CHAPTER VI

DISCUSSION

The important role of EBV-specific CTL (EBV-CTL) in controlling EBV infection has been documented in a number of studies (11, 13, 145, 146). In this study we analysed the EBV- specific T cell response in HIV-infected patients and compared the response with healthy donors. We also demonstrated the relationship between EBV-specific T cell response and cell-associated EBV-DNA by our newly-developed in-house real-time PCR.

All of HIV-infected patients and healthy controls were test for anti-VCA IgG by ELISA. The results showed significantly higher level of anti-VCA IgG in HIV-infected patients than that in healthy control (Table3). High anti-VCA IgG in HIV-infected patients was also shown by Stevens et al (147).They found an increase of anti-VCA IgG together with a decrease of anti-EBNA-1 IgG level in the patients who had high EBV viral load which indicated the impairment of latency immune control.

To quantitate the EBV-DNA, we developed an in-house real-time PCR. The PCR assay was based on amplification of 297 bp fragment of *EBNA-1*. In addition, we used LightCycler-based PCR which has been reported as sensitive, rapid and reliable method (148-151). Several specimens such as serum (152-154), plasma (84, 155), whole blood (156-158) and PBMC (159, 160) could be used to determine EBV load. Since monitoring cell-associated EBV-DNA from PBMC was reported as the effective way to predict the development of EBV PTLD in recipients of T cell–depleted transplants (161) we therefore developed real-time PCR for detection of EBV-DNA in PBMC of healthy donors and HIV-infected patients.

Our real-time based PCR was proved to be both sensitive (as few as 10 copies of EBV-DNA could be detected) and specific (no cross amplification of other Herpesviruses-DNA). Moreover, our assay showed to be accurate and reproducible with intra- and inter assay of less than 0.5 $\log_{10}$ range which was comparable to the assay developed by other group (151).

We were able to detect EBV-DNA in EBV-seropositive healthy donors ranging from <10 copies to 57 copies/μg DNA with a median <10 copies per μg DNA. This finding was consistent with previous report which showed absence or low level of

EBV-DNA in healthy control (162). On the other hand, the EBV loads in HIV-infected patients were ranging from <10 to 3,785 copies/μg DNA with a medium of 38 copies/μg DNA. Similar to other reports (163, 164), the results revealed a significantly high EBV viral load in HIV-infected patient compare to that in healthy control ($p<0.05$). The high EBV-DNA in HIV-infected donors might be due to impairment of EBV-specific $CD8^+$ T cell response (165).

We investigated the EBV-specific T cell responses in HIV-infected patients and in healthy control in order to understand the role of the T cell response. The results demonstrated that EBNA-3 was an immunodoninant protein. The immunogenicity of EBNA-3 has been reported by other scientists (166, 167). Whitney et al. studied the EBV-specific T cell responses in Asian population (26 nasopharyngeal carcinoma patients and 50 healthy donors) by peptide-based ELISpot. They found the strongest responses were to epitope in EBNA-3 protein. Similarly, Blanke et al. analysed the EBV-specific $CD8^+$ T cell responses in HLA B*3501-positive donors by peptide-based ELISpot and found that the epitope in EBNA-3 protein was immunodominant. Moreover, we found that the protein mediating the highest T cells responses in this study was EBNA-3b (E3b). This E3b-specific T cell response was strongest both in EBV-seropositive healthy donors and HIV-infected donors.

On the other hand, the $CD8^+$ T cell responses to E3a were frequently recognised by EBV-seropositive healthy donors than in HIV-infected donors. While E3a was recognised by 5 out of 10 (50%) EBV-seropositive healthy donors, only 1 out of 10 (10%) HIV-infected patient was able to recognise this protein. This may be due to inadequate EBV-specific immune responses and lead to escape mutation in these HIV-infected donors. Lacking of $CD8^+$ T cell response may results in loss control of EBV infection leading to high EBV-DNA load and EBV-associated malignancies.

When considering the correlation between EBV-specific CD8+ T cell responses and EBV-DNA load, the positive trend was observed in healthy EBV-seropositive group, in contrast, the negative correlation trend was found in HIV-infected donors. Similar to our observation, Legoff et al demonstrated the inverse correlation between the levels of cell-associated EBV-DNA and the EBV-specific $CD8^+$ T cells responses in longitudinal study of HIV-infected patients harbouring high EBV viral load (1,000 copies/10^6 PBMC) (165). Furthermore, they found impairment of EBV-specific $CD8^+$ T cell response in both intensity and spectrum to EBV-peptides of these patients when

compared with healthy control. Moreover an inverse correlation between number of functional EBV-specific $CD8^+$ T and EBV viral was also shown in HIV-infected donors progressing to non-Hodgkin lymphoma (168). They also demonstrated that these patients obtained the normal number of EBV-specific $CD8^+$ T cell comparing with other HIV-infected control patients. However, the T cell lost the capacity to produce IFN-γ in response to EBV-peptide. Taken together, these emphasized the important role of EBV-specific $CD8^+$ T in controlling EBV-infection.

Previously reports and our study demonstrated that EBV-specific T cell function was impaired or loss in HIV-infected patients (165, 168, 169), who ultimately might develope EBV-associated lymphoma. Reconstitution of EBV-specific T cell response might help prevent the patient at-risk of developing EBV-associated disease (9, 11, 170). Therefore, we studied the feasibly to establish EBV-CTL from HIV-infected patients and healthy donors and evaluate their cytotoxic function. Here, we were able to establish EBV-specific CTL from HIV-infected patients with success rate of 100 %. The 100% success rate was also found in establishment of EBV-specific CTL in patient with EBV-positive relapsed Hodgkin's Disease by Rokcrow et al (170). On the other hand, success rate in EBV-seropositive healthy donors was only 70%. The lower success rate in healthy donor might be influenced by many factors such as the low EBV-specific CTL precursor in healthy donor (171), the variation of EBV local strain when compared with B95-8 which used as EBV-source in establishing EBV-CTL (172, 173).

When EBV-specific CTL phenotype was analysed, we found that most of them had $CD8^+$ phenotype. Indeed, all EBV-specific CTL lines from HIV-infected patients were mainly of $CD8^+$ T cells phenotype (100%) whereas relatively high proportion of $CD4^+$ T cell could be observed in CTL lines established from EBV-seropositive healthy donors (Table 13). Mixture of $CD4^+$ and $CD8^+$ T cells was also shown in other studies establishing EBV-CTL from healthy donors and transplant patients (174, 175).

We evaluated cytotoxic function of the established EBV-CTL lines by Chromium release assay. The cytotoxic function of CTL lines deriving from HIV-infected donors was unexpectedly higher than those from healthy donors (46±13 vs. 23±17% at an E:T ratio of 6.25:1, table 14). The higher cytotoxic function in HIV-infected donors might be from the fact that the CTL lines obtained from these donors had higher proportion of $CD8^+$ T cells.

In conclusion, this study nicely demonstrated the importance of EBV-specific T cell responses in controlling EBV infection. Indeed, when donors were in immunosuppressed condition such as being infected with HIV, their EBV-specific T cell responses were suppressed leading to loss of EBV control and high EBV viral load. Reconstitution of EBV-specific T cell response either by adoptive T cell therapy or therapeutic vaccine may therefore protect these HIV-infected donors from progressive to EBV-associated diseases.