Immunological Study for Patients in Najaf Governorate with Specific Antibody Response

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2012

ABSTRACT

The present investigation was an attempt to obtain profiler of serological response to EPSTEINBARRVIRUS (EBV) infection in four patients with EBV-mononucleosis, and in two bone marrow transplant patients. Four forms of EBV antigens were studied for the detection of antibody, namely crude-extracts of infected human embryonic lung fibroblasts, soluble antigens, purified enveloped-virus particles and purified nucleocapsid, and the serological tests employed were complement fixation(CF), neutralization test(NT)and enzyme linked immunosorbant assay(ELISA).

دراســـــة مـناعيــة للمرضى في محافظة النجف الأشرف مع استجابة الأجسام المضادة المتخصصة

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الخلاصة

الدراسة الحالية محاولة للحصول على الاستجابة المصلية (للفيروس المضخم للخلايا) (EPSTEINBARRVIRUS) عدوى الفيروس المضخم للخلايا في أربعه مرضى، كريات الدم البيض، واثنين من المرضى في زرع نخاع العظام. تمت دراسة أربعة أشكال من مستضدات الفيروس المضخم للخلايا للكشف عن الأجسام المضادة، وهي خلاصات من المصابين بتليف الرئة الجنينية، ومستضدات القابلة للذوبان و جسيمات منقات لاغلفة الفيروسات ولـ nucleocapsid المنقات ؛ الاختبارات المصلية المستخدمة كانت تثبيت المتمم . واختبار تحييد وفحص إنزيم المناعي المرتبط (ELISA).

INTRODUCTION :

Opportunistic infections with EPSTEINBARRVIRUS (EBV) frequently cause serious complications in immunosuppressed patients who have undergone kidney transplantation or bone marrow grafting. In the bone marrow transplantation EBV pneumonia is probably the most frequent cause of death (Pango, 1997) . Whether or not the infection is primary or reactivated, serological evidence of diagnostic value is seldom found during the first four weeks after surgery. The majority of patients who acquired EBV antibody (primary infection) or show a significant change in EBV antibody titer (reactivated infection) do so over the subsequent 2-5 months (Craighead, 1991) . Latent infection in the man, little is known of the site in the body at which the virus is harbored and of the precise mechanisms whereby infection is reactivated in man, EBV probably persists in the circulating lymphocytes, since there is a risk of transmitting EBV infection in transfused blood from about 5% of sero_positive doners (Diosi *etal*. 1991). Primary infections which develop after bone marrow grafting probably also originate from transfused blood. In many virus infections the antibody response to different antigenic fractions of the virus particle do not develop synchronously.

Moreover, viral antibodies may begin to be detected at different times after infection depending on the sensitivity and specificity of the serological technique employed. It was found that in patients under going primary EBV infection, virus neutralizing antibody became detectable much later than antibody detected by complement fixation with crude cell extract antigen and shown to reach maximum titer(Anderson *etal*. 1999)in similar patients, CF antibody against purified enveloped-virus particle antigen appeared more slowly than CF antibody against a crude antigen prepared by freezing-thsving of EBV-infected cells (Anderson *etal.* 1999). Anderson also studied the pattern of antibody response in patients with reactivated EBV infection. In these cases there was no obvious delay in appearance of neutralizing antibody in relation to complement fixing antibody (against crude cell extract antigen), although the neutralizing antibody titer look longer time to reach a stable maximum value. Serological

studies in patients undergoing bone transplantation have been carried out mainly using the complement fixation test with crude viral antigen. Primary and reactivated infections are common in these patients and seroconversion is detected mainly between (2-3) months post-operation, and although in some cases as early as 13 days or as late as 120 days (Neiman *etal*. 1995) (Neiman *etal.* 1998). Patients with pre-existing antibody sometimes show a small decrease in CF antibody titer during the first week after surgery.

MATERIALS AND METHODS :

Tissue culture and virus: this was decided elsewhere (AZIZ TAG , *etal.* 1998).

