

PAPERS AND ORIGINALS

A case of Ebola virus infection

R T D EMOND, BRANDON EVANS, E T W BOWEN, G LLOYD

British Medical Journal, 1977, 2, 541-544

Summary

In November 1976 an investigator at the Microbiological Research Establishment accidentally inoculated himself while processing material from patients in Africa who had been suffering from a haemorrhagic fever of unknown cause. He developed an illness closely resembling Marburg disease, and a virus was isolated from his blood that resembled Marburg virus but was distinct serologically. The course of the illness was mild and may have been modified by treatment with human interferon and convalescent serum. Convalescence was protracted; there was evidence of bone-marrow depression and virus was excreted in low titre for some weeks. Recovery was complete. Infection was contained by barrier-nursing techniques using a negative-pressure plastic isolator and infection did not spread to attendant staff or to the community.

Introduction

In the late summer of 1967 a serious outbreak of an unknown infectious disease occurred in Germany and Yugoslavia. It affected 31 people, seven of whom died. A strange new RNA virus was isolated from the patients, and the source of the outbreak was traced to vervet monkeys (*Cercopithecus aethiops*) imported from Uganda. Since many of the cases were centred on the West German town of Marburg, the disease was designated Marburg disease.¹ The original outbreak subsided and no further cases were recognised until 1975, when a young man was

admitted to hospital in South Africa having recently travelled extensively in Rhodesia. This patient was found to have Marburg disease and infection spread to his travelling companion and to a nurse. The original patient died but the other two survived. The source of the infection was not determined.²

Just over a year later, in July to November 1976, a serious outbreak of haemorrhagic fever occurred in the Western Equatoria province of the Sudan and the adjacent Equateur Region of Zaire.³ Infection spread rapidly among the local people, particularly within the hospitals. There was an appallingly high death rate—30-80% in the Sudan⁴ and 89% in Zaire. In view of the severity of this outbreak specimens were sent to high-security laboratories in England, Belgium, and the United States of America for identification of the agent responsible. All three laboratories isolated a virus that resembled Marburg virus morphologically but was serologically distinct.⁵⁻⁷ The name Ebola was given to the prototype strain.

Case report

On the 5 November 1976 one of the investigators at the Microbiological Research Establishment, Porton Down, accidentally pricked his thumb through a protective rubber glove while transferring homogenised liver from a guinea-pig infected with this new virus. According to standard safety protocol he immediately removed the glove and immersed his thumb in hypochlorite solution then squeezed it vigorously. There was no bleeding and careful examination with a hand lens failed to reveal a puncture wound. He was kept under surveillance, and on the sixth day became ill.

CLINICAL COURSE

Shortly after midnight on 11 November his temperature rose to 37.4°C. During the early morning he complained of central abdominal pain and nausea. He did not vomit or have the headache or myalgia that had been a feature in other cases. Later that day he was seen at the Microbiological Research Establishment, where a blood sample was taken before he was transferred to the high-security infectious diseases unit at Coppetts Wood Hospital and placed in a Trexler negative-pressure plastic isolator.⁸

When he was admitted he felt physically exhausted and complained of anorexia, nausea, and constant central abdominal pain. There were no other symptoms. His temperature was 38°C with a relative bradycardia. He was alert and did not seem to be particularly ill.

Department of Infectious Diseases, Royal Free Hospital, London

R T D EMOND, FRCP, DTM&H, consultant physician
BRANDON EVANS, MB, MRCP, senior registrar

Microbiological Research Establishment, Porton, Salisbury, Wiltshire

E T W BOWEN, FIMLT, head of special pathogens unit
G LLOYD, MSC, member of special pathogens unit

Apart from slight abdominal tenderness there were no other abnormal findings. In view of the hazards to laboratory staff it was considered unwise to undertake haematological or biochemical studies until the results of the virological tests were known. Since it appeared highly probable that the illness was due to this virulent Marburg-like virus, treatment was started that same evening with human interferon, which had been prepared by stimulating peripheral lymphocytes with Sendai virus *in vitro*.⁹ Interferon was given by intramuscular injection in a dose of 3 million units every 12 hours for 14 days.

