行政院國家科學委員會專題研究計畫成果報告

計畫編號名稱：第八型人類皰疹病毒與愛滋病毒間互相活化的探討

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一、中文摘要

第八型人類皰疹病毒（Human herpesvirus 8; HHV8）最早由卡波希氏瘤（Kaposi's sarcoma）中發現，是目前人類皰疹病毒群中最新的成員。雖然此病毒與卡波希氏瘤及體腔淋巴瘤（body cavity lymphoma）有極強的相關性，顯示此病毒應有相當臨床角色，但實際上對此病毒在一般大眾的感染情況及可能引起疾病仍所知十分有限。為何卡波希氏瘤在愛滋病患身上會大量增加可能有兩種解釋，第一個可能是愛滋病患由於免疫系統遭受破壞，對惡性腫瘤監視清除能力下降，因此較容易出現卡波希氏瘤．另一個可能解釋是愛滋病毒可以激發第八型人類皰疹病毒的活性，因而導致較多的卡波希氏瘤．此外另一個重要的話題是第八型人類皰疹病毒會不會影響愛滋病毒的活性，導致愛滋病病程加速．因此這個計畫就是要了解這兩種病毒的交互作用。我們的結果發現這兩種病毒互相可活化對方。將帶有第八型人類皰疹病毒的細胞與帶愛滋病毒的細胞融合後，如先刺激帶愛滋病毒的細胞則第八型人類皰疹病毒的 RNA 製造增加，反之亦然。將 HIV-1 LTR-CAT 送進 BCBL-1 細胞後，LTR 的轉訊亦被將強，表示 HHV8 可活化 HIV-1 的 LTR。目前我們更進一步確定 LTR 上那個序列可被 HHV8 活化，也測試 HIV-1 的 Vpr 及 Tat 能否促進 HHV8 繁殖。

關鍵詞：愛滋病毒，第八型人類皰疹病毒，Vpr, Tat

Abstract

Human herpesvirus 8 was discovered through the etiology search of Kaposi's sarcoma. This virus was soon identified to belong to human herpesviruses. To date, HHV8 has been shown to be highly associated with some malignancies, such as Kaposi's sarcoma and body cavity lymphoma. The remarkable higher incidence of Kaposi's sarcoma in HIV-infected patients can be explained in two ways. One is that HIV infection leads to immune disruption and impaired tumor surveillance in the host. The immune dysfunction is the direct reason of occurrence of Kaposi's sarcoma and HIV plays an indirect role. Another possibility is HIV can turn on the replication of HHV8. Increased HHV8 activity in turn leads to the occurrence of Kaposi's sarcoma. In this aspect, HIV plays a more direct role. We have done a series of cell-fusion experiments and found that HHV8 could increase the RNA amounts of HIV-1. The reverse was also true. LTR-CAT produced significant more amounts of CAT when transfected into BCBL-1 cells and stimulated with TPA. This result demonstrated that HHV8 could transactivate HIV-1 LTR. We are currently exploring the cis-elements on HIV-1 LTR that is responsive to HHV8. Furthermore, Vpr and Tat are being tested for their ability to activate the replication of HHV8.

Keywords: HIV-1, HHV8, Vpr, Tat

二、緣由與目的

Kaposi's sarcoma (KS) is the most common neoplasm occurring in persons with the acquired immunodeficiency syndrome (AIDS); approximately 15 to 20% of AIDS patients develop this neoplasm, which is 20000-fold the KS risk of the general population [1]. Epidemiologic data indicate that AIDS-associated KS (AIDS-KS) may have an infectious etiology and that human immunodeficiency virus (HIV) is not the sole determinant of KS risk. First, the tumor also occurs in selected HIV-negative groups, including immunosuppressed transplant recipients and some African and Mediterranean populations. Second, even among HIV infected individuals, the risk for KS