Preparation of epsteinbarrvirus antigen:

Crude tissue culture-extract antigen was prepared as described by (Kettering e*tal*. 1999).

Soluble antigen:

A batch of crude antigen was prepared as above. This was then subjected to two cycles of centrifugation at 40.000 r.p.m. for 1 hour in the sw50 rotor of the Beckman L265B ultracentrifuge, discarding the deposit on each occasion. The final supernatant was stored at 70^{o} C in 0.5 ml aliquots. Control soluble antigen was prepared in the same way from uninfected tissue culture.

Purification of enveloped virion antigen:

To separate complete enveloped virus particles from the incomplete virus components, and from cell particulate debries present in the infected tissue culture supernatant and concentrated in parallel with complete virus by the polyethylene glycol (Adams , 1995). Purification was undertaken by sucrose-density-gradient ultracentrifugation (Gupta *etal*. 1999).

Nucleocapsid antigen:

A method described by (Predue *etal* . 1996) was followed.

Complement fixation test(CFT):

This was carried out as described by (Booth *etal*. 1991).

Plaque-neutralization test:

Neutralizing antibodies was measured in a plaque system (Plummer *etal*. 1986) with some modification of the technique.

Sera: four patients with epsteinbarrvirus mononucleosis confirmed by isolation of the virus from urine and by the presence of EBV-specific Igm antibody in the serum, were studied. Serial blood specimens were available from all patients, give sera from case1 and three each from case 2-4. Two patients given bone marrow transplant were also studied. They were aged 11 years and 16 years respectively, and had been given bone marrow transplants for aplastic anemia. All sera were inactivated for 30 minutes at 56ºC and stored at 20ºC. The sera were subsequently titrated in serial two-fold doubling dilution, for CF and NT antibody and examined, at a single 1:300 dilution, for the presence of antibody by ELISA test.

RESULTS :

(A) Naturally-infected patients:

In all four patients a very similar antibody response was demonstrated by the four antigens, in both the CF and ELISA tests. By CF test[Fig. 1 A-D], antibody was not detected before the second week after the onset of illness, but there was a rapid rise to peak titers during the third week, with little subsequent change in the final titers over some 3-8 months. All four antigens gave very similar titers of antibody over the course of illness although the soluble antigen consistently produced the highest levels. By ELISA [Fig. 1 A-D], there was probably a little delay in antibody appearance, as compared with CFT.there was again no detectable antibody at 2weeks but taken arise late in the third week to reach peak titers shortly afterwards. The results from case 1 and 2 showed a tendency for somewhat more rapid appearance of antibody to the soluble antigen, and some delay in the appearance of antibody to the enveloped-virion antigen, The neutralizing antibody was definitely delay in it´s appearance, in 3 out of 4 cases [Fig.1A-D] compared with the CF andELISA antibodes. In these three cases antidoby was not detected at 3 weeks after onset of symptoms. The antibody titers achieved ranged from 16-128, iower than by CFT.

(B) patients given abone marrow transplant:

