Clin Exp Immunol 2002; **128**:163–168

Inflammatory responses in Ebola virus-infected patients

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(Accepted for publication 20 December 2001)

SUMMARY

Ebola virus subtype Zaire (Ebo-Z) induces acute haemorrhagic fever and a 60–80% mortality rate in humans. Inflammatory responses were monitored in victims and survivors of Ebo-Z haemorrhagic fever during two recent outbreaks in Gabon. Survivors were characterized by a transient release in plasma of interleukin-1β (IL-1β), IL-6, tumour necrosis factor-α (TNFα), macrophage inflammatory protein-1α (MIP-1α) and MIP-1β early in the disease, followed by circulation of IL-1 receptor antagonist (IL-1RA) and soluble receptors for TNFα (sTNF-R) and IL-6 (sIL-6R) towards the end of the symptomatic phase and after recovery. Fatal infection was associated with moderate levels of TNFα and IL-6, and high levels of IL-10, IL-1RA and sTNF-R, in the days before death, while IL-1β was not detected and MIP-1α and MIP-1β concentrations were similar to those of endemic controls. Simultaneous massive activation of monocytes/macrophages, the main target of Ebo-Z, was suggested in fatal infection by elevated neopterin levels. Thus, presence of IL-1β and of elevated concentrations of IL-6 in plasma during the symptomatic phase can be used as markers of non-fatal infection, while release of IL-10 and of high levels of neopterin and IL-1RA in plasma as soon as a few days after the disease onset is indicative of a fatal outcome. In conclusion, recovery from Ebo-Z infection is associated with early and well-regulated inflammatory responses, which may be crucial in controlling viral replication and inducing specific immunity. In contrast, defective inflammatory responses and massive monocyte/macrophage activation were associated with fatal outcome.

Keywords haemorrhagic fever innate immunity human monocyte IL-10

INTRODUCTION

Several outbreaks of Ebola haemorrhagic fever occurred recently in Central Africa, causing severe disease and a 60–80% mortality rate in the Democratic Republic of Congo (DRC) (Kikwit, 1995, 316 cases) [1] and Gabon (Mekouka, late 1994, 49 cases; Mayibout, early 1996, 37 cases, and Booué, late 1996, 60 cases) [2]. Ebola virus, a non-segmented negative-stranded RNA virus of the *Filovirus* genus, comprises four subtypes [3]. Ebo-Z, which was initially isolated in 1976 in Zaire (DRC) [4], is the most pathogenic for humans and non-human primates, and caused the epidemics in DRC and Gabon [3–5]. During the two Gabonese outbreaks studied here, in Mayibout and Booué, Ebola disease was characterized by an onset 4–7 days after exposure to infected biological fluids, and by non-specific symptoms such as high fever, asthenia, abdominal pain, myalgia, arthralgia, diarrhoea and vomiting. Haemorrhagic signs including melaena, epistaxis, gingivorrhagia, petechiae, conjunctivitis and spontaneous bleeding, subsequently occurred in some patients, most of whom died 5–9 days after clinical onset [2].

The major cellular target of Ebola virus is the monocyte/macrophage lineage [6], but infection of endothelial cells occurs in the final stages of the disease [7]. Viral membrane-associated glycoprotein (GP) can bind to endothelial cells and induce endothelial cell death and vascular permeability [8], which suggests a major pathogenic role of GP. Adaptation of Ebo-Z to mice and guinea pigs is accompanied by increasing pathogenicity during serial passage, but not by changes in the GP gene [9]. In guinea pigs, adaptation leads to a disappearance of granulomatous inflammation of the liver [10]. We recently reported the existence of asymptomatic Ebo-Z infection; some close contacts of patients who were effectively infected by Ebo-Z never developed symptoms or antigenemia. These asymptomatic infections were characterized by transiently high levels of IL-1β, IL-6, TNFα, the β chemokine macrophage chemotactic protein-1, MIP-1α, and MIP-1β in plasma about one week after the first potentially infectious contact, followed two weeks later by the emergence of Ebo-

