An Epstein-Barr Virus-associated Superantigen

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Summary
More than 90% of adults are latently infected with Epstein-Barr virus (EBV), the causative
agent of infectious mononucleosis, a self-limiting lymphoproliferative disease characterized by
extensive T cell activation. Reactivation of this herpesvirus during immunosuppression is often
associated with oncogenesis. These considerations led us to analyze the early events that occur
after exposure of the immune system to EBV. Strong major histocompatibility complex
(MHC) class II-dependent, but not MHC-restricted, T cell proliferation was observed in vitro
in response to autologous, lytically infected EBV-transformed B cells. By measuring the ap-
pearance of the early activation marker CD69 on individual T cell Vβ subsets, we could dem-
onstrate selective activation of human Vβ13+ T cells. This was confirmed with murine T cell
hybridomas expressing various human BV genes. While EBV− Burkitt’s lymphoma cells were
nonstimulatory, they induced Vβ-restricted T cell activation after EBV infection. EBV specific
activation was also demonstrated in cord blood cells, excluding a recall-antigen response. Thus,
all of the characteristics of a superantigen-stimulated response are seen, indicating that induc-
tion of the EBV lytic cycle is associated with the expression of a superantigen in B cells. A
model is presented proposing a role for the superantigen in infection, latency, and oncogenesis.

EBV has a strong tropism for B lymphocytes and has the
capacity to activate them to proliferate continuously
(reviewed in 1, 2). Exposure to EBV during childhood re-
sults in no symptoms or only limited symptoms. Infection
during adolescence or early adulthood, however, can give
rise to infectious mononucleosis (IM)1 in ~50% of cases.
IM is a self-limiting lymphoproliferative disease character-
ized by virus shedding into the saliva, growth of infected B
cells, and a massive expansion of nonspecific T cells. Ini-
tially, both CD4+ and CD8+ T cells are activated; how-
ever, later on, the response comprises mainly atypical
CD8+ lymphoblastoid cells. Disease regression occurs grad-
ually, manifested by a decrease in virus shedding as well as
in the number of infected B cells. Presumably, this is medi-
ated by virus-specific CTL (3).

EBV is a highly successful virus, which has evolved to
inhabit and manipulate the immune system to its own ad-
vantage. The infection of antigen-presenting B cells, fol-
lowed by a rapid activation of nonimmune T cells, led us
to speculate that an EBV-associated superantigen might be
influencing the immune response during the establishment
of persistent infection.

1Abbreviations used in this paper: CBMC, cord blood mononuclear cells;
IM, infectious mononucleosis; LCL, lymphoblastoid cell line; MMTV,
murine mammary tumor virus; SEB, Staphylococcus enterotoxin B.

Superantigens are a class of pathogen-derived proteins
that elicit a powerful T cell response, activating whole fam-
ilies of T cells with identical or related TCR Vβ chains (re-
viewed in 4). Superantigens form a bridge between MHC
class II molecules on APC and a region on the TCR Vβ
chain outside of the unique antigen-binding domain. This
bridging transduces a signal to the T cell as well as the
APC, resulting in activation of both. The hallmarks of a su-
perantigen-induced T cell response are (a) a vigorous acti-
vation of primary T cells; (b) an MHC class II-dependent,
but not MHC-restricted, recognition; and (c) a TCR Vβ-
restricted response. Exposure to superantigens in vivo re-
results in skewing of the T cell repertoire, manifested either
by amplification of particular T cell Vβ families, or deletion
and/or anergy of specific subsets. A virally encoded super-
antigen could have irreversible, indirect effects on the hu-
man immune system by altering the response to pathogens,
leading to disease or autoimmunity. An example is pro-
vided by the recent demonstration that the murine mam-
mary tumor virus (MMTV) superantigen, Mls-1, predis-
poses mice to polyoma virus associated oncogenesis by
deleting the cytotoxic T cells that control polyoma virus
infection (5).

