Epstein–Barr virus (EBV) is a ubiquitous human lympho-cryptovirus (LCV) associated with cell proliferative disorders, such as infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma and chronic active EBV infection (Macsween & Crawford, 2003). More than 90 % of the world’s human population carries EBV asymptomatically as a life-long persistent infection (Cohen, 2000). EBV infection is usually latent but occasionally reactivation of EBV occurs. It has become accepted in recent decades that psychological stress can impair immune function by a complex network of the central nervous, endocrine and immune systems (Glaser & Kiecolt-Glaser, 2005). There has been an accumulation of literature on the psychosocial modulation of latent infections of various herpes viruses (Kiecolt-Glaser & Glaser, 1987) including EBV. For example, university students showed significant increases in antibody titres against EBV viral capsid antigens (VCA) during examination periods (Sarid et al., 2001) and high-loneliness students had significantly higher EBV antibody titres than low-loneliness students (Glaser et al., 1985); higher levels of EBV shedding into saliva have been detected in astronauts in association with the space flights (Payne et al., 1999; Stowe et al., 2001; Pierson et al., 2005).

Infections with LCVs closely related to human EBV are highly prevalent in Old World monkeys and apes (Ishida & Yamamoto, 1987) and reactivation of LCV in macaques under stress was also documented (Ishida et al., 1993). Chimpanzee’s immune system is very similar to that of humans and species-specific LCV (EBVcmp) exists (McGeoch et al., 2005). Nine male chimpanzees originally reared in solitary cages were set up to form a group. Plasma viral load of the lymphocryptovirus (LCV) of chimpanzee [Epstein–Barr virus chimpanzee (EBVcmp)] was measured by real-time PCR. In the group formation (Form) period, the first-ranking male showed an imminent increase in plasma EBVcmp load compared with 1 week before (pre-Form) and 3 months after (post-Form) group formation. Other upper-ranking males such as the second-, third- and fourth-male also showed the highest level of viral load in the Form period. The kinetics of EBVcmp load in the Form period were statistically different from other periods (against pre-Form, \( t = -4.878, P < 0.001 \); against post-Form, \( t = 6.434, P < 0.001 \)). The effect of the male dominance rank did not differ between the pre-Form and post-Form periods (\( t = -1.557, P = 0.12 \)). Reactivation of LCV (EBV) as an immunological stress marker for humans might also be applied to chimpanzees.
presence of IR1 of EBVcmp. The familiarization procedure started a month before group formation. The chimpanzees were reared in individual cages and every chimpanzee was exposed to the other eight chimpanzees one by one through the cage. One week before group formation, to reduce unfamiliarity among the subjects and to reduce conflicts during the group formation process, each male was put in an indoor cage in which the male could have visual contact with other subjects in the neighbouring cages. The facility consisted of individually separated indoor runs and an outdoor enclosure, which are connected by corridors. During the group formation period and afterwards, the subjects were placed in the outdoor enclosure for 1 h (13:30–14:30) everyday and for the rest of the day they stayed in the individual indoor runs.

Behavioural observations were conducted while the chimpanzees were in the outdoor enclosure from 13:30 to 14:30 for 13 days after the start of the group formation process. Throughout this study, the subjects’ behaviour was recorded by the all-occurrence sampling method (Altmann, 1974). Among the behavioural data, pant-grunt vocalization was adopted as the behavioural parameter to determine the rank of the chimpanzees (Noé and de Waal, 2003). To avoid the omission of pant-grunts, which we used for later confirmation, we designed a novel primer set (9-GGGTTCGCGTTGCTAGGC-3’ and 5’-GGGAT-CCTGGAT-GCGGAGTCA-3’). Using this set, we amplified part of the IR1 region of EBVcmp and calculated the viral load by real-time PCR assay.

Real-time PCR was performed using the LightCycler (Roche) to measure the concentration of EBVcmp in plasma. SYBR Green I was used to label PCR products following the manufacturer’s instruction (Roche). For the standard concentration of DNA, the Raji cell line was prepared for DNA extraction. A calibration curve was run in parallel with each analysis, using 10-fold serial dilutions of DNA extracted from an EBV-positive cell line (Raji) as a standard. Raji is a diploid cell line containing EBV viral genome 50 copies per cell (Nonoyama & Pagano, 1973). A conversion factor of 6.6 pg of DNA per diploid cell (Saiki

### Table 1. Nine chimpanzees of this study

<table>
<thead>
<tr>
<th>Identity</th>
<th>Dominance rank</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Origin</th>
<th>Separation from mother (days)</th>
<th>Rearing*</th>
<th>Group housing experience</th>
</tr>
</thead>
<tbody>
<tr>
<td>KE</td>
<td>1</td>
<td>19</td>
<td>57.4</td>
<td>Captivity</td>
<td>0</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>LN</td>
<td>2</td>
<td>34</td>
<td>75.4</td>
<td>Wild</td>
<td>–</td>
<td>Imported at 6 years of age</td>
<td>+</td>
</tr>
<tr>
<td>KZ</td>
<td>3</td>
<td>16</td>
<td>60.8</td>
<td>Captivity</td>
<td>390</td>
<td>Mother in a group</td>
<td>+</td>
</tr>
<tr>
<td>TK</td>
<td>4</td>
<td>16</td>
<td>56.3</td>
<td>Captivity</td>
<td>388</td>
<td>Mother in a group</td>
<td>+</td>
</tr>
<tr>
<td>JM</td>
<td>5</td>
<td>11</td>
<td>56.1</td>
<td>Captivity</td>
<td>2866</td>
<td>Mother</td>
<td>+</td>
</tr>
<tr>
<td>KA</td>
<td>6</td>
<td>14</td>
<td>51.0</td>
<td>Captivity</td>
<td>106</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>MK</td>
<td>7</td>
<td>12</td>
<td>56.8</td>
<td>Captivity</td>
<td>1558</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>NH</td>
<td>8</td>
<td>17</td>
<td>44.6</td>
<td>Captivity</td>
<td>205</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>MN</td>
<td>9</td>
<td>12</td>
<td>47.8</td>
<td>Captivity</td>
<td>3131</td>
<td>Human</td>
<td>+</td>
</tr>
</tbody>
</table>