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Z-specific IgG [11]. In another study, we described humoral and T-cell responses in symptomatic patients, and showed that recovery from Ebola disease during these outbreaks was associated with early and vigorous humoral responses directed mainly against the 110 kD nucleoprotein (NP), and the 40 kD and 35 kD viral proteins. Moreover, cytotoxic cell activation was observed among peripheral blood mononuclear cells (PBMC) of these patients at the end of the disease. In contrast, patients who died were characterized by defective humoral responses and early T-cell activation, followed by extensive intravascular apoptosis of T cells [12]. Inflammatory processes are key elements of innate and specific immune responses, and the prompt release of proinflammatory cytokines in patients with asymptomatic Ebo-Z infection suggests that this response may be involved in the control of viral replication and in the induction of specific immunity. Some data concerning inflammatory responses in Ebola virus-infected patients from Kikwit are available but are devoted mainly to fatally infected patients [13]. In order to define the role of inflammatory responses in the control of Ebola virus infection in survivors, and the possible involvement of inflammatory mediators in the pathophysiology in fatalities, we examined some parameters of the inflammatory response in plasma samples serially obtained from fatally and non-fatally infected patients.

PATIENTS AND METHODS

Patients and outbreaks

Specimens were obtained during two Ebola outbreaks [5]. The first epidemic (February 1996) occurred in Mayibout, an isolated village on the Ivindo river in dense tropical rainforest (north-east Gabon). The population has little access to health care. The patients were hospitalized in the nearest town, Makokou, seven hours by pirogue up the Ivindo river. Eighteen of the 20 primary cases had been in contact with a single chimpanzee found dead in the forest, presumably from Ebola infection, which they dismembered, cooked and ate. As we could not determine the source of infection in the other two primary cases, we excluded them from the study. Ten secondary cases and one tertiary case (infected by human–human contact) were observed but were not included in this study. The seven primary cases studied during this outbreak were thus, presumably infected by the same Ebo-Z strain and at the same point in time. A distinct epidemic occurred 6 months later in the town of Booué, 300 km from Mayibout. The primary case was a hunter from the surrounding rainforest. Two secondary cases were infected after contact with this patient, and the epidemic (57 other cases) resulted from subsequent human–human transmission within the community (between four and eight human–human transmissions of the virus before infection of the 10 patients included in this study). Except for the primary case, all patients reported a history of direct contact with the patients' biological fluids (blood, sweat, urine or faeces). Patients were hospitalized at Booué Medical Center where they received symptomatic treatments (paracetamol, nifuroxazide, ampicillin, Ringer-lactate perfusion). Mortality was similar in the two epidemics (66% in Mayibout and 75% in Booué) and was stable throughout the Booué outbreak, which lasted 5 months. Twelve healthy individuals, who lived in the same region but had had no contact with infected patients, served as uninfected controls. Eight survivors (three from Mayibout and five from Booué) and nine patients who died (four from Mayibout and five from Booué) were included in this study, although only two or three plasma samples were available from some patients (see legends).

Biological samples

Several blood samples were taken with the patients' verbal informed consent during the course of the disease and during recovery, and were transported immediately by air, on ice, to the International Center for Medical Research of Franceville (CIRMF) (less than 6 h after sampling). Plasma was separated and stored at –80°C until use. Ebo-Z infection was confirmed in patients with fulminating haemorrhagic fever by detection of circulating viral antigens (reagents kindly provided by the Centers for Disease Control (CDC), Atlanta, GA, USA) and viral RNA in PBMC by reverse transcription-polymerase chain reaction (RT-PCR) [14].