varies widely, with high rates observed in HIV-positive homosexual men and very low rates among HIV-infected hemophiliacs and children [1,2]. These and other data suggested that a second, sexual transmitted cofactor may be involved in KS etiology or pathogenesis. Extensive investigations to implicate known agents such as cytomegalovirus (CMV), human herpesvirus type 6 (HHV-6), HIV, human papilloma virus and Mycoplasma penetrans in KS etiology have failed. Recently, a new herpesvirus-like DNA sequence, termed KS-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8), has been identified in KS tissue by polymerase chain reaction (PCR)-based methods [3]. Emerging evidence suggests that this is an entirely unique herpesvirus and contains 270 kb, making it the largest known herpesvirus. These sequences are found in more than 90 percent of AIDS-KS tissues, and in the majority of HIV-negative KS cases as well [4-8]. Besides, HHV8 sequences have been found in Castleman's disease [9], a rare lymphoproliferative disorder often associated with KS, and in peripheral effusion lymphomas, unusual high-grade body cavity-based lymphomas in HIV-infected patients[10]. Viral DNA is generally absent from non-KS tumors and other tissue specimens from individuals lacking KS risk factors[11]. In AIDS patients who were HHV8 positive in peripheral blood mononuclear cells (PBMC) found by PCR but did not have KS at the time of testing, the risk of subsequent KS development was substantially higher than those in whom HHV8 was not detected [12].

The higher incidence of Kaposi's sarcoma in patients infected with HIV may be due to immune suppression which leads to impaired tumor surveillance. This view is supported by the frequent occurrence of various tumor in HIV-infected patients [13]. There also exists a possibility that HIV-1 will increase the replicative activity of HHV8 and leads to much higher incidence of Kaposi's sarcoma in HIV-infected victims. In line with this reasoning, Harrington et al. studied the effect of Tat protein on HHV8 [14]. By adding Tat to the medium, they found that HHV8 DNA was significantly increased in cells harboring this virus. Hence, HIV-1 may activate HHV8 at least through Tat protein. It is also likely that interaction between these two viruses may be more complicated. In this proposal, we want to test the impact of HIV-1 on HHV8 and vice versa. Cells harboring HIV-1 and HHV8, respectively, will be induced to fuse and the activity of both viruses be monitored. Although HHV8 and HIV-1 seem to infect different cells, HIV-1 infection may induce syncytial formation. Therefore, fusion experiment in this project may mimic in vivo scenario. Furthermore, fusion assay has the maximal potential to unveil the interaction between HHV8 and HIV-1. In contrast to the approach taken by Harrington et al. [14] who specifically test one viral protein (Tat in this case), fusion assay has the best chance to reveal all possible interactions between these two viruses, no matter what viral products mediate this interaction. Also, this approach can reveal two-way interaction. It is also highly desirable to see if HHV8 can activate HIV replication and affect the speed of progression to AIDS. The results coming out from this project should have implication on both clinical and basic understanding of these two viruses.

三、結果

(一) HHV8 與 HIV 可以互相活化

HHV8 在分類上隸屬於γ-herpesvirus 的一員，其特徵是以淋巴細胞為潛伏感染之宿主；然而卡波西氏瘤(Kapopsi's sarcoma，簡稱KS)為一內皮贅瘤，因此，HHV8 由潛伏期到細胞溶解期的 變不僅是代表病毒增殖，同時也可能是造成KS生成

