

Prolonged Illness after Infectious Mononucleosis Is Associated with Altered Immunity but Not with Increased Viral Load

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Background. Primary Epstein-Barr virus (EBV) infection causes a spectrum of characteristics that range from asymptomatic seroconversion to severe infectious mononucleosis (IM), sometimes with prolonged symptoms and disability. We examined the relationships between clinical course, number of viral copies, and immunological parameters in a prospective cohort of subjects with recent IM.

Methods. Eight case patients with at least 6 months of disabling symptoms and 31 matched control subjects who had recovered promptly were included. Symptom scores were recorded at regular intervals over the course of 12 months. Cellular EBV load, EBV-specific antibody responses, lymphocyte subsets, and EBV-specific interferon (IFN)- γ induction were measured.

Results. In case patients with prolonged illness, the severity of acute-phase symptoms was greater, the development of anti-EBV nuclear antigen-1 immunoglobulin G was more rapid, and the time to development of the peak IFN- γ response to the majority of latent-cycle EBV peptides was generally slower than those in control subjects. However, in both groups, neither viral nor immune parameters correlated with the severity or duration of symptoms.

Conclusions. The resolution of symptomatic IM is not determined by control of viremia, nor is it easily explained by altered host responses to EBV infection. The detailed determinants of delayed recovery remain to be elucidated.

In industrialized countries, 40%–65% of episodes of primary Epstein-Barr virus (EBV) infection occur during early childhood and are asymptomatic [1, 2]. By contrast, primary infection in teenagers or adults often causes symptomatic infectious mononucleosis (IM), which is characterized by sore throat, fever, fatigue, and headache. On examination, these symptoms are often found to be accompanied by pharyngitis, lymphade-

nopathy, and splenomegaly, as well as an atypical lymphocytosis in peripheral blood.

Most cases of acute IM resolve within several weeks without sequelae, but some individuals have prolonged and disabling illness. Risk factors for the development of chronic illness after IM are poorly understood, but they may reflect the uncontrolled proliferation of EBV in infected B, T, or NK cells. For instance, primary infection in children with a congenital [3] or acquired [4, 5] impairment of T cell competence may result in chronic, active EBV infection (CAEBV), with ultimately fatal lymphoid malignancy or organ failure. Such patients have very high viral copy numbers in peripheral blood and markedly elevated titers of antibodies against EBV viral capsid antigen (VCA) but often no antibodies against EBV nuclear antigens (EBNA).

In previously healthy young adults, the kinetics of recovery from acute IM has been examined in 2 prospective cohort studies [6, 7]. Both studies revealed that

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almost one-half of the group had substantial ongoing symptoms 2 months after onset and that ~10% had disabling symptoms marked by fatigue lasting ≥ 6 months. These subjects did not have clinical features to implicate the recognized patterns of CAEBV disease or other chronic sequelae. Hence, the determinants of this protracted clinical course are unknown.

In an effective humoral and cellular response during typical IM, dramatic lymphocytosis is frequently evident in peripheral blood. The majority of these cells are EBV-specific CD8⁺ T lymphocytes [8, 9], which appear to be the major mechanism of host control of viral replication [10]. Thus, resolution of the symptoms of acute IM is thought to correlate with the onset of a broad cytotoxic T lymphocyte (CTL) response to latent antigens, seroconversion to EBNA-1, and control of viral replication [11, 12]. A report that examined EBV-specific immune responses in 2 previously healthy adults who had widely divergent durations of clinical IM suggested that the development of a broadly directed CTL response was coincident with the resolution of symptoms [13]. Accordingly, the present study was conducted to characterize the immunological and virological associations of the severity and duration of IM in a cohort of previously healthy young adults with primary EBV infection.

SUBJECTS, MATERIALS, AND METHODS

Subjects. Participants in an ongoing prospective cohort—the Dubbo Infection Outcomes Study—were enrolled after having presented to their general practitioner with symptoms of IM and after the detection of IgM antibodies against EBV VCA. These provisional serological diagnoses were confirmed in 50 subjects by the testing of longitudinally collected serum samples [14].