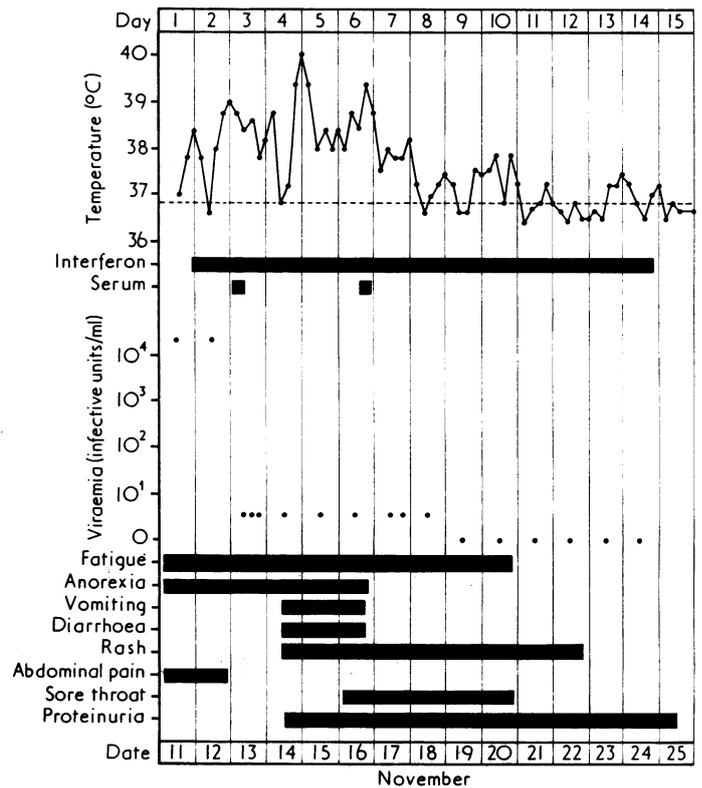
The next morning his temperature was normal and he was free from symptoms, but later in the evening his temperature rose again to 39°C. Apart from loss of appetite there were no other symptoms. By this time direct electron microscopy had shown Marburg-like virus particles in the patient's blood. In view of this finding it was thought advisable to give the patient convalescent serum. Since the new virus was serologically distinct from the original Marburg virus it was necessary to obtain the serum from people convalescing after the recent African outbreak. 450 ml serum obtained from Zaire was heated at 60°C for one hour to inactivate virus and tested for hepatitis B surface antigen and antibody (HBsAg and HBsAb). The serum was given by slow intravenous infusion over a period of four hours from 1.30 am on 13 November. Blood samples were taken at frequent intervals to ascertain virus and antibody levels.

On 13 November the patient had no appetite, but was otherwise free from symptoms. Examination showed an inflamed throat, but exudate was not present. Some small lymph nodes were palpable in the neck and axillae, though these were not tender. A few erythematous maculopapular lesions were noted on his back over the shoulders. The muscles were not tender. The cardiovascular system, respiratory system, and abdomen were normal. Urine was free from protein and output was satisfactory.

During the early morning of the fourth day of illness, 14 November, his temperature fell to normal after a profuse bout of sweating. At this stage he still felt relatively well and the only change was an extension of the rash over the chest wall. About midday he had a sudden violent bout of shivering followed by a sharp rise in temperature to 40°C. This was accompanied by nausea, retching, and a single episode of vomiting. Since admission he had been constipated, but at this point he had a loose bowel action. His mental state began to change and over the next 24 hours there was striking deterioration in concentration and memory. Protein was detected in his urine for the first time and persisted thereafter until the fever subsided. Over the next 72 hours, when the illness was at its height, there was severe malaise and extreme weakness. Profuse watery diarrhoea developed and continued for two days accompanied by persistent vomiting. The rash spread to all parts of his body and ultimately became confluent. There was no bleeding into the skin or mucous membranes. The throat remained inflamed and a few small patches of thrush were detected. The abdomen was slightly distended, but there was no tenderness or guarding. He was mildly dehydrated and the urinary output was falling. Metoclopramide was prescribed for the vomiting and Lomotil for the diarrhoea.

