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Reduced Levels of Protein Tyrosine Phosphatase CD45 Protect Mice from the Lethal Effects of Ebola Virus Infection

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SUMMARY

Ebola virus (EBOV) infection of humans is a lethal but accidental dead-end event. Understanding resistance to EBOV in other species may help establish the basis of susceptibility differences among its hosts. Although rodents are resistant to EBOV, a murine-adapted variant is lethal when injected intraperitoneally into mice. We find that mice expressing reduced levels of the tyrosine phosphatase CD45 are protected against EBOV, whereas wild-type, CD45-deficient, or enzymatically inactive CD45-expressing mice succumbed to infection. Protection was dependent on $CD8^+$ T cells and interferon γ. Reduced CD45-expressing mice retained greater control of gene expression and immune cell proliferation following EBOV infection, which contributed to reduced apoptosis, enhanced viral clearance, and increased protection against the virus. Together, these findings suggest that host susceptibility to EBOV is dependent on the delicate balance of immune homeostasis, which, as demonstrated here, can be determined by the levels of a single regulator.

INTRODUCTION

Ebola virus (EBOV), a member of the family *Filoviridae*, is a single-stranded, negative-sense RNA virus that can cause acute hemorrhagic fever and rapid death within 7–14 days following infection in humans and nonhuman primates (Hoenen et al., 2006). There are at least five different species of EBOV—Zaire, Sudan, Ivory Coast, Reston, and the recently identified Bundibugyo virus (Towner et al., 2008)—having different patterns of pathogenicity in nonhuman primates and man (for example, EBOV-Reston causes no disease in man, whereas EBOV-Zaire is highly lethal). Several animal models have been developed to study the pathogenesis of filoviruses, including mouse, guinea pig, hamster and nonhuman primate models (Bowen et al., 1978; Bray et al., 1998; Connolly et al., 1999; Ryabchikova et al., 1996). Adult mice are resistant to naturally occurring isolates of EBOV. However, a mouse-adapted variant of the Ebola Zaire virus is lethal when injected via the intraperitoneal route of infection, but not other routes (Bray et al., 1998). The natural reservoir of EBOV is unknown, but it is clear that the infection of nonhuman primates and humans is an accidental dead-end event and that none of the physiologic abnormalities that occur during its course are the result of evolutionary adaptation of the virus to humans (Warfield et al., 2009). The resistance of other species such as rodents may allow for determination of the differences in the susceptibility between different accidental hosts. The interaction between the virus and immune cells plays a critical role in the pathogenesis of this disease. During the early stage of infection, the virus infects cells of the monocytic lineage, including dendritic cells (DCs), monocytes, and macrophages, whereupon these cells die or become nonfunctional and lose their ability to stimulate antigen-specific T cells (Mohamadzadeh et al., 2007). As the disease progresses, there is increased release of proinflammatory cytokines, lymphocyte apoptosis, and spread of the virus to nonlymphocytic cell types such as hepatocytes (Mohamadzadeh, 2009; Mohamadzadeh et al., 2007). Thus, multiple mechanisms have been shown to contribute to EBOV pathogenesis.

Protein tyrosine phosphatases (PTPs) are critical regulators of signal transduction and include a set of 107 genes within the human genome (Alonso et al., 2004). Among these is CD45, an abundant protein expressed in nucleated cells of hematopoietic origin (Thomas, 1989). The primary role of CD45 is regulation of antigen-receptor signaling of T and B cells via dephosphorylation of the Src family kinases (SFKs) (Alexander, 2000; Hermiston et al., 2003; Thomas and Brown, 1999). CD45 can act both as a positive and negative regulator of SFKs, depending on the nature of the stimulus, the cell type involved, and the developmental stage of the cells (Alexander, 2000; Hermiston et al., 2003; Thomas and Brown, 1999). In addition to its role in regulating intracellular signaling, CD45 is thought to play a role in mediating cell-cell contact by modulating integrin-mediated adhesion (Roach et al., 1997). Thus, CD45 is a pleiotropic mediator of lymphocyte function.

We recently observed that mice expressing intermediate levels of CD45 were protected against the highly virulent Ames strain of *B. anthracis*, a gram-positive spore-forming bacterium (Panchal et al., 2009). To explore the beneficial effects of

Table 1. Genotype of the Transgenic and Heterozygous Mice and Corresponding Percentage of CD45 Expression

Genotype	% CD45 Expression[b]	Text Designation
$KO^{-/-}$	0	$CD45^{0\%}$
$B^{+/-}$	11	$CD45^{11\%}$
$B^{+/+}$	22	$CD45^{22\%}$
$F^{+/-}$	36	$CD45^{36\%}$
$het^{+/-}$	62	$CD45^{62\%}$
$F^{+/-}$ $E^{+/-}$	77	$CD45^{77\%}$
$WT^{+/+}$	100	$CD45^{100\%}$
$CSV10^{+/-a}$	62	$CSV10^{+/-}$
$CSV10^{+/+a}$	100	$CSV10^{+/+}$

E indicates the endogenous allele of normal mice.
[a] Cysteine-to-serine point mutation (C817S) in founder mouse V10 inactivating the CD45 phosphatase activity.
[b] All expression levels represent means with variation of ± 3%.

modulating CD45 levels in other disease models and to investigate whether evolutionarily distinct pathogens share a convergent host pathway that could serve as a common therapeutic target, mice expressing differential levels of CD45 were challenged with EBOV. In this study, we show that reduced CD45 expression levels protect mice from the lethal effects of EBOV infection. Mechanistic studies of mice challenged with EBOV revealed that reduced CD45 expression levels retained the ability to properly regulate early gene expression and inflammation, as well as generated a robust protective immunity response. All of these concerted changes contributed to reduced apoptosis and rapid viral clearance, leading to the survival of the mice.