At enrollment, the date of onset of symptoms was recorded. At each visit, detailed self-report and interview assessments of physical and psychological health were recorded, and a blood sample was collected. The severity and duration of symptoms were monitored using the Somatic and Psychological Health Report, a 34-item self-report questionnaire [15, 16]. An empirically derived subscale—termed the “SOMA-6”—records physical symptoms, including muscle pain, tired muscles, and prolonged tiredness after activity, as well as the need to sleep longer, poor sleep, and poor concentration [17]. The reliability and construct validity of the instrument in the identification of persisting states of fatigue have been demonstrated [18]. A SOMA score of ≥ 3 (out of a possible 12) correlates with physician-rated assessments of significant disability [18]. In addition, subjects reported functional impairment associated with the illness on the Brief Disability Questionnaire, including the number of “days in bed in the last month” and “days out of role in the last month” (i.e., days in which subjects were unable to perform their normal work or other activities) [19].

Subjects were assessed at baseline, 2–3 weeks, 4–6 weeks, and 3 months. In those subjects who had persistent symptoms for

>3 months, structured medical and psychiatric assessments, as well as laboratory investigations to exclude CAEBV or unrelated causes of illness, were undertaken, and additional follow-up was continued at 3-month intervals until recovery. A late follow-up sample (≥ 12 months) was collected, when possible, from all subjects.

Eight subjects had illness that persisted for >6 months. For comparisons of cellular and humoral immune responses, these 8 case patients were matched by HLA-A and -B genotype (with at least 2, and up to 4, matched HLA alleles) to a total of 17 control subjects from the cohort of those who had recovered promptly (within 6 weeks of the onset of symptoms of IM). Because the number of stored specimens was limited, an additional 14 control subjects, matched for sex and age (but not for HLA genotype), were also selected for comparisons of EBV load.

The study protocol was approved by the relevant institutional review boards. Written, informed consent was provided by all subjects.

Specimens. Peripheral blood mononuclear cells (PBMCs) were separated by density-gradient centrifugation (Lymphoprep; AXIS-SHIELD) and cryopreserved in RPMI 1640 (Trace) with 10% dimethyl sulfoxide (Sigma) and 50% autologous plasma, and aliquots were stored in the vapor phase of liquid nitrogen. Serum and plasma were also separated and stored in aliquots at -80°C .

Antibody ELISAs. Serum anti-VCA IgG and IgM and anti-EBNA-1 IgG antibodies were measured by sandwich ELISA, using commercially available assays (PanBio). IgG avidity was measured in parallel, using urea to dissociate low-avidity immune complexes, as described elsewhere [14]. The sample:assay cutoff optical density ratio was used as a surrogate for antibody titer [20, 21].

Flow-cytometric analysis. CD3⁺, CD8⁺, and HLA-DR⁺ antigens were identified on PBMCs with phycoerythrin-, peridinin-chlorophyll-protein-, or fluorescein isothiocyanate-labeled antibodies, using standard methods, with a FACSCalibur instrument and Cellquest software (all from BD Biosciences), as described elsewhere [22].

Interferon (IFN)- γ enzyme-linked immunospot (ELISPOT) assay. Assays for the production of IFN- γ were performed as described elsewhere [14] in 96-well nitrocellulose-base plates (Multiscreen; Millipore) coated with capture anti-human IFN- γ monoclonal antibody (MabTech). PBMCs were cultured for 24 h at 2.5×10^5 cells/well in RPMI 1640 with 10% fetal calf serum with appropriate HLA-restricted peptides at 10 $\mu\text{g}/\text{mL}$, either without stimulus or with phytohemagglutinin (PHA) at 10 $\mu\text{g}/\text{mL}$. Detection was completed with biotinylated detector anti-human IFN- γ monoclonal antibody (MabTech) with streptavidin-alkaline phosphatase (Sigma) and bromochlorodolyl phosphate/nitroblue tetrazolium substrate (Sigma). Spots were counted using a computer-assisted ELISPOT analyzer

Table 1. Characteristics of subjects and illness at enrollment.

Subject (age in years, sex)	Symptom score ^a	HLA genotype			
		HLA-A		HLA-B	
Case patients					
1 (17, F)	6	2	2	8	40
2 (16, M)	8	2	3	7	7
3 (23, F)	11	23	24	7	44
4 (23, M)	4	3	1	8	35
5 (49, F)	7	2	1	35	44
6 (19, F)	5	3	24	35	35
7 (18, M)	11	2	23	7	35
8 (17, F)	3	2	26	35	44
Control subjects					
1 (18, F)	1	3	24	13	15
2 (17, M)	0	2	2	7	8
3 (33, F)	3	1	29	8	44
4 (19, F)	1	24	26	7	7
5 (18, F)	6	3	11	35	51
6 (19, F)	5	2	2	44	15
7 (19, M)	9	24	24	7	38
8 (17, M)	2	2	1	44	37
9 (16, F)	10	3	11	7	35
10 (18, M)	3	3	24	35	38
11 (16, M)	9	2	3	7	57
12 (19, F)	1	2	23	7	44
13 (34, F)	5	2	2	7	8
14 (18, M)	0	2	3	27	35
15 (19, F)	2	3	1	8	35
16 (19, F)	3	1	1	8	14
17 (48, M)	3	1	11	8	35

NOTE. F, female; M, male.