*Human rearing: mother–infant separation because of mother’s rejection (younger than 1 year).
et al., 1988) was calculated for the quantity of EBV. The cycling programme was as follows: incubation at 95 °C for 10 min was followed by 50 cycles of amplification at 95 °C for 15 s, 62 °C for 10 s, 72 °C for 12 s and then 86 °C for 1 s. The assay was performed in triplicate and the average value of the three experiments was used in further statistical analyses.

The data of EBVcmp was log-transformed in order to fit a normal distribution and was analysed by the General Linear model. The male dominance rank, sampling periods and interaction between these two variables were fitted as independent terms. All analyses were two-tailed and the significance level was set at 0.05.

The results of titres of antibodies against EBVcmp-VCA did not show trends between the titres and the sampling period or the rank (Fig. 1).

The kinetics of EBVcmp load in plasma are shown in Fig. 2. In the group formation period, the value of the plasma level of EBVcmp load of the newly qualified first-ranking male (alpha-male) showed an immediate increase compared with the pre-Form and post-Form periods. Other upper-ranking males, such as the second-, third- and fourth-male also showed the highest level of viral load in the group formation period. In the meantime, lower-ranking males showed different patterns; the sixth-male demonstrated a gradual decrease as group formation progressed. The seventh-male experienced the lowest viral load in the group formation period. The higher the rank, the higher the viral load in the Form period became. The viral loads in the fifth-, eighth- and ninth-male fluctuated throughout the three periods. The interaction between the male dominance rank and the sampling period was significant ($P<0.0001$). In the graph, the slope of the Form period was statistically different from other sampling periods (against pre-Form, $t=-4.878$, $P<0.001$; against post-Form, $t=6.434$, $P<0.001$). The effect of the male dominance rank did not differ between the pre- and post-Form periods ($t=-1.557$, $P=0.12$).

The level of EBVcmp viral load in plasma was significantly higher in the dominants than in the subordinates during group formation. This result indicates that the viral reactivation occurred more intensely among high-ranking males. The relationship between the dominance hierarchy of animals in a group and psychological stress, especially from an endocrinological point of view, has been widely argued. It has been shown that socially high-ranking individuals (dominants) are more psychologically stressed than the low-ranking ones (subordinates) in some species including chimpanzees (Creel, 2001; Muller & Wrangham, 2004), where the dominants assert intensive aggression towards their subordinates. Thus, it has been suggested that dominants are exposed to more severe psychological stresses because they must maintain their rank and status. The instability of rank in animal groups is known to enhance this tendency (Sapolsky, 2005).

It has been well documented that psychological stresses caused by higher dominance not only increase stress hormone levels, but also depress the immune status. The dominant mouse showed a significantly higher reactivation of latently infected herpes simplex virus type 1 than the subordinate mice (Padgett et al., 1998). Suppression of immunity in the dominant chimpanzees was demonstrated using immunoglobulin levels, where higher-ranking subjects showed lower levels of immunoglobulins (Masataka et al., 1990). Such immune status in the dominants probably results in the reactivation of latently infected viruses. It is confirmed that glucocorticoid hormones can reactivate latent EBV in vitro and can also enhance the lytic
replication of the virus in cells superinfected with infectious EBV (Glaser et al., 1995).

Group formation referred to in this work might illustrate the situation where the ranking relationships among the chimpanzees were unstable, which in itself could have increased the psychological stress, especially among the chimpanzees that attained the dominant positions. The dominant chimpanzees could be threatened by the challenges from the subordinates, who did not have so much to lose as to feel further psychological stresses. This may explain why the subordinates did not experience the rise in EBVcmp viral load after the group formation process, unlike the dominants. Three months after group formation, the viral load in the dominants decreased and reached the levels before the group formation process. This might indicate that psychological stress reduced over the period of the experiment as each chimpanzee established its rank and became accustomed to the new environment it had achieved.

In our study, the kinetics of antibody titres against EBVcmp-VCA did not synchronize with that of EBVcmp viral loads. It is conceivable that the elevation of the plasma viral load precedes the rise in the antibody titre, since the reactivation of latent LCV triggers the elevation in antibody titres when the viral antigens are synthesized and released from the infected cells. The elevation in antibody titres might have occurred after the Form period, when we only observed the increase in viral loads. Similar discrepancies regarding different methods for detecting EBV reactivation were reported in several studies of humans (Gärtnert et al., 2000; Luderer et al., 2005; Hoffmann et al., 2010). Furthermore, it is suggested that for healthy subjects serology runs the risk of underestimating the frequency of asymptomatic EBV reactivations (Maurmann et al., 2003).

This study demonstrates a distinct rise in EBVcmp among dominant chimpanzees and suggests that reactivation of LCV (EBV) as an immunological stress marker might possibly be applied to chimpanzees as well as humans. Since each Old World monkey and ape species harbours its own LCV, viral load of LCV could be a general tool for monitoring psychological stresses among them.

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References