On the sixth day of illness, 16 November, a further 330 ml of convalescent serum from the Sudan, pretreated in the same manner, was infused and followed by Hartmann's solution to correct the dehydration. Next day his urinary output fell to its lowest volume of 830 ml despite adequate fluid replacement and a satisfactory blood pressure. At this stage his appetite began to return, but swallowing induced pain in the throat and behind the sternum. Examination showed extensive candidiasis. Diarrhoea and vomiting had become less frequent and ceased on the 18 November. The thrush responded to treatment with amphotericin B lozenges and the dysphagia settled within a few days. The erythematous stage of the rash began to fade on the 19 November, disclosing a petechial element over the limbs. On the same day he complained of stiffness of the small joints of his hands and to a lesser extent of the wrists and knees.

After 20 November his general condition improved. His fever subsided to a low level, his energy began to return, and there was dramatic improvement in his interest and ability to concentrate, though he could barely recollect the acute phase of his illness. The joint symptoms did not persist. The temperature returned to normal on 22 November but there was a further slight flicker of fever on the next two days, after which the temperature remained normal (see figure). Output of urine was normal by 23 November. At this stage it was decided to take specimens for clearance tests at weekly intervals and it was arbitrarily agreed that three negative sets of cultures from throat swab, blood, urine, and faeces would be an acceptable standard for discharging the patient from isolation. The discovery of virus in semen was not thought to justify further isolation, especially as the patient fully appreciated the implications. Subsequently he made an uneventful but slow recovery over 10 weeks.



Clinical course of disease.

At the end of the acute stage of the illness he had lost a considerable amount of weight, which he regained slowly during convalescence. The rate of growth of hair slowed during the acute illness and during convalescence there was considerable loss of hair from his scalp. There were no other clinical complications. Electrocardiograms taken during the acute stage between days 5 and 9 were normal, though the amplitudes of the T-waves were lower than in a recording made on 27 January during convalescence. Blood urea, and sugar concentrations and liver function were normal during convalescence. The HBsAg and HBsAb tests on blood were negative. The result of a chest radiograph was normal. During the early period of convalescence the haemoglobin level and the white blood cell counts were depressed and did not recover fully until 8 February 1977, three months after the onset of illness (table I).

TABLE I—Results of haematological and biochemical investigations

| | 11 Jan | 8 Feb | 15 Feb |
|--|--------|-------|--------|
| Haemoglobin (g/dl) | 11.1 | 13.2 | 13.2 |
| Packed cell volume (%) | 36 | 40 | 38 |
| Mean cell haemoglobin concentration (g/dl) | 31 | 33 | 34 |
| White blood count ($\times 10^9/l$) | 3.6 | 4.525 | 4.275 |
| Platelets ($\times 10^9/l$) | 203 | 190 | |
| Serum aspartate aminotransferase (IU/l) | <10 | | <10 |
| Serum alanine aminotransferase (IU/l) | <10 | | <10 |
| Alkaline phosphatase (IU/l) | 35 | | |
| Total proteins (g/l) | 75 | 72.7 | 72.9 |
| Urea (mmol/l) | 4.37 | | |
| Sugar (mmol/l) | 5.58 | | |

Conversion: SI to traditional units—Urea: 1 mmol/l \approx 6 mg/100 ml. Sugar: 1 mmol/l \approx 18 mg/100 ml.

The Trexler negative-pressure plastic isolator and the techniques used for disposing of waste proved to be effective in preventing spread of Ebola virus from the patient to attendant staff and to the general community. Of the 24 nurses who were directly concerned in the care of the patient, six became ill with acute respiratory infections, which lasted on average two days. Four of the five doctors looking after the patient developed a 'flu-like illness with some gastrointestinal symptoms. At onset these illnesses caused concern but the problems

invariably resolved within two or three days and antibody studies later showed no evidence of Ebola virus infection among either medical or nursing staff.

VIROLOGICAL INVESTIGATIONS

The first specimen of blood was collected about 14 hours after the patient became feverish; this was six days after the accident. This blood specimen was examined by electron microscopy and virus particles were seen which were similar to those of Ebola virus. Guinea-pigs inoculated with this blood specimen developed a febrile illness and electronmicroscope examination of their blood and tissues showed particles which were again similar to those of Ebola virus. These observations are consistent with an infection due to Ebola virus.