^a Possible range, 0–12.

(Autoimmun Diagnostika). Results were calculated as the mean of triplicate wells, expressed as the number of IFN- γ spot forming cells per 1×10^6 cells, with the background counts subtracted. In all assays designated as valid, 2.5×10^5 PBMCs demonstrated >300 sfc in response to PHA.

Real-time polymerase chain reaction (PCR) quantitation of EBV DNA load. DNA was extracted from 5×10^6 PBMCs (Qiagen). PCR primers were from the BALF5 gene sequence encoding EBV DNA polymerase, as described elsewhere [23]. Amplifications with 200 ng of sample DNA were performed on a Rotorgene 2000 thermal cycler (Corbett Research). A standard curve was constructed using DNA from the Raji cell line, which has a known-input viral copy number. The assay sensitivity was $1.0 \log_{10}$ DNA copies/ 10^6 PBMCs.

Statistical analysis. Because the interval between the onset of symptoms and enrollment varied between individuals, the sampling points for each subject were categorized for analysis into 3-week intervals until 3 months after onset ($0 \leq 3$, $3 \leq 6$, $6 \leq 9$, and $9 \leq 12$ weeks) and into 3-month intervals thereafter ($3 \leq 6$, $6 \leq 9$, $9 \leq 12$, and >12 months).

Unpaired *t* tests were used to compare age distributions and the time between the onset of symptoms and enrollment. The nonparametric Mann-Whitney *U* test was applied to symptom and disability data. Two-way analysis of variance (ANOVA) was applied to \log_{10} -transformed EBV load, antibody, and cellular response data. The kinetics of development of anti-EBNA-1 IgG were compared using Kaplan-Meier survival curves followed by a log-rank χ^2 statistic. Prism software (version 3.0; GraphPad) was used for all analyses.

RESULTS

Demographics and illness characteristics. The age and sex distributions of the case patients (mean age, 23 years; male:female ratio [M:F], 0.6:1) were not significantly different from those of the HLA-matched control subjects (mean age, 21 years; M:F, 0.7:1), the additional 14 age- and sex-matched control subjects (mean age, 21 years; M:F, 0.7:1), or the combined control group (mean age, 20 years; M:F, 0.6:1) (table 1). These 39 subjects had enrolled in the cohort an average of 27 days after the onset of symptoms (range, 13–49 days). This interval was not significantly different between the case patients and the control subjects.

At enrollment, the case patients had a mean symptom score of 7 (range, 3–11; figure 1), had been in bed for a mean of 13 days (range, 7–30 days), and had been out of role for a mean of 21 days (range, 14–31 days). By contrast, in the HLA-matched control subjects, the mean symptom score at enrollment was 4 (range, 0–10), they had been in bed for a mean of 6 days (range, 0–14 days), and they had been out of role for a mean of 14 days (range, 0–30 days). These differences in baseline symptom severity and degree of disability between the case patients and the control subjects were significant ($P = .022$ and $P = .039$, respectively). As expected, the time spent

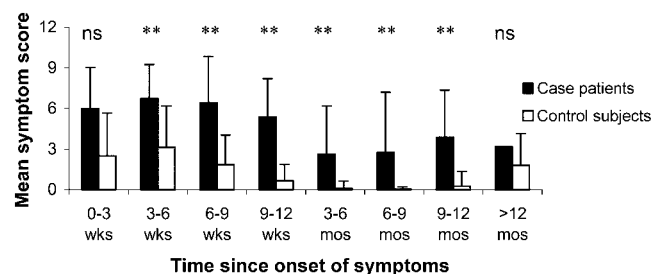


Figure 1. Relative symptom severity over the course of the illness after acute infectious mononucleosis. Subjects who had prolonged illness (case patients; $n = 8$) also had significantly worse symptoms during the acute phase of infection than did those who recovered from the illness more promptly (control subjects; $n = 31$). Bars are mean symptom scores (\pm SD) with the degree of difference between case patients and control subjects shown by “ns” (not significant) or $**P < .01$ (analysis of variance). mos, months; wks, weeks.