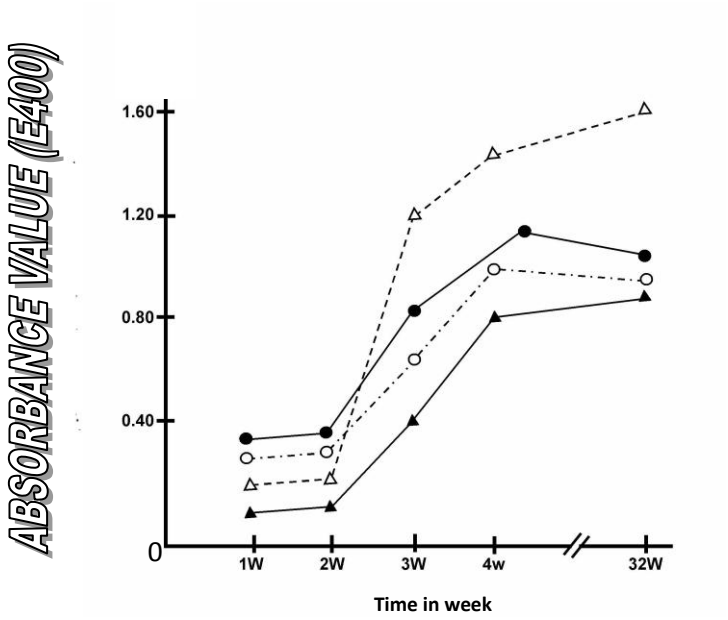
Patient 1 :this patient [Fig.2]remained seronegative by CF,ELISA and neutralizing antibody test, for 3 weeks after bone marrow transplantation. CF antibodies against all four antigens then appeared sometime after 3 weeks, and achieved relatively low level peak titers [16withthe crude, nucleocapsid and soluble antigen] by 7 weeks. There was consistent fall in antibody titer occurred over the next 5 weeks. This was followed by are turn to original peak titers by the antibody against the crude and soluble antigens, and by a boost in the antibody against the enveloped- viron antigen to 32.

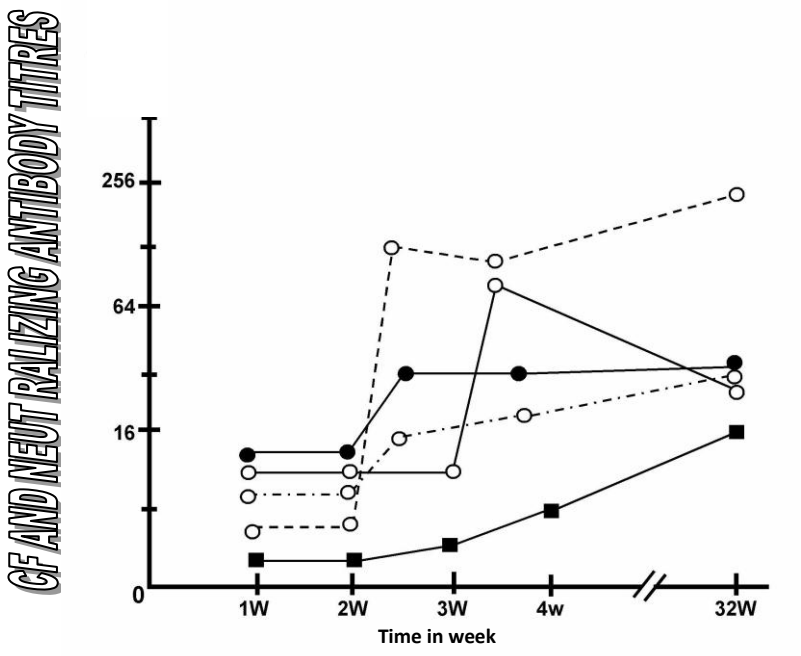
Neutralizing antibody [Fig.2] also appeared after 3 weeks and rose sharply to a peak titer at about 7 weeks in parallel with CF antibodies, and then maintained a peak titer of 32-64 throughout the following 16 weeks. Unexpectedly, when compared with EBV mononeucleosis patients, there was some delay in the CF antibody against enveloped-virion antigen.

At 12 weeks the highest titer was achieved by neutralizing antibody, followed by soluble nucleocapsid and enveloped-virion

The next serm available from this patient was about 1 year later .All that time the neutralizing and CF antibodies were preset at more or less the same high level [256] except for the nucleocapsid CF antibody which was present at a titer 8-fold less than the others. This differential was maintained steadily over the next 4 months. This difference was however, not seen by ELISA, All four antibodies maintained more or less the same high titers. The greater sensitivity of ELISA for antibody detection was shown in patient 2 who was apparently seronegative before operation but was proved, by ELISA, to be seroposive , this was therefore a reactivation, probably accounting for the much higher antibody titers achieved by patient2. The neutralizing antibody titer exceeded that of the CF antibodies. ELISA tests [Fig.2] with all four antigens showed all four antibodies to develop in paralled and in step with the CFand neutralizing antibodies. They appeared after 3 weeks and reached peak titers by 7 weeks , and