Virus isolations and serological studies were also made on specimens of blood collected daily during the acute phase of the illness and on blood, urine, faeces, throat swabs, and seminal fluid collected during the convalescent phase. The highest levels of virus in the blood ($10^{4.5}$ guinea-pig infective units/ml) were recorded on the first and second day of the illness. After the start of interferon treatment and serotherapy, the level dropped dramatically to 3-10 guinea-pig infective units/ml and remained at this level until the viraemia disappeared on the ninth day of illness (table II).

No virus was isolated from faeces, urine, and throat swabs collected between days 14 and 27. Ebola virus was, however, isolated from specimens of seminal fluid collected on days 39 and 61 but not on days 76, 92, and 110.

After the infusion of 450 ml of convalescent serum (fluorescent antibody titre of 1/128-1/256) on day 2 circulating antibody levels of 1/16 were recorded in the patient's blood from days 3 to 9. This increased to 1/32 on day 10 and gradually increased to a fluorescent antibody titre of 1/128 by day 34. The patient was then subjected to plasmapheresis between 16 and 25 February 1977. A total of seven units of plasma was taken, which resulted in the fluorescent antibody level dropping from 1/128 to 1/32, and a specimen of blood collected on 5 May 1977 had a fluorescent antibody titre of 1/16.

Discussion

The nature of the accident and the absence of a visible puncture mark emphasise the invasiveness of Ebola virus and the high susceptibility of man. Although the new Ebola virus is serologically distinct from the original Marburg virus, the pattern of illness in our patient closely followed the course of Marburg disease as described in Germany and South Africa. The course and duration of the illness were similar and a characteristic clinical syndrome was produced by the exanthem, excessive fatigue, and considerable gastrointestinal disturbance. There were, however, some minor differences, notably the absence of headache and myalgia, which were prominent in Marburg and Johannesburg. The rash emerged after the standard prodromal period and had the morbilliform appearance described in the previous outbreaks of Marburg disease. The evolution of the rash differed from measles in that the lesions appeared first over

the back and not on the head and neck. A painful throat was not a feature of the early stage but developed later when there was frank evidence of candidiasis.

While the course of the illness was milder than expected from reports elsewhere, the pattern and duration of symptoms were not modified. The relatively mild course of the illness and the absence of haemorrhage might have been determined by treatment with interferon and convalescent serum, but the value of these preparations could not be accurately assessed from experience with one patient. Treatment was started with interferon 20 hours after the onset of illness and convalescent serum was first given 47 hours after onset. There was no obvious clinical improvement after treatment, but there was a striking fall in the level of circulating virus. On the first day of illness a blood sample was found to contain $10^{4.5}$ guinea-pig infective units/ml; on the day after starting treatment with interferon there was no change in the amount of virus, but on the next day, after infusion of serum, the level in the blood dropped to $10^{0.5}$ guinea-pig infective units/ml. Since there is known to be a time lag before interferon produces an effect on virus levels it is not possible to assess the relative effectiveness of the two preparations in clearing the blood. Subsequently virus was detected in low titre in the bloodstream throughout the acute stage of the illness but disappeared on the 9th day of illness, before the temperature had returned to normal (see figure). The second infusion of serum had no effect on the amount of virus. The antibody levels achieved in the patient's blood after infusion were consistent with the dilution of the convalescent serum (table II). The oliguria and proteinuria present at the height of the illness could have been attributed to deposition of immune complexes in the kidney, especially in view of the transient arthralgia at the end of the acute stage, but these features were recorded in severe cases during the original Marburg outbreak, when no serum was given.

Treatment of the convalescent serum to ensure safety presented serious problems. Marburg virus has been shown to persist in the body for several months after the acute illness, though it has not been shown in the circulating blood. Marburg virus is relatively resistant to heat but is inactivated in serum maintained at 60°C for 60 minutes.¹⁰ The Ebola convalescent serum was therefore treated at this temperature for 60 minutes to ensure safety. The serum was also tested for HBsAg and HBsAb because carriers are common in many parts of tropical Africa. During convalescence the patient's blood was found to be negative for HBsAg and HBsAb.