Detection of circulating viral antigen and specific IgG in plasma of Ebola-infected patients

Ebola virus circulating antigen and virus-specific IgG were detected using reagents kindly provided by the Special Pathogen Branch of the CDC. Briefly, 'maxisorp' plates (Nunc, Denmark) were coated overnight with a cocktail of seven monoclonal antibodies against Ebola Zaïre, or with a normal mouse ascitic fluid; several dilutions of each plasma were incubated on each coating (1 h, 37°C), followed by rabbit anti-Ebola Zaïre polyclonal serum (1 h, 37°C) and peroxidase (HRP)-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories, KPL, MD, USA) (1 h, 37°C). TMB substrate (KPL) was used and results are expressed as antigen titre. Specific IgG was detected by coating plates overnight with Ebola-Zaïre antigen; several dilutions of each plasma were incubated for 1 h at 37°C, followed by incubation with HRP-conjugated anti-human IgG (γ-specific) (Sigma, st Quentin Fallavier, France) for 1 h at 37°C. Results are expressed as IgG titre.

ELISA

IL-1β, TNFα, alpha-interferon (IFNα), interleukin-12 (IL-12), MIP-1α, MIP-1β, IL-1RA, TNFα soluble receptor-1 (sTNF-RI) and TNFα soluble receptor-2 (sTNF-RII) were assayed in plasma from Ebola virus-infected subjects and endemic controls using commercial kits from R & D Systems (R & D, Abingdon, UK). IL-6, and soluble receptors for IL-6 (sIL-6R), interleukin-8 (IL-8) and neopterin, were also measured by commercial kits (Immunotech, Marseille, France). Plasma IL-1β and TNFα levels were confirmed using antibody pairs and recombinant cytokines (Pharmingen, San Diego, CA, USA). IL-10 was quantified using two different commercial kits (Immunotech and Amersham, Les Ulis, France). Serum amyloid A (SAA) was detected with kits from Biosource (Camarillo, CA, USA). Finally, cortisol was quantified using a VIDAS® automat (Biomérieux, France).

Nitric oxide (NO) assay

NO_2^-, a stable metabolite of NO, was measured in the plasma of Ebola virus-infected subjects with a commercial colorimetric assay (Boehringer Mannheim, Germany) according to the manufacturer's instructions. Briefly, plasma was diluted twice in potassium phosphate buffer, loaded on an ultrafilter with a cut-off of 10 000 kD (Macrosep, Filtron, Northborough, MA, USA) and centrifuged for 45 min at 2000 ***g***. The ultrafiltrate was collected and tested according to the manufacturer's instructions. The detection limit of the assay was 0·3 μM. All assays were carried out on plasma.

Statistical analysis

Student's *t*-test was used to analyse differences between datasets for the assays. Statistical data were obtained using Excel software (Microsoft Corporation, Redmond, USA).

RESULTS

Description of patients

The nine fatalities and eight survivors included in this study presented a similar sex ratio (1·1 and 0·9, respectively) and mean age (23·2 ± 15·6 years and 23·1 ± 11·1 years, respectively). As the time of infection of patients was not precisely known for the Booué outbreak, the incubation period has been calculated only for the Mayibout epidemic; it was 7·8 ± 0·9 days and 8·4 ± 1·3 days for fatalities (n = 12) and survivors (n = 5), respectively. Finally, the duration of the symptomatic phase (during both outbreaks) was similar between fatalities and survivors (7·5 ± 2·5 days and 9 ± 3·1 days, respectively). As the incubation period was determined only for some patients, we decided to separate fatalities into four groups according to the time after onset of symptoms (1 ± 1 day, 4 ± 1 days, 6 ± 1 days and 8 ± 0·5 days, corresponding to 8 ± 1 days, 5 ± 1 days, 3 ± 1 days and 1 ± 0·5 day before death). Survivors were divided into two groups during the symptomatic phase (1–4 days and 5–8 days after onset of symptoms) and two groups during recovery (2–4 days and 6–10 days after disappearance of symptoms).

