EB-VIRUS ANTIBODIES IN POST-TRANSFUSION MONONUCLEOSIS AND CARDIOPULMONARY BYPASS

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SINCE the initial reports (Bergstrom and Dahlstrom, 1957; Battle and Hewlett, 1958), there have been many studies of a febrile haematological syndrome, known as post-transfusion mononucleosis or PTM, occurring after blood transfusion and resembling infectious mononucleosis.

Clinical findings include fever, atypical lymphocytes in the peripheral blood, and splenomegaly, and, less frequently, hepatomegaly, abnormal liver function tests, rash, pharyngitis, lymphadenopathy, lymphocytosis, neutropenia and eosinophilia. The syndrome has been seen most commonly after the massive blood exchanges necessary in open-heart surgery and cardiopulmonary bypass. The incidence in different series varies from 3 to 67 per cent., depending on the definition used; the higher incidences are reported when atypical lymphocytes and fever are used as the sole criteria for diagnosis and when frequent haematological examinations are made. Cytomegalovirus (CMV) has been implicated as the agent responsible for the majority of cases, possibly transmitted with the transfused blood (*Lancet*, 1969).

The herpes-type or Epstein-Barr virus (HTV, EBV), a newer member of the herpesvirus group, was first detected (Epstein, Achong and Barr, 1964) in a culture of Burkitt lymphoma cells. This virus may be responsible for the classical (heterophile-antibody-positive) variety of infectious mononucleosis (Diehl *et al.*, 1968; Evans, Niederman and McCollum, 1968; Henle, Henle and Diehl, 1968; Niederman *et al.*, 1968). The evidence for this has been reviewed in a previous publication (Stevens, Pry and Manaker, 1970). Infection with EBV in man appears to lead to chronic latent infection of leucocytes and possibly other cells.

The present study was undertaken to determine (1) whether EBV could be associated with cases of PTM with or without evidence of CMV infection, (2) whether there is any serological evidence of subclinical activation of infection, reinfection or superinfection with EBV in patients following cardiopulmonary bypass, (3) whether a rise in antibodies to EBV or CMV could lead to the appearance of reciprocal cross-reacting antibodies, as has been shown to occur between other herpes viruses in human and experimental infections, and (4) whether EBV antibody was protective against PTM whether related to CMV, EBV or other agents.

MATERIALS AND METHODS

The indirect immunofluorescence test for detection of antibodies to EBV antigens was performed as described previously (Henle and Henle, 1966; Stevens *et al.*). Only an eight-fold or greater rise in antibody titre was regarded as significant, as previously determined by studies with coded replicate samples; all patients with classical infectious mononucleosis

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have shown greater than eight-fold changes in titre from pre-illness to post-illness specimens (Stevens *et al.*). All tests were performed on coded specimens without patient identification or knowledge of relation of serum sample to time of surgery.

The patients studied underwent cardiopulmonary bypass for open-heart surgery at the University of California at Los Angeles Medical Center and the Wadsworth Veterans Administration Hospital, Los Angeles, during the 13 mth from Oct. 1967 to Nov. 1968. These patients were all adults with rheumatic, congenital or arteriosclerotic heart disease, and underwent a variety of surgical procedures. Serum samples were available from them before or after surgery or at both times. Clinical, virological and serological studies of CMV infection occurring in some of these patients have been published elsewhere (Kantor and Johnson, 1970).

FIGURE.—Distribution of EBV antibody titres in patients before surgery.

RESULTS

Pre-surgical patient population. Pre-surgery serum samples were collected during the week before surgery in 20 cases and 8–84 days before surgery in four cases. The geometric mean titre of antibody to EBV was 569. All the patients had antibodies to a titre of 40 or greater, distributed as shown in the figure. The frequency of antibody was not significantly different from that found in 68 randomly selected healthy adult blood donors studied concurrently, 97 per cent. of whom had antibodies. The geometric mean titre of antibody to EBV in the latter group, however, was 109.

Dilution effect of blood exchange. It was felt that serum specimens obtained during the 1st wk after surgery might show an increase or decrease of titre due to dilution with donor blood resulting from the bypass procedure, but that no significant reactive antibody changes even of the anamnestic type would have occurred at this time. Among 15 patients providing paired serum samples, collected before and within 1 wk after surgery, eight showed no change in titre, four showed a four-fold drop, two showed a four-fold rise, and one showed a 16-fold fall in titre. Since there appeared to be no significant rise in serum antibody titre due to the operative procedure itself, any subsequent changes in titre could be attributed to the patient's own antibody response.

Changes in antibody titre and development of PTM. Of 42 patients studied serologically, seven developed two or more symptoms of PTM between 1 wk and 4 mth after surgery. None of them, however, showed a significant rise in antibody to EBV.

Serum samples were available both before and after surgery from 23 patients. All had antibodies to EBV before surgery, so that no seroconversions could be demonstrated. In 16 of these patients post-surgery sera were obtained more than 1 wk after surgery (range 8–169 days); nine showed no change in EBV antibody titre, three had a four-fold drop in titre, one had a four-fold drop in titre, which later returned to the pre-operative level, and two had a four-fold rise in titre, which returned to the pre-operative value during the period of study. One patient had a 16-fold rise in titre between his pre-operative serum and a single specimen obtained 106 days after surgery; he did not develop any symptoms or signs of PTM.