PATIENT NO.1





INTERVALS AFTER INFECTION

Crude cell – extract antigen test **soluble antigen test**

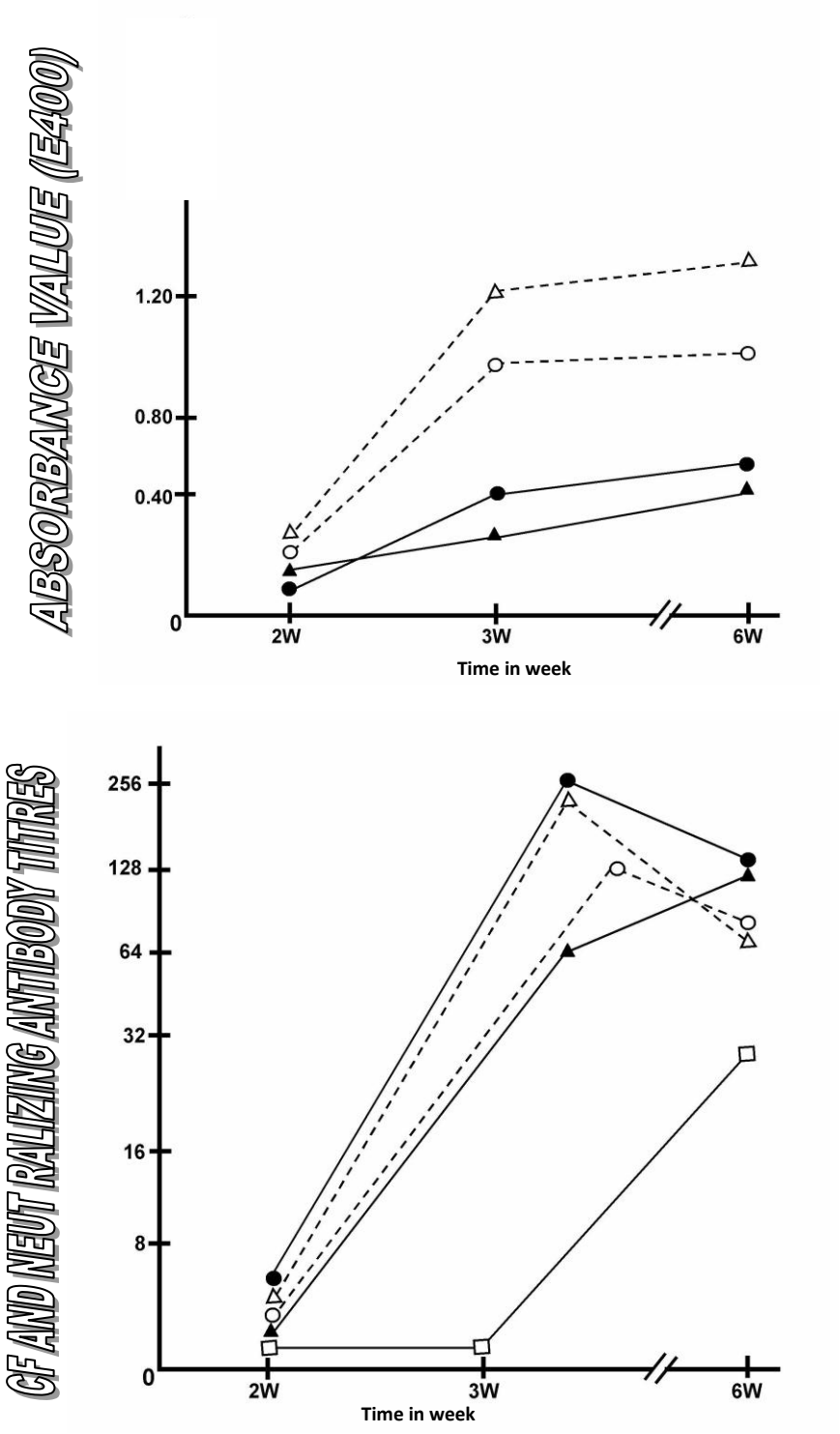
Enveloped – virion antigen test **nucleo capsid antigen test**

