data indicate that polyclonal sera, including convalescent, hyperimmune and vaccinated sera, represent a mixture of neutralizing, enhancing (ADE), and non-neutralizing, non-enhancing antibodies [22,32,33]. In this regard, it might be possible that the presence of ADE antibodies counterbalances the activity of neutralizing antibodies in the sera [22] and that the use of polyclonal sera might have a limited potential for prophylaxis and treatment of EBOV infection. In addition, neutralizing and ADE antibodies in a polyclonal serum directed to one species of EBOV have little cross-reactivity to the other species [22,33–35]. The repertory of these antibodies may vary depending on the EBOV species, and the characteristics of this array of antibodies depend essentially on their classes and epitopes [9,22]. Thus, to reduce the potential risks and inherent disadvantages in the use of whole polyclonal sera for Ebola HF therapies, approaches using monoclonal antobodies (mAbs) should be more promising.

Table 1. Efficacy of passive immunization with polyclonal antibodies.

Origin of antibody	Treatment	Recipient	Day treatment received	Survival rate (%; survival/total)	Remarks	Ref.
Human (convalescent)	Serum and interferon	Human	3 and 6 after onset of symptoms	100 (1/1)	Moderated viremia?	[23]
	Blood and concomitant drugs	Human	4, 7, 9, 11, 13 or 15 after onset of symptoms	87.5 (7/8)	Moderated viremia?	[24]
Equine (hyperimmune)	Purified IgG	Hamadryas baboon	0 (-2 or 0 h)	67 (2/3) or 100 (2/2), respectively	Prolonged survival	[28,30]
		Hamadryas baboon	0 (-2, 0, 0.5, 1 or 2 h)	Up to 100 (2/2, 10/10, 11/14, 5/5 or 2/7, respectively)*	Prolonged survival	[28,29]
		Cynomolgus monkey	-2, 0, or 0 and 5	0 (0/6), 33 (1/3) or 0 (0/3), respectively	Moderated viremia Prolonged survival	[27,31]
		Mouse (BALB/c)	0	Up to 25 (5/20)*	Prolonged survival	[27]
		Guinea pig	0, 0 and 3 or 4	100 (10/10), 100 (10/10) or 0 (0/5), respectively	Prolonged survival	[27]
Goat (hyperimmune)	Purified IgG	Guinea pig	-3, -2 -1, 0 (4 h), 1, 2, 3, 4 or 5	? (some at low challenge dose)	Larger prophylaxis index	[28]
Mouse (infected)	Serum or purified IgG	Mouse (BALB/c)	-1 or 1	Up to 100 (5/5)*	Moderated viremia, virus replication in surviving mice	[26]

**Survival rates varied depending on the antibody doses, titers and/or times of treatments.*

Passive immunization with mAbs

In recent years, passive transfer of human or humanized mAbs specific to viral proteins has been tested in clinical studies, providing models for the use of mAbs for prophylaxis or treatment of infectious diseases [36,101]. In particular, a humanized mAb specific to respiratory syncytial virus (RSV) F protein is already approved by the US FDA and being used in clinical cases. In this section, we review the studies toward the development of mAb therapies for EBOV infection.

Previous studies reported EBOV GP-specific mAbs, including neutralizing antibodies [20,35,37–40]. Several neutralizing epitopes of mAbs have already been identified on GP molecules [35,39,40]. Passive immunization with mAbs was evaluated in mice [34,35,40], guinea pigs [34,41] and cynomolgus monkeys [42] (Table 2).

In an earlier study [40], GP-specific mAbs were classified into five groups by competitive binding assay, and mAbs belonging to four of the five groups protected mice dose-dependently, when given 1 day before or 1–2 days after virus challenge. However, the extents of protective efficacy were different among the groups and not correlated with *in vitro* neutralizing activities of the mAbs.