Detection of viral antigen, specific IgG and mediators involved in the inflammatory response in plasma of Ebola-infected patients

Circulating antigen titres were similar between fatalities and survivors during the first days of the disease (Table 1). Afterwards, circulating antigen rose until death in fatalities, while a diminution, then a disappearance of antigen load coincident with recovery, were observed in survivors (data previously reported in [12]). While specific IgG were never detected in fatally-infected patients, high levels of Ebola virus-specific IgG were found in survivors as soon as the symptoms appeared (Table 1) (data previously reported in [12]). IL-1β was never detected in patients who died, whereas moderate but significant levels of TNFα and IL-6 were found in the days before death (Table 1). In survivors, concentrations of IL-1β and IL-6 were significantly more elevated during the symptomatic phase of the disease compared with fatalities and controls, and weak levels of TNFα were also detected. After clinical recovery, these cytokines disappeared from the circulation. IL-1RA, sTNF-RI and sTNF-RII release in plasma increased significantly during the last 5 days before death in fatalities. Increasing levels of IL-1RA, sIL-6R, sTNF-RI and sTNF-RII were also observed during the symptomatic phase in survivors (then declined during recovery), but concentrations of IL-1RA and sTNFRs were significantly lower than values observed in fatalities. IFNα, IL-12 and IL-8 were undetectable in all the plasma samples tested (not shown). Plasma MIP-1α and MIP-1β

Table 1. Inflammatory and anti-inflammatory mediators in plasma of Ebola virus-infected patients

		Fatalities (days after onset [*days before death*])				Survivors			
						Symptoms (days after onset)		Recovery (days after symptoms)	
	Controls (n = 12)	1 ± 1 (*8 ± 1*)	4 ± 1 (*5 ± 1*)	6 ± 1 (*3 ± 1*)	8 ± 0·5 (*1 ± 0·5*)	1–4	5–8	2–4	6–10
Antigen titres	0	1728 ± 800*	3070 ± 850†	4096 ± 1000†	4096 ± 1000†	1600 ± 800*	250 ± 225*	2 ± 0·9	0
IgG titres	<100	<100	<100	<100	<100	1900 ± 1000†	4300 ± 1100†	>6400*	>6400*
IL-1β	<10	<10	<10	<10	<10	28·3 ± 12·2†	15·2 ± 10†	<10	<10
TNFα	<20	<20	40 ± 20	130 ± 75*	164 ± 130*	70 ± 26*	56 ± 28	<20	<20
IL-6	<10	20 ± 20	100 ± 10*	140 ± 50*	190 ± 100*	480 ± 180†	490 ± 190†	80 ± 80	<20
IL-1RA	1·6 ± 0·3	1·4 ± 1·4	8·6 ± 3·7†	10·3 ± 5·1†	13·5 ± 3·6†	2·5 ± 0·4	3·8 ± 1·6*	1·5 ± 0·6	0·5 ± 0·1
sTNF-RI	1·8 ± 0·4	1·8 ± 0·5	7·3 ± 3·5*	14·9 ± 5·8†	18·1 ± 9†	2·5 ± 0·3	6·3 ± 3·3*	2·3 ± 0·8	2·1 ± 0·5
sTNF-RII	5 ± 0·8	6·3 ± 3·5	19·5 ± 8·6*	157 ± 5·5*	22 ± 6·9*	10·3 ± 0·6*	16·3 ± 5·9*	9·9 ± 2·4*	7·6 ± 2·3
SIL-6R	20 ± 3·4	41 ± 25	53·5 ± 30	38 ± 12·5	43·5 ± 22	19·7 ± 2	47 ± 23·1	51·8 ± 22·8	31·25 ± 5·3
MIP-1α	0·2 ± 0·1	0·2 ± 0·2	0·4 ± 0·4	0·1 ± 0·1	0·4 ± 0·3	0·6 ± 0·2	0·6 ± 0·2	0·3 ± 0·2	0·1 ± 0·1
MIP-1β	0·9 ± 0·4	0·8 ± 0·6	1 ± 0·8	0·5 ± 0·5	0·9 ± 0·3	3 ± 1·6†	1·7 ± 1·2†	0·5 ± 0·3	0·1 ± 0·1
Neopterin	2·2 ± 0·4	17 ± 7	70 ± 35†	100 ± 57†	137 ± 22†	10 ± 1·7*	28 ± 12*	14·8 ± 3*	10·5 ± 5
SAA	<5	180 ± 50*	600 ± 250*	350 ± 125*	64 ± 35	307 ± 81*	592 ± 150*	83 ± 43*	26 ± 6
NO_2^-	20 ± 12	73 ± 35	77 ± 29	60 ± 27	160 ± 60*	73 ± 40	130 ± 85*	136 ± 91*	27 ± 4
Cortisol	92 ± 44	102 ± 40	301 ± 138*	325 ± 100*	392 ± 29*	292 ± 39*	446 ± 149*	208 ± 66*	83 ± 18
IL-10	<0·02	0·05 ± 0·01	1·02 ± 0·54†	0·9 ± 0·32†	1·18 ± 0·63†	0·07 ± 0·06	<0·02	<0·02	<0·02