Neutrali zing antibody (plaque – reduction test)

Fig . IA

CF , ELISA and neutral zing antibody response in patients naturally infected with EBV (patients No 1-4)

PATIENT NO.2



INTERVALS AFTER INFECTION

Fig . 1B

Crude cell – extract antigen test

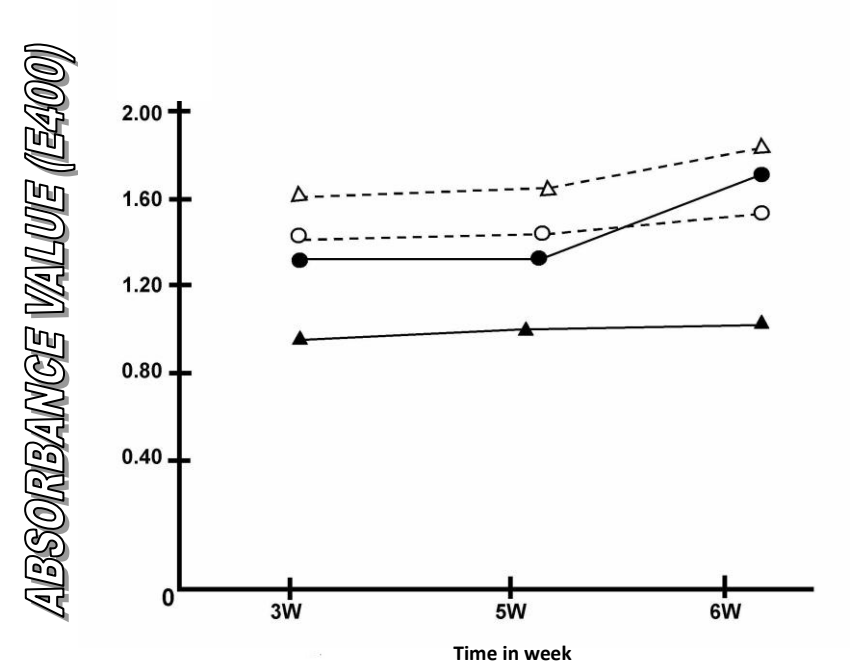
soluble antigen test

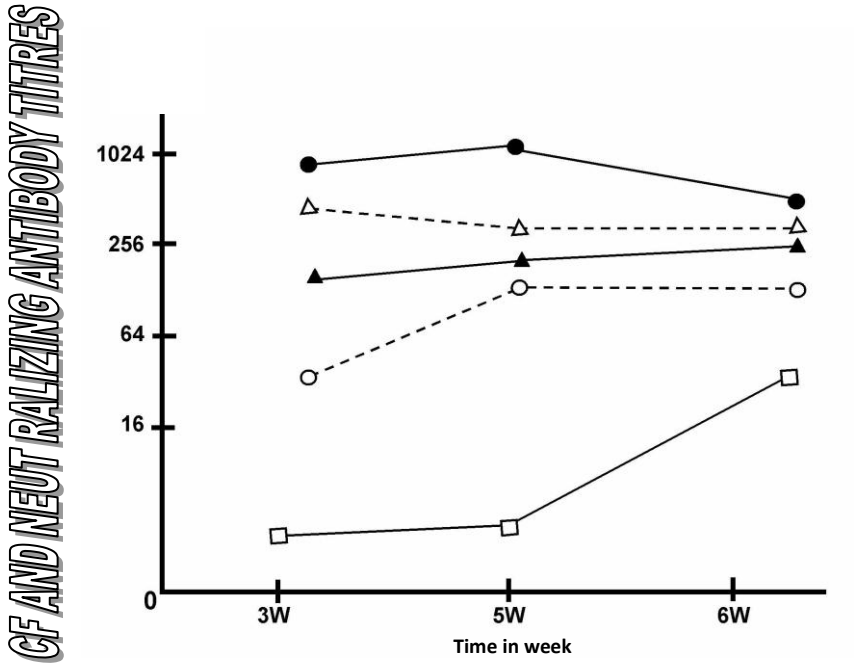
Enveloped – virion antigen test

nucleo capsid antigen test

Neutrali zing antibody (plaque – reduction test)