Blood examination during convalescence showed evidence of bone-marrow depression with a low haemoglobin concentration and low white blood cell count. These features were shown during the original outbreak of Marburg disease and were attributed to the activity of the virus. Interferon also causes bone-marrow depression affecting the stem cells of the granulocytes¹¹⁻¹³ and synthesis of haemoglobin.¹⁴ Furthermore, interferon causes immunodepression¹⁵ and may have contributed to the severity of

TABLE II—Results of virological investigations throughout course of illness

| Day of sample (from onset of illness) | Details and remarks | Activity of circulating antibody (Fluorescent antibody titre) | Recovery of infective virus (guinea-pig intraperitoneal infective units/ml or g of sample tested) | |
|--|--|--|--|---|
| | | | Positive | Negative |
| 1 | | | Blood, $10^{4.5}$ | |
| 2 | Before transfusion of 450 ml convalescent plasma | <1/2 | Blood, $10^{4.5}$ | |
| 3 | 11 am, 6 pm, 11 pm | 1/16 | Blood, 3-10 | |
| 4 | Morning | 1/16 | Blood, 3-10 | |
| 5 | Morning | 1/16 | Blood, 3-10 | |
| 6 | Before transfusion of 330 ml convalescent plasma | 1/16 | Blood, 3-10 | |
| 7 | Morning and afternoon | 1/16 | Blood, 3-10 | |
| 8 | Morning | 1/8 | Blood, 3-10 | |
| 9 | Morning | 1/16 | | Blood |
| 10, 11, 12, 13 | Morning | 1/32 | | Blood |
| 14, 16, 20 | Morning | 1/64 | | Blood, faeces, urine, throat swab |
| 23, 27 | Morning | | | Blood, faeces, urine, throat swab |
| 34 | Morning | Not done | | Blood |
| 39 | Morning | 1/128 | Seminal fluid, 3-10 | |
| 61 | Morning | 1/128 | Seminal fluid, 3-10 | |
| 76 | Morning | 1/128 | | Blood, urine |
| 92, 110 | Morning | Not done | | Blood, urine, seminal fluid Urine, seminal fluid |

the thrush in our patient. Liver function tests during convalescence showed no evidence of liver damage.

In the early stage of the illness facilities were not available for conducting haematological or biochemical studies safely, so efforts were concentrated on establishing the virological diagnosis; in the late stage of the illness, when provision had been made for routine tests,¹⁶ they were not required for the management of the patient, though they proved useful for assessing the extent of damage during convalescence. Fortunately there was no bleeding and the use of prophylactic heparin was not considered to be necessary.

Once the haemoglobin and white blood cell levels had returned to normal plasmapheresis was performed to obtain a supply of convalescent serum.

We thank Professor K Cantell for supplying the interferon and Professor A J Zuckerman for advising on its use; the World Health Organisation team in the Southern Sudan and the International Commission team in Zaire for supplying the convalescent sera used in treatment; Dr D A Rutter at the Microbiological Research Establishment, Porton, for the haematological and blood chemistry studies; and Dr Patricia A Webb at the Center for Disease Control, Atlanta, for some serological studies. Finally we would like to express our apprecia-

tion of the support given by the staff of The Royal Free Hospital and the various health departments.