Results are expressed in ng/ml, except for circulating viral antigen and Ebola virus-specific IgG in titres (last dilution of plasma positive for Ebola antigen or specific IgG), IL-1β, TNFα, and IL-6 in pg/ml and NO_2^- in μM, as mean ± s.d. of individual values according to the time after disease onset among fatalities (1 ± 1 days, n = 3; 4 ± 1 days, n = 7; 6 ± 1 days, n = 9; 8 ± 0·5 days, n = 8) and the symptomatic phase (1–4 days after disease onset, n = 7; 5–8 days after, n = 8) and recovery among survivors (2–4 days after symptoms ended, n = 6; 6–10 days after, n = 5). The time before death (8 ± 1 days; 5 ± 1 days; 3 ± 1 days; 1 ± 0·5 days) is shown in italics for fatalities. Mean ± s.d. of individual values from 12 endemic controls is also given. Significant differences (P < 0·05) between levels observed in a group of patients and controls are indicated by * and between levels measured in one group of patients and those observed in the other group and controls, by †.

levels were, respectively, moderately and significantly increased in survivors at the beginning of the symptomatic phase and then fell below the detection limit of the assays. In contrast, MIP-1α and MIP-1β levels in patients who died were similar to those observed in controls. Low but significant levels of neopterin were found in survivors during the symptomatic phase. In fatalities, neopterin plasma concentrations increased markedly during the 5 days before death, and were significantly higher than those measured in survivors. Elevated levels of SAA were detected during the disease course in survivors and patients who died. Interestingly, SAA plasma concentrations returned to baseline levels during recovery and were greatly reduced on the last day before death. Finally, high but inconsistent levels of NO were found in survivors and in patients who died, at the end of the disease course and also in the early recovery phase. In both survivors and patients who died, increasing concentrations of cortisol were seen during the symptomatic phase, falling during the recovery phase. Finally, IL-10 was barely detectable in survivors, while high and significant levels were found in all patients who died.

Potential markers for prognosis in Ebola virus-infected patients

Host response parameters, which were significantly different between the two groups of patients (Table 1), could be used as potential markers for prognosis in Ebola virus-infected patients. Thus, detection of specific IgG during the disease is clearly indicative of a non-fatal outcome. High levels of IL-6 and, particularly, detection of IL-1β in the plasma of patients during the symptomatic phase could represent markers of non-fatal Ebola virus infection. Elevated plasma concentrations of IL-1RA or neopterin from a few days after disease onset are potential markers for fatal outcome, while high levels of sTNF-RI are only significant the last days before death. Finally, detection of IL-10 in plasma represents a powerful marker for prognosis of fatal infection, as this cytokine was not significantly detected in plasma from survivors.

DISCUSSION

We compared inflammatory responses in survivors and victims of Ebo-Z infection. Significant levels of IL-1β, TNFα, IL-6, MIP-1α and MIP-1β were detected in plasma during the symptomatic phase of the disease in survivors. These results, together with the high levels of proinflammatory cytokines detected 4–7 days after infection in asymptomatic Ebo-Z-infected subjects [11], suggest that proinflammatory cytokine release may be triggered shortly after infection in survivors. Moderate levels of TNFα and IL-6 have also been reported during the symptomatic phase of Ebola disease among survivors in Kikwit [13]. These results point to inflammatory cell activation a few days after viral infection in survivors. The weak but significant rise in neopterin plasma levels observed during the symptomatic phase points to monocyte/macrophage activation [15] and suggests that these cells, which are the main target of Ebola virus [16], are probably involved in the inflammatory process. Thus, non-fatal Ebo-Z infection is associated with early inflammatory responses.