PATIENT NO.3





INTERVALS AFTER INFECTION

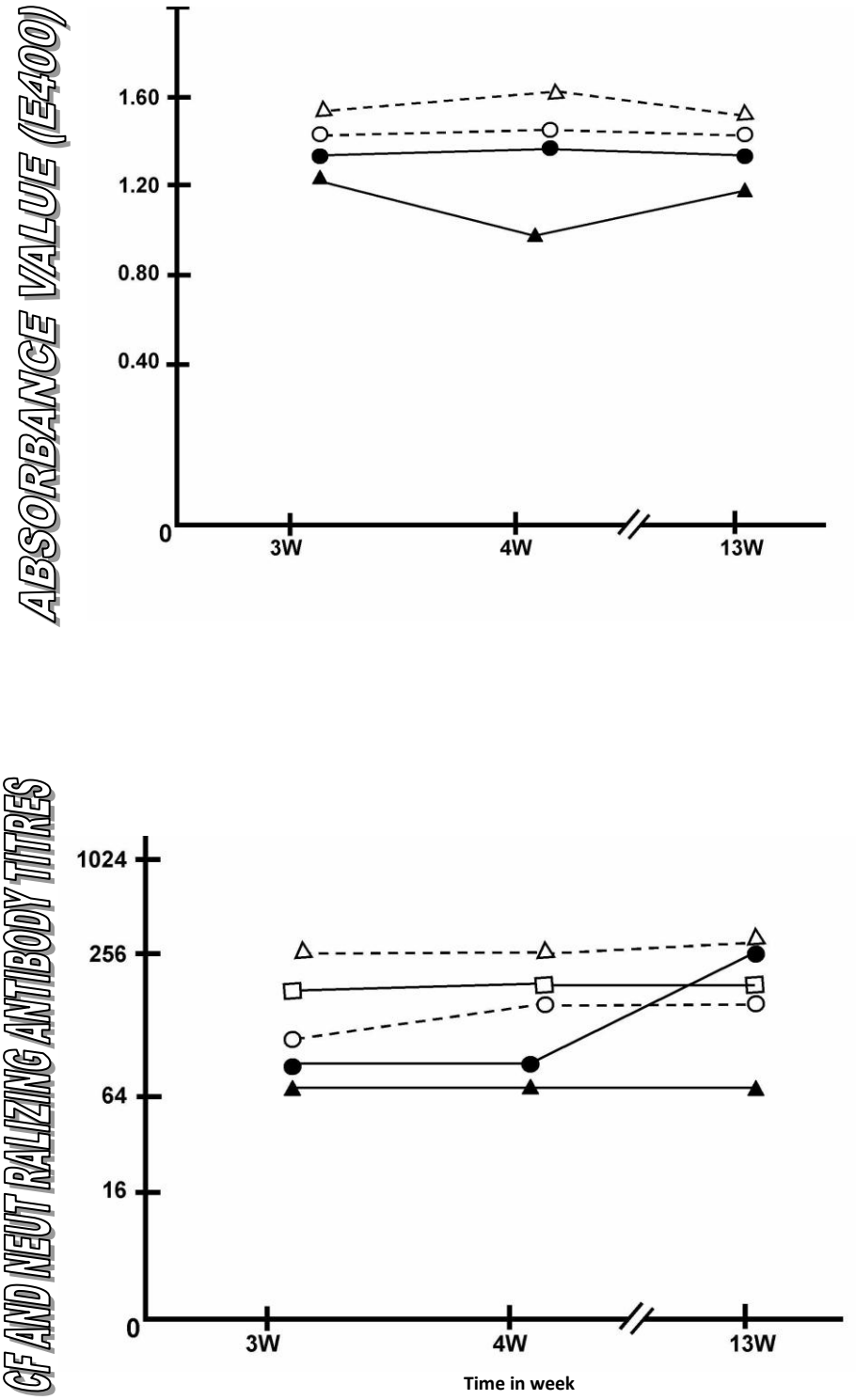
Fig . 1C

Crude cell – extract antigen test — **soluble antigen test**

Enveloped – virion antigen test — **nucleo capsid antigen test**

zing antibody (plaque – reduction test)

PATIENT NO.4



INTERVALS AFTER INFECTION

Fig . 1D

Crude cell – extract antigen test

soluble antigen test

Enveloped – virion antigen test

nucleo capsid antigen test

Neutrali zing antibody (plaque – reduction test)