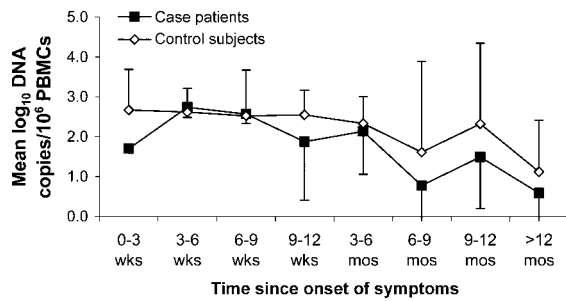


Figure 2. Changes in Epstein-Barr virus cellular load during the course of the illness after acute infectious mononucleosis. Data are the mean (\pm SD) for the groups at each time point. There were no significant differences in EBV load between the 8 case patients and the 17 age- and sex-matched control subjects who recovered promptly. mos, months; PBMCs, peripheral blood mononuclear cells; wks, weeks.

out of role correlated with the reported number of days in bed during the same period of time ($P = .003$) and with symptom severity ($P = .007$).

The mean duration of the entire illness (i.e., from the onset of symptoms to recovery [when the symptom score was <3]) was 34 weeks for the case patients (range, 24–52 weeks), compared with a mean of 8 weeks for the HLA-matched control subjects (range, 2–18 weeks) ($P < .001$). The mean of the total number of days in bed for the case patients was 21 days (range, 7–54 days), whereas the HLA-matched control subjects had a mean of 5 days (range, 0–16 days) ($P = .002$). The groups also differed in the total number of days out of role over the course of the whole illness period, with the case patients having a mean of 38 days (range, 14–74 days), and the HLA-matched control subjects having a mean of 14 days (range, 0–39 days) ($P = .003$).

EBV cellular load. There were no significant differences in EBV copy numbers between the case patients and the control subjects at any time (figure 2). The levels recorded were generally stable over the course of the initial 3 months, with a mean of $2.4 \log_{10}$ DNA copies/ 1×10^6 PBMCs. The range of values recorded was wide: 1.0–4.2 \log_{10} DNA copies/ 1×10^6 PBMCs. This was followed by a gradual decrease in copy number in a similar pattern for the case patients and the control subjects. By 12 months after the onset of acute IM, the case patients had a mean of $0.6 \log_{10}$ DNA copies/ 1×10^6 PBMCs, and the control subjects had a mean of $1.3 \log_{10}$ DNA copies/ 1×10^6 PBMCs. Serum EBV loads were also measured in a subset of subjects ($n = 11$)—all were low or undetectable (mean, 4 copies/mL; range, 0–24 copies/mL).

Humoral immune responses. Both IgG and IgM antibodies against EBV VCA were detectable in serum from all subjects at enrollment. There was no significant difference between the case patients and the control subjects in the mean sample:cutoff

optical density ratios, which provide a reasonable estimate of the level of anti-VCA IgG antibodies over time (data not shown). There was also no difference in the rate of avidity maturation of anti-VCA IgG between the case patients and the control subjects (data not shown). By contrast, the case patients developed anti-EBNA-1 IgG somewhat more quickly and at higher levels than did the control subjects (figure 3A and 3B). However, for individual subjects, there was no correlation between the timing of the appearance of anti-EBNA-1 IgG antibodies, the decrease in viral copy number, and the resolution of symptoms.

T lymphocyte subpopulations. Prominent but variable expansions of CD8⁺ T lymphocytes and the activated (HLA-DR⁺) subset of these cells were evident in a significant proportion of subjects at early time points, which decreased over time (figure 4). The observed pattern was consistent with that expected