This early response may be important for the control of viral spread. An important role for type I interferons has recently been suggested in the mouse model of Ebola virus infection [17]. The presence of IFNα in the plasma of some Ebola virus-infected survivors from Kikwit has been described [13], but we were not able to detect this cytokine in our samples. In addition, proinflammatory cytokines are known to directly inhibit replication of many viruses, to activate the cytotoxic functions of macrophages and to facilitate antibody-dependent cell-mediated cytoxicity [18]. These cytokines are also involved in the induction of specific immune responses by providing co-stimulatory signals for naive T cells [19], and by contributing to the induction of humoral responses [20], while the release of MIP-1α and MIP-1β may have a role in the chemotaxis of T cells [21]. In keeping with this view, high levels of specific IgG were detected as soon as the onset of the disease in survivors [12,22] and were mainly directed against NP, VP40 and VP35 [12]. This strong and early humoral response is probably involved in the control of Ebola virus infection, as observed in animal models [23,24]. We have also shown that cytotoxic T cells appeared in the blood during clinical recovery [12]. In addition to the humoral response, induction of cellular immunity is probably very important for protection [25–27]. An inflammatory response was followed by the release of IL-1RA, sTNF-R and sIL-6R during the symptomatic phase and early convalescence in survivors. IL-1RA and sTNF-RI are antagonistic towards the biological effects of IL-1β and TNFα, respectively, and down-regulate inflammatory responses [28,29]. In contrast, sTNF-RII and sIL-6R potentiate the effect of their respective ligands by slowly releasing them [30,31]; this may have occurred in the survivors, given their elevated sTNF-RII:sTNF-RI ratio and the increasing concentration of sIL-6R observed during the symptomatic phase.

In contrast, IL-1β, IL-6, MIP-1α and MIP-1β were barely detectable in patients who died, and TNFα was released in plasma only during the days preceding death. Similarly, weak levels of TNFα and IL-6 have been observed in fatally-infected patients in Kikwit [13], while histological studies of fatally-infected humans and monkeys have shown a lack of inflammatory cells in infected tissues [32,33]. Furthermore, the disappearance of inflammatory responses has been observed during adaptation, which increases pathogenicity and mortality, in Ebo-Z-infected guinea pigs [10]. The high plasma levels of anti-inflammatory molecules found in the patients who died may be involved in the lack of early inflammatory responses. Although glucocorticoids (GC) suppress IL-1β, TNFα and IL-6 production [34], the inflammatory responses observed during the symptomatic phase in survivors, despite similar cortisol plasma concentrations, suggest that these elevated levels were related to acute infection. More importantly, high plasma levels of IL-10 were associated with fatal outcome, an observation previously reported in fatally-infected patients in Kikwit [13]. IL-10 is mainly produced by monocytes/macrophages and T cells; it inhibits both proinflammatory cytokine release by inflammatory cells [35] and activation of T-helper lymphocytes either by a direct effect [36] or by suppressing activation of antigen-presenting cells [37]. Furthermore, IL-10 inhibits the microbicidal activity of macrophages [35]. Thus, the lack of proinflammatory cytokine release in fatally-infected patients may be related to the elevated IL-10 concentrations found during the symptomatic phase. Interestingly, these defective inflammatory responses were associated with impaired T-cell activation and a lack of specific IgG production in patients who died (Table 1) [12].