的關鍵。雖然已證實：在培養 BCBL-1 時加入 HIV 的調控蛋白 Tat，可增加細胞中 HHV8 DNA 的複製，但單一蛋白的作用並不能完全概括 HIV 和 HHV8 的交互影響。我們嘗試以 PHA 和 PMA 刺激 ACH2 或 U1 細胞中潛伏的 HIV 後與 BCBL-1 的細胞融合之方法，來探討 HIV 在細胞內增殖時是否對潛伏的 HHV8 具有激發作用；另外，利用經 TPA 刺激的 BCBL-1 分別與 ACH2 和 U1 的細胞融合，以偵測 HHV8 活化 HIV 的情形。從以往的研究中發現：在卡波西氏瘤(Kapopsi's sarcoma，簡稱 KS)病理組織中可偵測出高量的 IL-6 (interleukin-6)、bFGF (basic fibroblast growth factor)、PDGF (platelet-derived growth factor) (15-18)；在以 nude mice 為實驗動物的研究中也指出：inflammatory cytokine 誘導 KS 細胞產生 bFGF，並形成典型的 spindle-shaped 腫瘤細胞(19,20)；此外，許多證據都顯示 KS 的產生與 cytokine 的異常表現有關(21)。而 HHV8 本身即帶有一連串的 inflammatory cytokine 同源基因，vIL-6 基因是其中之一。vIL-6 目前已知屬於細胞溶解前期(early lytic phase)表現的基因(22)。在 HIV 活化 HHV8 的實驗設計中，以 vIL-6 基因的表現程度為指標，應更能適切反應 HIV 在 HHV8 病理發生學上的可能角色。由 Fig.1 的結果可看出，當 ACH1 中的 HIV 經刺激而活化後，BCBL-1/ACH2 融合細胞的 HHV8 vIL-6 表現量顯著增加。至於 U1 細胞在未刺激的情況下與 BCBL-1 融合，即能誘導 vIL-6 表現，可能是分化程度不同的細胞株對細胞融合作用之反應有所差異的結果。Tat-responsive target sequence 簡稱 TAR，是位於 HIV-1 transcript 的 5' 端，具 stem-loop 結構。Tat 在與 TAR RNA 結合後，能進一步促進 HIV 的轉訊作用(23)。BCB1-1 在刺激後，不論是在 ACH2/BCBL-1 或 U1/BCBL-1 融合細胞中，TRA RNA 表現量皆有增加的現象。綜合以上的觀察結果，可以得知 HIV-1 和 HHV8 之間的確有相互活化的交互作用發生。

四、討論

HIV-1 在受感染細胞中的表現是藉由病毒 LTR(long terminal repeat) 上的 cis-regulatory element，和宿主細胞的

錄因子所產生的交互作用。位於轉錄始點上游的 LTR 可以區分成數個功能性區域，其中包括 錄因子 NF-κB 和 Sp1 的辨識位置(24)。我們目前已將全長的 LTR 和其 deletion mutant(Fig.2)接於帶有 CAT 報告基因的質體上，並 染(transfect)經 TPA 前處理的 BCBL-1 細胞，以便進一步瞭解 HHV8 活化 HIV 的分子機制。初步結果(Fig.3(a))顯示：HHV8 能作用在 括 NF-κB 及 Sp1 辨識序列在內的 LTR，促使 CAT 基因表現。有研究報告指出：人類巨細胞病毒(human cytomegalovirus，簡稱 HCMV) 的 IE2-82 基因產物主要作用在 HIV LTR 的 -120 至-20 位置之間的幾個區段，HCMV 可能藉此正向調控 HIV 的生命週期，導致同時感染 HCMV 和 HIV 的個體其臨床症狀之惡化(25)。由於 NF-κB 和 Sp1 辨識序列正位於-105 和-43 之間，意謂著 LTR 的 NF-κB 和 Sp1 site 在 HHV8 和 HCMV 活化 HIV 的作用上扮演相當重要的角色。

TPA 為一腫瘤促進劑(tumor promoter)，能促使基因表現和包括 HIV-1 在內的病毒複製。目前已知 TPA 可透過兩種途徑來影響細胞因子，藉以增加 HIV LTR 的活性。一是 TPA 先誘導 PKC (protein kinase C)的活化，使 NF-κB 和 IκB 解離，進而與 LTR 上的 NF-κB sit 結合，在 Tat 蛋白的協同作用下，提高 LTR 的活性(26)。另一途徑是 TPA 會調節細胞合成數種 TAR RNA 結合蛋白(TAR RNA binding protein，簡稱 TRBP) 來活化 LTR(20)。不過新證據顯示：細胞若以 TPA 處理 24 小時以上，反而會抑制 PKC 的活性，並降低 HIV LTR 的基礎表現(27)。為了釐清在本實驗中所使用的條件下，TPA 對 HIV LTR 何直接影響，故選擇無病毒感染的 Jurkat 細胞株為對照組。將 Jurkat 和 BCBL-1 做相同處理，即 TPA 作用 72 小時後，再進行 HIV LTR 及其 deletion mutant 的 染。在比較經 TPA 前處理和未處理的 Jurkat 於 HIV LTR 活性