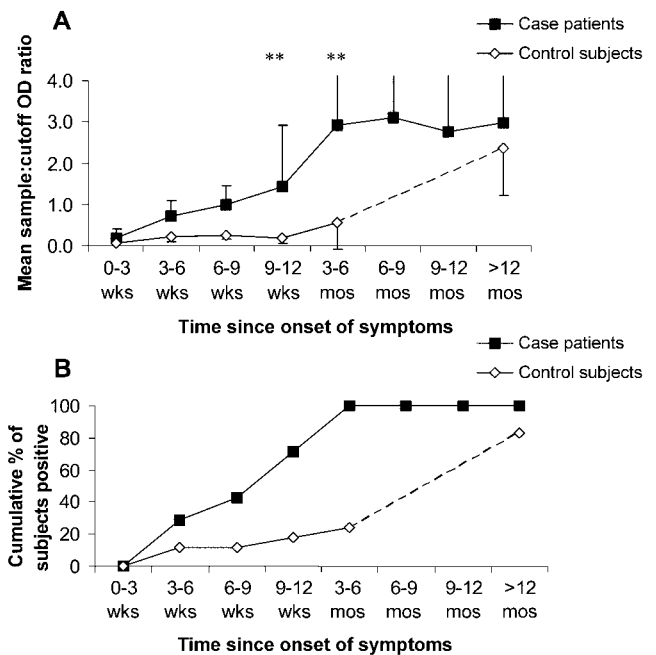


Figure 3. Levels and kinetics of development of anti-Epstein-Barr virus nuclear antigen-1 (EBNA-1) IgG in subjects with prolonged illness (case patients; $n = 8$) and those with prompt recovery (control subjects; $n = 17$) after acute infectious mononucleosis (IM). *A*, Levels of anti-EBNA-1 IgG, shown as mean \pm SD (error bars) are given above for the case patients and below for the control subjects). The case patients had significantly higher antibody levels than the control subjects ($P < .01$, analysis of variance). The differences were significant at 9–12 weeks (** $P < .01$, protected *t* test) and at 3–6 months (** $P < .01$) after the onset of symptoms. The interpolated (dashed) line indicates that samples from control subjects were rarely available between 6 and 12 months. *B*, Kinetics of the development of anti-EBNA-1 IgG antibodies in the case patients and the control subjects. The case patients developed anti-EBNA-1 IgG significantly earlier than did the control subjects ($P < .01$, Kaplan-Meier χ^2 test). The differences were significant at 9–12 weeks (** $P < .01$) and at 3–6 months (** $P < .01$) after the onset of symptoms. mos, months; OD, optical density; wks, weeks.

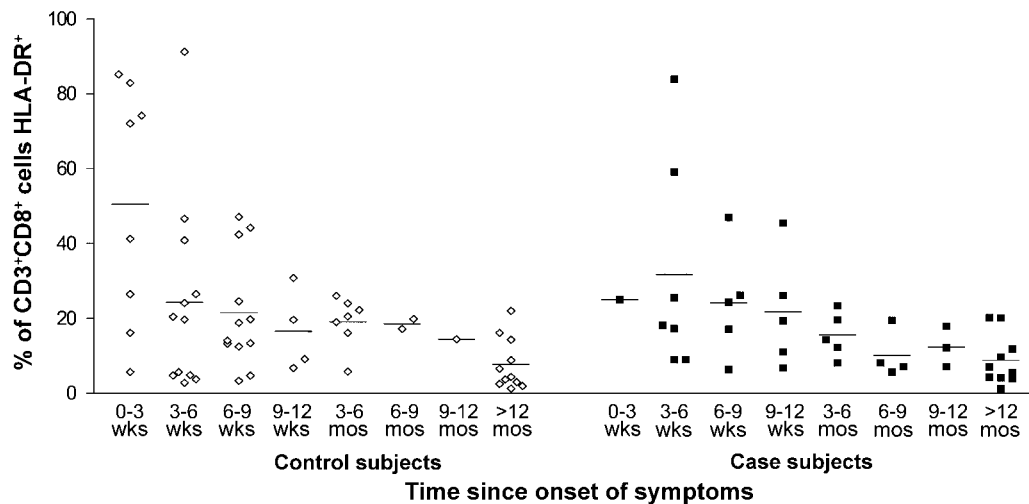


Figure 4. Absence of difference in the proportions of activated CD8⁺ T cells over the duration of infection between the case patients and the control subjects. The expected pattern of a rapid decrease then a steady further decrease in activated CD8⁺ T cells after acute Epstein-Barr virus infection was seen in both the control subjects (*white squares*) and the case patients (*black squares*). The bar indicates median percentage of CD3⁺CD8⁺ peripheral blood mononuclear cells that also expressed HLA-DR. mos, months; wks, weeks.

during the early resolution phase of primary EBV infection. There were no significant differences between the case patients and the control subjects in these patterns.