The increasing and very high neopterin plasma levels observed during the symptomatic phase suggest that monocytes/macrophages are strongly activated during fatal Ebola infection. Neopterin is mainly secreted by activated monocytes/macrophages in response to IFNγ [15], which is in keeping

with the elevated IFNγ concentrations found in fatally-infected patients [12,13]. The increasing concentrations of TNFα, IL-6, sTNF-R, IL-1RA and IL-10 observed in the final stages of the disease were probably produced by these activated monocytes/macrophages. The latter are primary targets of Ebola virus and are abundantly infected during the last days before death in humans and monkeys [33,38]. Furthermore, *in vitro* infection of monocytes/macrophages by Ebo-Z or Marburg virus induces cell activation and TNFα release [6,39]. Together, these data suggest that the increasing activation of monocytes/macrophages detected during fatal Ebola disease results from their viral infection, although the high IFNγ plasma levels observed in these patients were probably also involved. This strong activation of monocytes/macrophages may be involved in the massive apoptosis of T lymphocytes, which occurs during fatal infection [12], via expression and/or secretion of molecules such as TNFα or Fas-ligand. Indeed, lymphocyte apoptosis is associated with massive macrophage infection in the lymph nodes of Ebo-Z or Marburg virus-infected monkeys [40].

A role for TNFα in the pathogenesis of Ebola haemorrhagic fever has been suggested by results obtained with experimental models [41]. However, TNFα plasma levels observed in the last days before death (150 ± 70 pg/ml) were lower than those detected 1 week after exposure to Ebo-Z in asymptomatic subjects (700 pg/ml) [11] and those in septic patients (>1500 pg/ml) [42]. In addition, IL-1β and IL-12, both of which are important mediators of cytokine-induced shock, were below the detection limit of the assays used here in patients who died. Finally, the plasma concentrations of IL-1RA and sTNF-RI, known to inhibit the biological activity of IL-1β [28] and TNFα [30], were markedly elevated during the last days before death. Together, these results suggest that terminal shock and death from Ebo-Z infection are unlikely to be due to excessive release of inflammatory cytokines alone, as in septic patients. Nevertheless, TNFα may be involved in the pathogenesis of Ebola virus infection by increasing endothelial permeability [6].

This study shows that survival from Ebola haemorrhagic fever is associated with early and well-regulated inflammatory responses. This, together with the prompt release of proinflammatory cytokines reported in asymptomatic Ebola virus-infected subjects, suggests that innate responses are crucial for controlling the infection. In addition, these results suggest that the presence of specific IgG, IL-1β, and of high levels of IL-6 in plasma during the symptomatic phase indicates a non-fatal infection. In sharp contrast, fatally-infected patients were characterized by a lack of an early inflammatory response; massive infection of monocytes/macrophages seemed to induce the release of anti-inflammatory products (which probably contributed to the suppression of inflammatory responses) and also of mediators potentially involved in the marked pathological changes observed in the final stages. Neopterin, IL-10, IL-1RA and, to a lesser extent, sTNF-RI are potential markers for the prognosis of fatal outcome when detected in plasma from a few days after symptom onset. The involvement of early viral load in the outcome is unclear, as we were not able to determine viral titres in the plasma and had no information about the viral load during the incubation period. However, this lack of innate response was unlikely to be related to viral replication, as early inflammatory responses were detected in survivors despite a similar level of antigenemia at the beginning of the symptomatic phase (Table 1) [12,22]. Furthermore, no mutations were observed in the nucleotide sequences of the GP, NP, VP40 and 24 kD viral protein (VP24) genes between survivors and patients who died [43]. Some viral proteins, such as soluble glycoprotein-1 (which potentially plays a pathogenic role) and glycoprotein-2 (which contains an immunosuppressive sequence) [44], could have an important role in the outcome of a defective inflammatory response. Further investigations are necessary to identify the early events leading to the two forms of immune response and to the outcome in Ebo-Z-infected patients.

ACKNOWLEDGEMENTS

We thank C. Tevi-Benissan and P. Tshipamba for their assistance with fieldwork, A. Pendy, B. Pambo and P. Obiang for access to patient specimens, and R. Nabias for the Vidas® assay. We are also grateful to S. P. Fisher-Hoch and J. B. McCormick for helpful discussions, and P. E. Rollin and T. G. Ksiazek for providing Ebola-specific reagents. Finally, we thank D. Young for checking the English. CIRMF is supported by the Republic of Gabon, ELF-GABON, and the French Ministry of Foreign Affairs. SB was supported by a grant from the Ministère de la Recherche et de l'Enseignement Supérieur (France).

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