References

- 1 Martini, G A, *Postgraduate Medical Journal*, 1973, **49**, 542.
- 2 Gear, J S S, *et al*, *British Medical Journal*, 1975, **4**, 489.
- 3 *Weekly Epidemiological Record*, 1976, **51**, 325.
- 4 Simpson, D, personal communication, 1977.
- 5 Johnson, K M, *et al*, *Lancet*, 1977, **1**, 569.
- 6 Bowen, E T W, *et al*, *Lancet*, 1977, **1**, 571.
- 7 Pattyn, S, *et al*, *Lancet*, 1977, **1**, 573.
- 8 Emond, R T D, *Postgraduate Medical Journal*, 1976, **52**, 563.
- 9 *British Medical Journal*, 1976, **1**, 64.
- 10 Bowen, E T W, *British Journal of Experimental Pathology*, 1969, **50**, 400.
- 11 Fleming, W A, McNeill, T A, and Killin, T, *Immunology*, 1973, **23**, 429.
- 12 Nissen, C, *et al*, *Lancet*, 1977, **1**, 203.
- 13 McNeill, T A, and Gresser, I, *Nature, New Biology*, 1973, **244** (II), 173.
- 14 Falcoff, E, *et al*, *Journal of Virology*, 1973, **12**, 421.
- 15 Johnson, H M, Smith, B G, and Baron, S, *Journal of Immunology*, 1975, **114**, 403.
- 16 Rutter, D A, *British Medical Journal*, 1977, **2**, 24.

(Accepted 22 June 1977)

Prolonged remission maintenance in acute myeloid leukaemia

A S D SPIERS, J M GOLDMAN, D CATOVSKY, CHRISTINE COSTELLO, D A G GALTON, C S PITCHER

British Medical Journal, 1977, **2**, 544-547

Summary

Twenty-five patients with acute myeloid leukaemia were treated with three quadruple drug combinations in predetermined rotation: TRAP (thioguanine, daunorubicin, cytarabine, prednisolone); COAP (cyclophosphamide, vincristine, cytarabine, prednisolone); and POMP (prednisolone, vincristine, methotrexate, mercaptopurine). Fifteen patients (60%) achieved complete remission and five (20%) partial remission. For maintenance, five-day courses of drugs were administered every 14 to 21 days and doses were increased to tolerance. The median length of complete remission was 66 weeks. In eight patients remission maintenance treatment was discontinued and some remained in complete remission for over two years.

In this series the remission induction rate was comparable with that reported for other regimens and complete remission lasted longer with this intensive maintenance regimen than with others. Nevertheless, the

TRAP programme must still be regarded as only palliative treatment for acute myeloid leukaemia.

Introduction

Many regimens are used in the treatment of acute myeloid leukaemia (AML), but none has shown unique superiority.¹⁻³ Intensive⁴ treatments have not proved greatly superior to non-intensive⁵ regimens. Complex remission maintenance using multiple drugs⁶ may be little better than simpler⁷ regimens. Remission-induction programmes have incorporated single drugs⁸ and combinations of seven⁹ or eight¹⁰ antileukaemic agents administered simultaneously. The complete remission rate in adults with AML has varied from 9.5% to 79% in different series.³⁻¹¹ Higher complete remission rates have been reported for small groups of patients at specialised centres¹¹⁻¹² than for larger groups treated at many hospitals, where rates have varied from 9.5% of 200 adults³ to 34% of 301 adults.¹³ The importance of the choice of drugs and the intensity of treatment are outweighed by uncontrolled factors including patient selection and the differing capabilities of different institutions to give supportive care during the induction of remission. The complete remission rate in adults with AML has seldom exceeded 50% in a multicentre study and 65% in a specialised centre.

Attainment of complete remission in AML slightly improves survival. In large series the median duration of complete remission has varied from five to 11 months³⁻¹³⁻¹⁵; median survival has been longer but has seldom exceeded 13 months.³ Immunotherapy administered during remission of AML¹⁶⁻¹⁷ seems to prolong the short duration of survival after relapse but does not prolong the duration of complete remission. No regimen for remission maintenance in AML is definitely superior, and the advisability of attempting to maintain remission at all has been questioned.

Medical Research Council Leukaemia Unit, Royal Postgraduate Medical School, London W12 0HS

A S D SPIERS, MD, FRACP, consultant physician (now professor, Section of Medical Oncology, University Hospital, Boston University Medical Centre, Boston)

J M GOLDMAN, BM, MRCP, consultant physician

D CATOVSKY, MD, MRCPATH, consultant physician

D A G GALTON, MD, FRCP, honorary director

CHRISTINE COSTELLO, MB, MRCP, research fellow

Stoke Mandeville Hospital, Aylesbury, Buckinghamshire HP21 8AL
C S PITCHER, DM, FRCP, consultant haematologist