EBV-specific IFN- γ production from T lymphocytes. Individual case patients and HLA-matched control subjects demonstrated high initial responses that decreased over time, whereas other subjects maintained responses. As was expected, the magnitude of the IFN- γ response to lytic antigens was consistently higher than that produced in response to latent antigens [24]. The magnitude of the highest response to lytic peptides for each subject and the number of weeks after the onset of symptoms required to reach this peak response were not significantly different between groups (figure 5). The peak response and its kinetics of onset did not correlate with either EBV load or the resolution of symptoms.

Individual patterns of response to latent-cycle peptides also varied widely in magnitude and breadth in both the case patients and the control subjects. The kinetics of the onset of a response to any one of the latent-cycle peptides was not different between the case patients and the control subjects (figure 6A). Of those tested by 3–6 weeks after the onset of symptoms, all case patients and all except 1 control subject had a cellular IFN- γ response to at least 1 latent peptide. By contrast, there was significantly slower development of a broad T lymphocyte response to latent-cycle antigens in the case patients, compared with that in the control subjects (figure 6B). By 3–6 weeks after the onset of symptoms, 12 (75%) of 16 control subjects had developed T lymphocyte responses to $\geq 50\%$ of the peptides tested, whereas only 5 (63%) of 8 case patients had developed similar responses. By the time of the late follow-up, all subjects had developed a broad cellular response to latent antigens.

Further analysis of these data that considered peptides grouped according to EBV viral protein families (EBNA-3 and latent membrane protein) did not reveal more substantive differences in the kinetics of the development of a response to multiple epitopes (data not shown).

There was no significant difference between the case patients and the control subjects in the magnitude of the peak response to individual latent peptides. However, for 9 (56%) of 16 peptides tested, the control subjects developed more-prompt peak responses (figure 7), whereas the kinetics were comparable for 1 peptide (6%) and were slower than those of the case patients for the 6 remaining peptides (38%). In contrast to the analysis of the response patterns by subject, this analysis by individual peptide revealed statistically significant differences ($P < .01$, 2-way ANOVA).

DISCUSSION

Host control of EBV replication during acute IM is believed to be primarily dependent on the generation of broadly directed CTL responses [25]. Hence, symptomatic IM is characterized by large, virus-driven expansions of CD8⁺ T lymphocytes [1] that decrease in parallel with the decrease in circulating EBV load [9]. Subjects who have an asymptomatic seroconversion to EBV have been reported to have EBV loads comparable to those measured in subjects with typical, symptomatic IM and well above those measured in healthy seropositive subjects [26]. Consistent with this finding, no correlation between cell-associated EBV load and the severity of IM symptoms was found in the present study. Nevertheless, the EBV loads recorded in the subjects in the study during acute IM and after recovery are in close accord with those

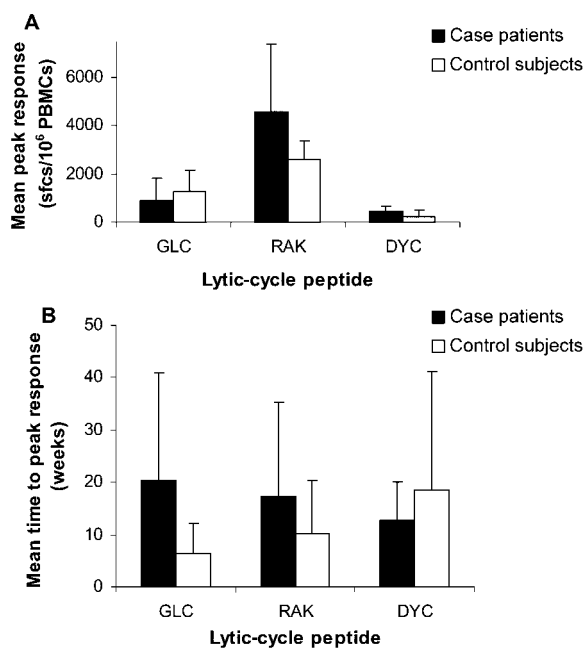


Figure 5. Magnitude and time to development of the peak interferon (IFN)- γ response to lytic peptides. *A*, Magnitude histogram of the mean no. of spot-forming cells per 1×10^6 peripheral blood mononuclear cells (PBMCs) (\pm SD) in 8 subjects with prolonged illness (case patients) and in the control subjects who recovered promptly. *B*, Time (in weeks after the onset of symptoms) to the development of the peak IFN- γ response. Data are group means (\pm SDs). Neither the magnitude of the peak response to lytic-cycle peptides nor the time to development of this response was significantly different between the case patients and the control subjects.

reported elsewhere [23, 27]. The serum EBV loads in the present study were either nondetectable or were very low, which argues against the possibility that exaggerated lytic-cycle replication in circulating B cells could be contributing to the severity or persistence of symptoms, because no increase in the number of free viral copies was evident [28]. Thus, lytic viral replication per se does not appear to be responsible for protracted symptoms in IM. However, given that prolonged shedding in saliva after IM has recently been documented, localized replication in the tonsils remains plausible [29].