On the other hand, we tested two mAbs, 133/16.3 and 226/8.1, which recognized different epitopes [35]. A single treatment at 1 day before or 2 days after virus challenge resulted in complete protection of the animals. We further demonstrated that treatment, even on day 3 or 4 following challenge, was still effective at providing partial protection. Interestingly, the mice treated with mAb 133/3.16 before virus challenge (day: -1) displayed no apparent IgG antibody responses to the virus after challenge, suggesting so-called 'sterile immunity', while all of the surviving mice that had been treated with the same antibody on day 2, 3 or 4 developed serum IgG antibody responses, indicating incomplete neutralization of the challenge virus. However, in a guinea pig model, regardless of the time of treatment, only partial protection was observed, although sterile immunity was suggested in some of the surviving animals [34]. Passive transfer of mAbs specific to Marburg virus GP also gave a partial protection of guinea pigs from challenge with a lethal dose of the virus [43].

A panel of recombinant human mAbs to EBOV antigens was created by phage display libraries constructed from RNA of donors who

Table 2. Efficacy of passive immunization with monoclonal antibody.

Origin of mAb	Recipient	Day treatment received	Survival rate (%; survival/total)	Remarks	Ref.
Mouse	Mouse (BALB/c and C57BL/6)	-1	Up to 100 (10/10)*		[40]
		1	60–100 (6/10–10/10)*		
		2	10–90 (1/10–9/10)*		
Mouse	Mouse (BALB/c)	-1 and 2	Up to 100 (5/5)*	Sterile protection?	[34]
		-1 or 2	75–88 (6/8-7/8)*	Virus replication in surviving mice	
		3 or 4	0–38 (0/8–3/8)*	Virus replication in surviving mice	
	Guinea pig	-1 and 2,	0–33 (1/3)*	Prolonged survival	[34]
		-1	0–67 (2/3)*	Prolonged survival	
		1	0–33 (1/3)*	Prolonged survival	
		2	0–33 (1/3)*	Prolonged survival	
Human (recombinant)	Guinea pig	0 (0 h)	Up to 100 (3/3)*	Moderated viremia	[41]
		0 (-1 h)	100 (5/5)	Moderated viremia	
		0 (1 h)	80 (4/5)	Moderated viremia	
		0 (6 h)	0 (0/5)		
Human (recombinant)	Rhesus macaque	-1 and 4	0 (0/4)	Prolonged survival?	[42]

**Survival rates varied depending on the challenge doses and titers and/or epitopes of mAbs.*
mAb: Monoclonal antibody.

had recovered from EBOV infection [20,38]. From this panel, a recombinant human neutralizing antibody, KZ52, was isolated and its therapeutic potential was tested in guinea pigs [41] and rhesus macaques [42]. In guinea pigs, administration of this antibody protected the animals if given 1 h before or 1 h after infection with a lethal dose of the virus, but not when given 6 h later. However, in rhesus macaques, three of four monkeys treated with this antibody 1 day before and 4 days after virus challenge died in 9–10 days, as was the case with an untreated monkey. The remaining monkey also died at 28 days post-infection. Although these data suggested the limited potential of this neutralizing antibody in protecting monkeys from EBOV infection, it was noted that another mAb, mAb cocktails, or combined treatments with mAbs and other compounds should be evaluated in a primate model [42].

It is noted that the protective potential of neutralizing mAbs, depends on their specific epitopes. Most of the epitopes identified by synthetic peptide mapping localized to a hypervariable region [40] including a so-called mucin-like domain that does not seem to be essential for GP function [7]. While antibodies to this region protected mice [40], their protective efficacy in other animal models has yet to be evaluated. Other epitopes for protective antibodies have been found in different regions of GP [35]. Interestingly, the protective effects seen *in vivo* did not correlate with the *in vitro* neutralizing activity of the antibodies tested [34,40], suggesting different mechanisms of the neutralization by these antibodies. Nevertheless, we assume that antibodies that directly block the essential functions of GP (e.g., receptor binding or membrane fusion) inhibit virus infection more efficiently *in vivo*.

Other targets for anti-Ebola therapies

Potential candidates for anti-Ebola drugs (i.e., inhibitors of viral proteins) have been discussed and tested mainly *in vitro*. The target mechanisms of these inhibitors are membrane fusion, transcription, replication and assembly [2], but none of them were shown to provide sufficient protection or a cure *in vivo*. Instead it seems important to focus on the interaction of EBOV with the immune system.

Monocytes/macrophages and dendritic cells (DCs) were identified as early and sustained targets of EBOV and their importance in