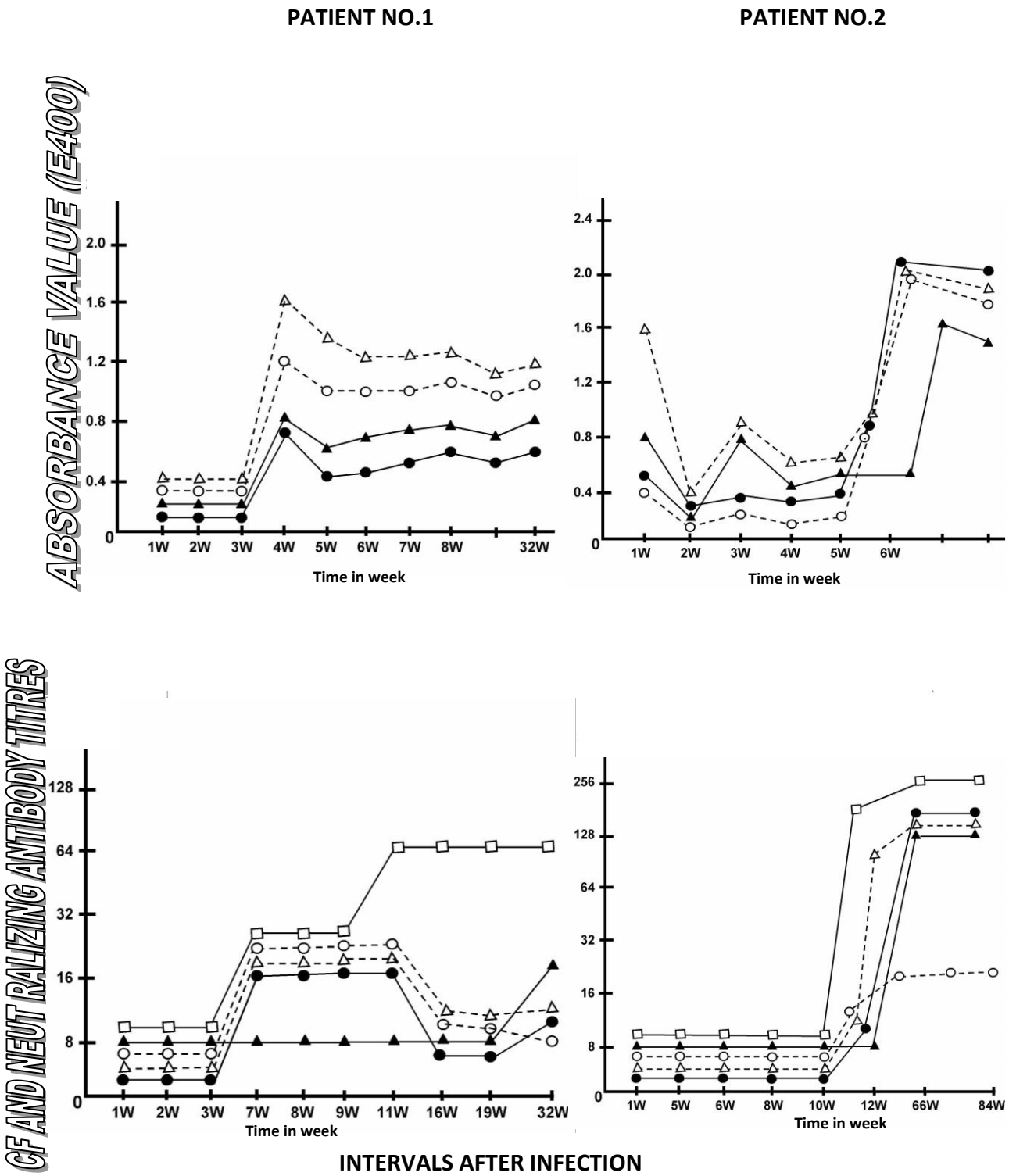


Fig . 2

CF , ELISA and neutrali zing antibody response in patients naturally infected with EBV (patients No 1-2)

were then maintained for the following 16 weeks. However, the ELISA tests did not reveal transient drop in titer, at 16 weeks shown by CF antibodies. Patient 2: this patient [Fig. 2]showed no detectable CF neutralizing antibodies for about 10 weeks after transplantation , however antibodies were detected , against all four antigens ,by ELISA at the same time of operation. These droped to zero levels shortly after operation but were again detectable at low levels during the rest 5of the initial 10 weeks period, after 10 weeks there was a sudden large rise in all antibodies ,neutralizing ,ELISA and CF, although.

DISCUSSION:

All for EBV antigens the curde cell associated antigen the soluble antigen, the purified enveloped –virion antigen and the nucleocapsid antigen , reacted well in both the CF and the indirect ELISA tests.

In the present study, CF antibody titers to the soluble and the crude antigens were closely similar, this is in contrast to the results of (Cremer *etal* . 1997) who reported low titers with the soluble antigen as compared with the crude antigen. Possibly the improved physical disruption of the infected cells by alkaline buffer plus sonication relassed greater quantities of soluble antigenic components that might overwise have remained adherent to particulate cellular material. The neutralization test was also relatively poor for detecting antibody in the acute serum specimens and recognized as many apparently primary infections as did CFT. In this case, strain-spesificity is perhaps more important as an explanation (Weller *etal* . 