Subjects with asymptomatic primary EBV infection do not develop the prominent CD8⁺ T lymphocyte expansions that are typical of symptomatic IM [26]. Thus, it is possible that the severity of symptoms during acute IM is directly related to the degree of the host immune response to the virus, which features T lymphocyte expansion, activation, and cytokine production. Data from primary EBV infection in infants, which is generally believed to be associated with minimal lymphocytosis [30, 31], are consistent with this notion. However, these early studies were neither systematic nor prospective. By contrast, that hypothesis is not supported by the findings reported here—the magnitude of the expansion in the peripheral blood CD8⁺ T

lymphocyte populations (CD8⁺ or CD8⁺DR⁺) did not correlate with reported symptom severity at baseline. It should be noted, however, that this symptom record queried subjects about illness manifestations “over the past few weeks,” whereas the blood sample for flow-cytometric analysis provided a snapshot of the circulating leukocytes at the end of that time period.

Similarly, the longitudinal course of CD8⁺ lymphocytosis did not predict the extended duration of symptoms. In all case patients and control subjects with an expanded population of activated T lymphocytes, levels returned to those typical of healthy individuals, regardless of the duration of symptoms. Again, it is important to note that the true peak of the CD8⁺ lymphocytosis is likely to have occurred before enrollment into the study, because the mean duration of illness from the onset of symptoms to enrollment was 27 days. Hence, no comment can be made on the relationship between the magnitude of the T lymphocytosis during the acute phase and the subsequent duration of illness.

The CTL response is considered to be the principal mechanism of host control of EBV replication during convalescence [10, 25, 32], whereas humoral responses, including the development of neutralizing antibodies against the gp350/220 com-

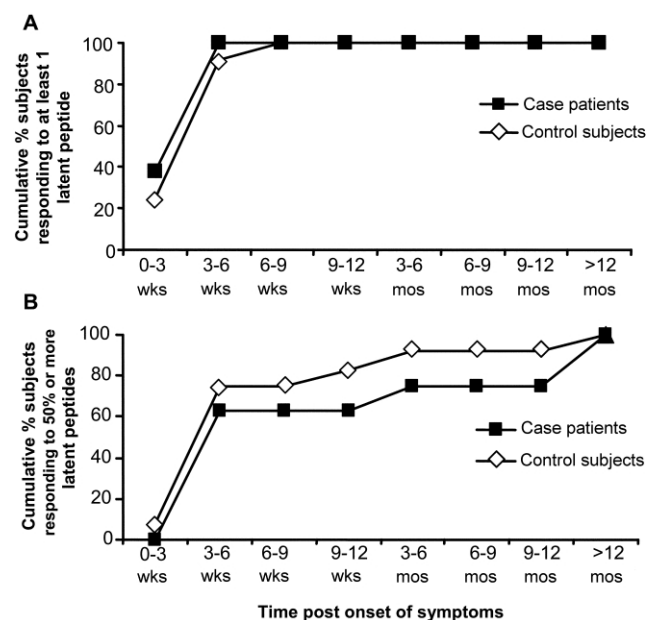


Figure 6. Kinetics of the development of a broad T lymphocyte response to latent epitopes. *A*, Kinetics of the onset of a response to any one latent-cycle peptide in 8 subjects with prolonged illness (case patients) and 17 HLA-matched control subjects who recovered promptly. No significant difference in the time to the onset of response was evident between the case patients and the control subjects. *B*, Cumulative proportion of the groups who had developed a broad T lymphocyte response to stimulation with latent-cycle peptides (defined as $\geq 50\%$ of peptides tested). Slower development of a broad latent antigen response was evident in the case patients ($P = .016$, analysis of variance). The significant difference occurred only at the 9–12-week time point.