表現上的差異時(Fig. 3(b))，我們觀察到前者在包含 NF-κB 和 Sp1 site 之 動子的活性表現上有些微下降的趨勢；此結果和以往的研究報告一致，同時也再次驗證 HHV8 有活化 HIV 的作用，而非 TPA 所造成的現象。

因 HIV 感染所引發的伺機性感染一直是臨床學上亟待解決的重要課題，而卡波希氏瘤在愛滋病患族群的高發生率，更說明了 HIV 和 HHV8 之間存在著密切的關聯。藉由揭櫫 HIV 和 HHV8 在分子生物學層次上的交互作用，或可提供基因治療發展上之基礎。

五、計畫成果自評

已確定 HIV-1 與 HHV8 之交互作用。也確定交互作用透過 HIV-1 LTR。成果達到原先的預期。

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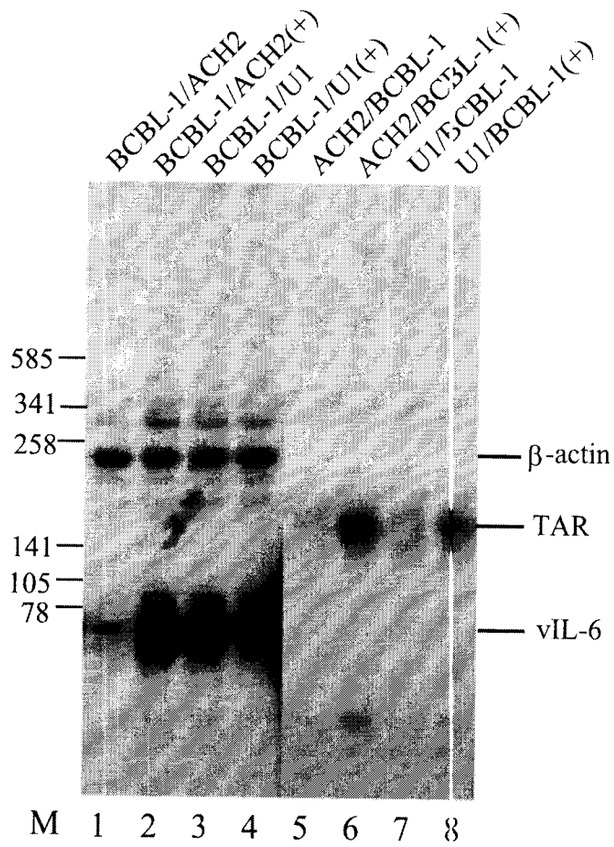


Fig.1 Revelation of the interaction between HHV8 and HIV. 50 μg of total RNA was isolated from cell (BCBL-1, lane1-4; ACH2, lane 5, 6; U1, lane7, 8) fused with uninduced (lane 1, 3, 5, 7) or induced (ACH2, lane 2; U1, lane 4; BCBL-1,lane6, 8) and incubated with 3x10^5 probe to perform RPA. M: size maker. Protecting probes are indicated at the right of the panel.