1982), although low sensitivity undoudtedly also plays apart. In all patients with primary infection the pattern of antibody response against the four EBV antigens was essentially the same, by both CF and ELISA tests . in naturally infected patients with EBV mononucleosis, antibody generally appeared 2-3 weeks after onset of illness, rapidly achieved peak titers and the levels were maintained for more than 6 months, and similar findings were obtained by both CF and ELISA TESTS .IN GENERAL ,The highest titers was found against the soluble antigen and the lowest against enveloped –virion antigen .the later antibody also differed in being the slowest to develop, being delay usually by about one week behind the others .the enveloped –virion antibody corresponded in its appearance closely with the neutralizing antibody .the later was also delayed in appearance until 4-6 weeks after the onset of illness and achieved relatively by low titers. These findings confirmed the results obtained by (Schmitz *etal* . 1997) in a study of eight patients with acute EBV infection .

Essentially the same basic pattern of antibody response was seen in the bone marrow patients. Strikingly, there was no delay in the appearance of neutralizing antibody and also it achieved unusually high titer, as high as or higher than the CF antibody titer. The exceptional behavior of neutralizing antibody demonstrated either by the NT or by CF-enveloped virion test, in the bone marrow patients

would be due to diminished activity of the immune mechanism because of immunosuppressive therapy allowing greater opportunity for maturation and release of virus from the infected cells.

Some support of this hypothesis comes from serological studies of active herpes simplex virus [HSV] infections in man, with different viral antigenic fractions (Martin *etal* . 1994).

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