RESULTS

Mice Expressing Reduced CD45 Levels Survive Ebola Challenge

The pathogenesis of the mouse-adapted EBOV-Zaire infections in mice largely resembles that in guinea pigs and nonhuman primates (Bradfute et al., 2007, 2008; Bray et al., 1998), although subtle differences in coagulopathy have also been observed (Gibb et al., 2001). Therefore, to explore the role of reduced CD45 expression in EBOV pathogenesis, we utilized mice engineered to express a range of CD45 levels (11%, 22%, 36%, 62%, and 77% of the wild-type level) and mice with a point mutation C817S, inactivating CD45 phosphatase activity but expressing 62% ($CSV10^{+/-}$) or 100% ($CSV10^{+/+}$) of the wild-type CD45 protein, were utilized (Table 1). These cell-surface CD45 expression levels were determined by flow cytometry using erythrocyte-depleted blood cells (Figure 1A) and based on comparison of mean fluorescence intensity with wild-type controls. The different mice groups were challenged with > 3000 LD_{50} of mouse-adapted EBOV. Mice expressing 11%–77% CD45 levels were protected from lethal EBOV challenge, with a survival rate of 90%–100%. In contrast, the $CD45^{100\%}$, $CD45^{0\%}$, $CSV10^{+/-}$, and $CSV10^{+/+}$ mice did not survive EBOV challenge (Figure 1B). However, a delay in the mean time to death was observed in $CD45^{0\%}$ and CSV10 (+/− and +/+) mice.

Functional Consequences of Reduced CD45 Expression

To investigate whether reduced CD45 expression affects the entry, replication, or budding of the virus, splenocytes harvested from $CD45^{100\%}$, $CD45^{62\%}$ and $CD45^{0\%}$ mice were infected ex vivo with EBOV, and viral titers were measured by plaque assay. As shown in Figure 1C, similar viral titers were obtained from splenocytes expressing different levels of CD45. Thus, changes in CD45 expression levels had no effect on viral replication.

The control and clearance of EBOV infection has been shown to correlate with effective humoral and T-cell-mediated responses, as mice vaccinated with Ebola virus-like particles (VLPs) require both B cells and $CD8^+$ T cells for protection against mouse-adapted EBOV infection (Warfield et al., 2005). Accordingly, mice that survived EBOV challenge developed EBOV-specific antibodies (Figure 1D), and upon rechallenge with EBOV, a 100% survival rate (20/20) was observed for mice expressing a range of reduced CD45 levels (data not shown). Furthermore, $CD8^+$ T cell responses against defined EBOV, GP, NP, and VP40 epitopes were observed by day 7 post-EBOV infection (data not shown).

Protective Immunity in EBOV-Infected $CD45^{62\%}$ Mice

To understand the role of reduced CD45 expression during EBOV infection, we performed a time course study to monitor functional and cellular changes at both the transcript and protein levels (Figure 2A). Chemo/cytokine analysis of blood plasma indicated that MCP-1, FGF, IFN-γ, IL-4, IL-10, and IL-12 were induced upon viral infection (day 3 and/or 5) in both the $CD45^{62\%}$ and $CD45^{100\%}$ mice (Figure 2B). A significant decrease ($p < 0.05$) in IL-4 levels was observed 3 days after infection in $CD45^{62\%}$ mice versus $CD45^{100\%}$ mice, and the levels of this cytokine were abolished by day 5 in both groups of mice. The levels of the regulatory cytokine IL-10 at day 3 were higher in $CD45^{100\%}$ compared to $CD45^{62\%}$ and completely abrogated by day 5 postinfection in $CD45^{100\%}$ mice. Thus, these data suggested that the initiation of optimal immunity in $CD45^{62\%}$ mice was achieved by regulated expression of IL-10 upon EBOV infection, which otherwise can suppress ongoing antiviral immunity (Couper et al., 2008).

Moreover, staining of splenocytes showed a significant increase ($p < 0.05$) in the percentage of $CD11b^+$ $CD11c^-$ macrophages and $Ly6G^+$ granulocytes at 5 days after EBOV challenge in $CD45^{62\%}$ versus $CD45^{100\%}$ mice (Figure 2C). In blood, the percentage of activated $CD8^+$ $CD44^{high+}$ T cells increased dramatically ($p < 0.05$) at 5 days after challenge in $CD45^{62\%}$ mice when compared to $CD45^{100\%}$ mice (Figure 2D). The $CD45^{62\%}$ mice had constitutively elevated percentages of activated T cells, and these increased levels were maintained through day 5 in both spleen and blood. The time course study suggested that the immune responses in $CD45^{62\%}$ mice were altered following EBOV infection, resulting in an increased percentage of macrophages and granulocytes. Collectively, these data suggested that reduced CD45 levels might heighten the homeostasis of immune cells in favor of protective immunity during filoviral infection.

Viral Clearance and Reduced Apoptosis in EBOV-Infected $CD45^{62\%}$ Mice

EBOV clearance is controlled by functional innate and adaptive immune responses (Mohamadzadeh et al., 2007). Accordingly,