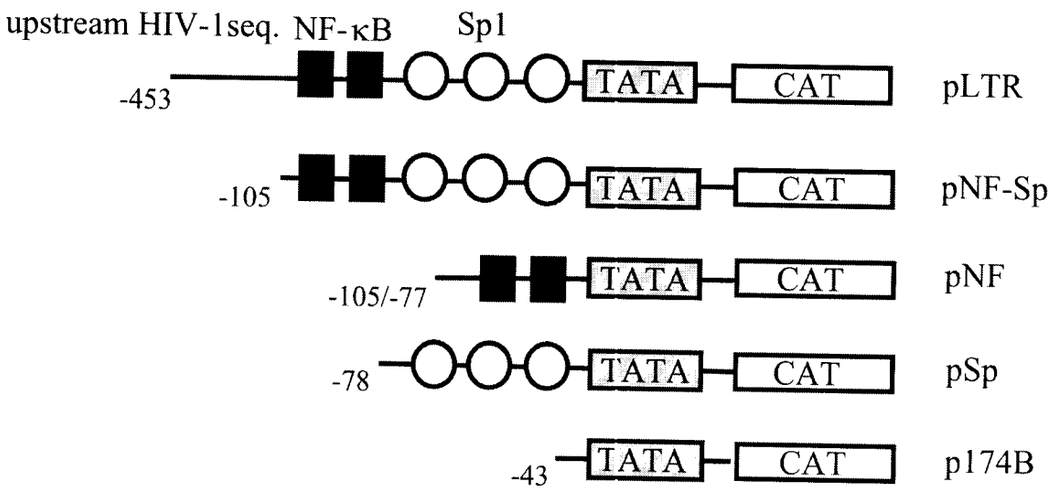
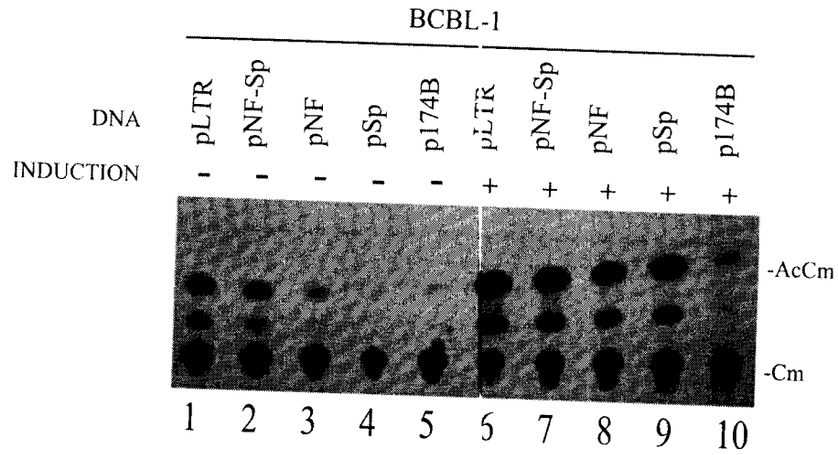


Fig.2 Schematic representation of plasmid constructs.

(a)



(b)

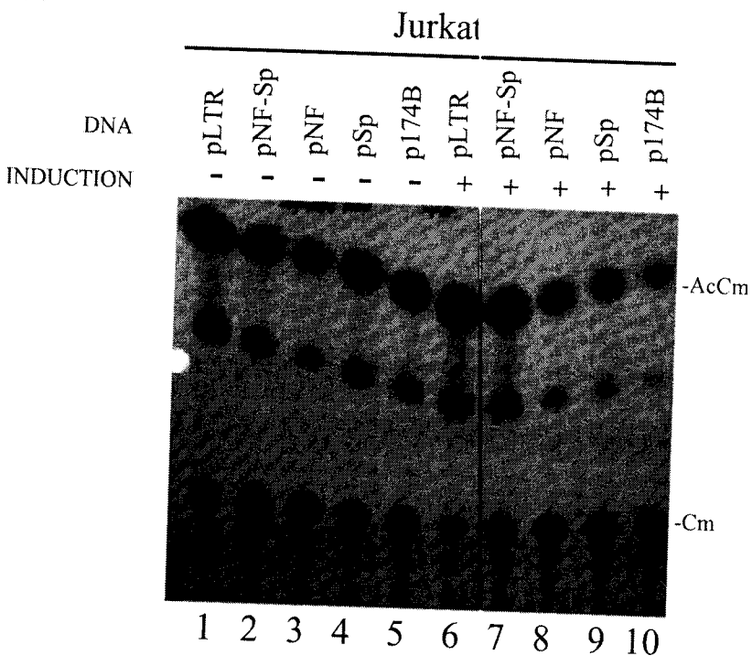


Fig. 3 Identification of the HHV8 target sequences in the HIV-1 LTR. pLTR or deletion mutants were transfected into BCBL-1(a) and Jurkat (b) without (lane 1-5) or with (lane 6-10) durg treatment. CAT activity was assayed 48 hr after transfection.