Sera were available from 19 patients only in the post-operative phase. None of them showed a greater than four-fold change in antibody titre during the post-operative period; there were no unusually high titres and no sero-negative individuals. The distribution curve of all the post-operative serum titres, and of the peak titres in patients providing two or more post-operative specimens, was not significantly different from that of the pre-operative serum titres.

Of the seven patients who developed PTM, CMV was associated with the illness in five. Virus was isolated from the urine in four cases at the time of illness, and one of these cases, who possessed CMV complement-fixing (CF) antibody before surgery, also showed a rise in antibody titre. The fifth patient also showed a rising CF antibody titre to CMV and had typical intranuclear inclusion bodies in cells of the urinary sediment, but since the urine was toxic for tissue culture cells CMV isolation was not achieved. In three of these five CMV-associated cases serial post-operative serum specimens were available; none showed any significant change in EBV antibody titre. In the two remaining PTM cases, neither CMV nor EBV was incriminated. From one of them only a single, post-operative serum specimen was obtained for antibody testing during the episode of PTM, and this had an EBV immunofluorescent antibody titre of 2560 and a CMV CF antibody titre of 128; both titres equalled the highest titres seen in patients in the post-operative period, and CMV was not isolated from the urine. The other patient provided a pre-operative specimen of serum, another specimen within 1 wk after operation and several later specimens during and after his episode of PTM (associated with atypical lymphocytes, hepatosplenomegaly, and abnormal liver function tests). Both EBV and CMV antibodies were present in high titre before surgery, and persisted without significant change post-operatively. The Sabin-Feldman dye test for toxoplasmosis and the heterophile-agglutination slide test were negative in all cases (Kantor and Johnson).

Evidence of reciprocal changes in CMV-EBV antibody titres. In all, seven patients in the course of this study, including two patients who developed PTM, had two-fold or greater rises in CMV CF antibody titre. None of these patients had a significant change in their EBV antibody titre.

DISCUSSION

There was no serological evidence in this series of patients that EBV caused any of the cases of PTM. There was no significant change in EBV antibody titre in patients followed serially after surgery, or when sera obtained before and after operation were compared. However, all our patients who were studied pre-operatively possessed antibodies to EBV, not a surprising finding as previous sero-epidemiological studies have demonstrated nearly universal seropositivity in adults when sensitive antibody assay methods are used (Henle and Henle, 1966; Gerber and Birch, 1967; Porter, Wimberly and Benyesh-Melnick, 1969). Perhaps, unlike CMV, EBV can cause PTM only in EBV-seronegative individuals. It has previously been shown (Paloheimo *et al.*, 1968), and substantiated in this group of patients, that many patients show rises of antibody to CMV without any signs of PTM. In this study, however, there was no evidence of such subclinical activation of infection with EBV. Attempts to isolate EBV from these patients would not be diagnostically informative, because EBV can be isolated from leucocyte cultures made from the peripheral blood of healthy adults with EBV antibodies, indicating only a latent, probably lifetime, infection.

The large blood exchanges that occur with the bypass procedure failed to change EBV antibody titres significantly, regardless of pre-operative antibody levels. It was also of interest that a rise in antibody to CMV, due to clinical or subclinical infection, did not affect the EBV antibody titre. On the other hand, in classical heterophile-antibody-positive infectious mononucleosis, considered to be due to EBV infection, anti-CMV macroglobulin has been demonstrated in at least some cases (Hanshaw, 1969). These findings could be helpful diagnostically. Further studies with antisera prepared in animals are needed to elucidate the antigenic relationship of these two agents.

As this study was completed, a paper (Gerber *et al.*, 1969) appeared concerning CF antibodies to EBV and CMV in patients with PTM. The authors examined only individuals without EBV antibodies before surgery, i.e., five of the 54 patients available for study. Of these five patients, two of whom were children, four showed persistent EBV antibody seroconversion after surgery. One of the latter developed symptoms of PTM and also a positive heterophile-antibody test, which is rare in PTM. Another developed antibodies to CMV, as well as to EBV, after surgery and had fever without other symptoms of PTM. These authors did not say whether any patients with antibodies to EBV before surgery developed PTM, or what percentage of the total number of cases of PTM among their 54 patients the one heterophile-antibody-positive case represented.

It appears (Gerber *et al.*; the present study) that EBV is an uncommon cause of PTM, if a cause at all. PTM associated with CMV infection may occur in patients having antibodies to CMV before transfusion, as seen in previous studies. If this is indeed reinfection, the antigenic heterogeneity of CMV (Weller, Hanshaw and Scott, 1960) may account for the apparent lack of protection of antibody. To determine whether EBV antibodies are

protective against EBV-associated PTM, further studies of patients with antibodies to EBV before surgery are required. Assuming that both CMV and EBV can cause PTM, CMV causing the heterophile-antibody-negative cases and EBV the less common heterophile-antibody-positive cases, there are still other cases, such as one mentioned in this study, in which there is no evidence of active infections with either CMV or EBV.

SUMMARY

The possible role of EB virus in post-transfusion mononucleosis (PTM) was investigated serologically. Sera were obtained from patients before and after cardiopulmonary bypass for open-heart surgery, and tested for antibodies by immunofluorescence. No association of EB virus with cases of PTM was found; nor was there evidence of activation of infection, reinfection or super infection with EB virus resulting from the massive blood exchange.

It appears that cytomegalovirus is implicated aetiologically in most cases of PTM, while EB virus infection is rarely associated with this disease. There was no evidence of reciprocal cross-reactivity between EB virus and CMV antibodies.

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