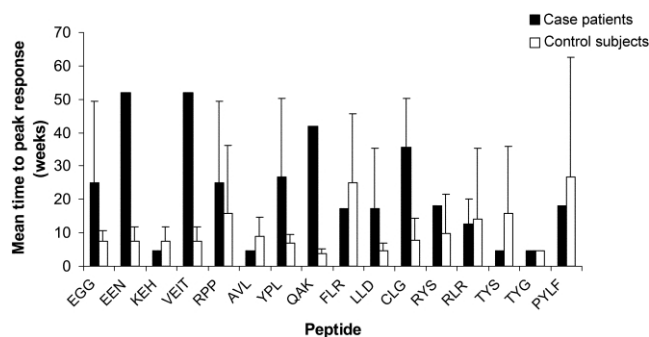


Figure 7. Time (in weeks after the onset of symptoms) to the development of the peak interferon- γ response of T lymphocytes stimulated with latent-cycle peptides. Data are group means (\pm SDs).

ponent of the membrane antigen, are typically delayed in appearance and are believed to be of limited importance [33]. In response to peptides derived from the EBV lytic-cycle proteins BMLF1 and BRLF1, both the case patients and the control subjects in the present study generally demonstrated strong IFN- γ responses, as detected by ELISPOT assay. There was no correlation between the kinetics of onset or the magnitude of this CTL response and the severity of symptoms at baseline. In contrast to the results of our previous report [13], no clear association between the dynamics of development of a broad CTL response against latent-cycle antigens and the resolution of symptoms was observed, although the case patients tended to develop their peak IFN- γ response to a latent peptide more slowly than did the control subjects.

Perhaps surprisingly, the most significant difference in host response found in the present study between the case patients and the control subjects was in the kinetics of development and the level of IgG anti-EBNA-1 antibody production. This antibody response was detectable earlier and at higher levels in the control subjects than in the case patients. This contrasts with the classical studies by Henle et al. [34], which led to the dogma that the development of anti-EBNA antibodies was associated with the convalescent period after IM. Given that the mean duration of illness for the case patients was markedly longer than that for the control subjects, this finding suggests that the development of anti-EBNA-1 antibodies should not be considered a marker of convalescence but simply a delayed humoral response to primary infection. Because the expression of the 6 nuclear antigens (EBNA-1–6) marks the establishment of latent infection in B lymphocytes, this finding suggests that the dynamics of EBV infection in vivo and the release of soluble antigens into the circulation may have been more rapid in the case patients, thereby triggering a more brisk humoral response. However, the baseline EBV loads did not differ significantly between the case patients and the control subjects, although the degree of variation in this parameter between subjects may

have precluded any reliable exclusion of a significant difference between groups. In addition, analysis of the kinetics of the peak CTL response to individual latent antigens determined that their response was somewhat slower in the case patients. In combination, these data therefore suggest that the integrated pattern of host response to latent antigens may be altered in subjects with prolonged illness after IM. In particular, the development of humoral rather than cellular responses may reflect a Th2 bias in the CD4⁺ T cell regulation of the early response. This bias may be favored by viral factors, such as the production of the viral homologue of interleukin (IL)-10 encoded by *bcf-1* [35], or by host factors—for example, polymorphisms in relevant cytokine genes, including that for IL-10—that have been shown to be linked to susceptibility to symptomatic EBV infection [36].

The principal conclusion that can be drawn from these data are that the sometimes prolonged duration of symptoms associated with IM in previously healthy adults cannot be easily explained by differences in circulating EBV loads or by alterations in host responses to EBV. Interestingly, data from the present cohort demonstrate that personality style (e.g., neuroticism) and psychological disorder (e.g., depression) do not predict prolonged illness (I.H., T. Davenport, D.W., U. Vollmer-Conna, B.C., S. Vernon, W. Reeves, and A. Lloyd, unpublished data). Differences in the magnitude and duration of the cytokine response could explain the ongoing symptoms. Indeed, correlations between the production of the proinflammatory cytokines IL-1 and IL-6 and acute-phase symptoms have been demonstrated in this cohort [37]. However, we have recently found that the levels of proinflammatory cytokines did not remain elevated in the case patients (U. Vollmer-Conna, B.C., I.H., T. Davenport, D.W., R. Nisenbaum, S. Vernon, W. Reeves, and A. Lloyd, unpublished data). Accordingly, we propose that alternative neurobiological mechanisms triggered during the severe, acute illness and sustained in the absence of ongoing peripheral inflammation underpin prolonged illness after EBV infection.

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