Superantigens are evolutionarily conserved in certain
pathogens, and it is presumed that they confer a selective
advantage for the microbe. It has been well documented
that stimulation of the host's immune system by the prototypic viral superantigen, Mls, is essential for transmission of infectious MMTV (6–8). Therefore, we set out to determine whether superantigen stimulation of the human immune system could likewise play a role in establishing persistent EBV infection and disease.

Materials and Methods

**Proliferation Assays.** Peripheral blood B cells from healthy adult volunteers were transformed to create lymphoblastoid cell lines (LCL), using viral supernatant from B95-8 cells, which produce a common laboratory strain of EBV (9, 10), or IM-1, an EBV strain isolated from an IM patient (a gift from M.A. Epstein, University of Oxford, England). LCL were stimulated with PMA (10 ng/ml) for 24 h to induce the lytic cycle, and were then treated with mitomycin C (100 µg/ml) for 1 h to arrest cellular division. After extensive washing with PBS, LCL were added to freshly isolated autologous PBMC or allogeneic umbilical cord blood mononuclear cells (CBMC). PBMC and CBMC were isolated by density gradient separation on Ficoll-Paque Plus (Pharmacia, Uppsala, Sweden). All cells were cultured in complete RPMI media (GIBCO BRL, Gaithersburg, MD), supplemented with 10% FCS (Intergen, Purchase, NY), at 37°C with 5% CO2. Each assay was carried out in quadruplicate wells of a 96-well plate with 10^5 responders/well, using stimulator to responder ratios of 1:3 and 1:10. Stimulation was compared to treatment with PHA (2 µg/ml). After an incubation period of 48 h, the cells were pulsed with [3H]thymidine (1 µCi/well) and harvested 8–12 h later to assess DNA synthesis as a measure of cellular proliferation.

**Similar assays were performed using as APC two EBV Burkitt’s lymphoma cell lines, BL-2 and BL-41 (11), both before and subsequent to productive infection with B95-8 supernatant. APC were treated with PMA and mitomycin C and plated with allogeneic PBMC or CBMC at APC/responder ratios of 1:1, 1:3, or 1:10.**

**MHC Class II-blocking Experiments.** Proliferation assays were performed as described above, with the following modifications: before the addition of responder cells, in some of the wells, LCL were preincubated for 1 h with D1-12 (80 µg/ml), an mAb that recognizes all HLA DR molecules (12), or the same concentration of an isotypic control mAb 116-13.1, anti-lyt-2. The mAb remained in the cultures for the entire 72 h. Each assay was set up in quadruplicate wells of a 96-well plate with 10^5 responders/well, using stimulator to responder ratios of 1:3 and 1:10. Stimulation was compared to treatment with PHA (2 µg/ml). After an incubation period of 48 h, the cells were pulsed with [3H]thymidine (1 µCi/well) and harvested 8–12 h later to assess DNA synthesis as a measure of cellular proliferation.

**Results**

**Peripheral Blood T Cells Proliferate in Response to Autologous, Lysically Infected EBV-transformed B Cells.** To investigate whether a vitally associated superantigen influences the establishment of persistent EBV infection, a classical T cell proliferation assay was performed. In this assay, lysically infected, EBV-transformed B LCL were used as APC, and autologous PBMC from healthy adult donors served as responders. This constituted an attempt to reproduce in vitro some of the initial events that occur during IM, leading to the massive T cell activation. It has not been possible to decipher these primary steps in vivo using IM patient blood samples, most likely because noticeable clinical symptoms
often do not appear until after the disease has progressed to the lymphoproliferative stage. At that point, there is significant bystander T cell activation, resulting from cytokine production and the generally activated immune status, which can obscure EBV-specific T cell activation.

LCL were established in vitro by infection of B cells with two different strains of EBV: B95-8, a common laboratory strain, and IM-1, a viral isolate derived from the peripheral blood of an IM patient. Since EBV is generally maintained in a latent form in LCL, we stimulated the cell lines for 24 h with the phorbol ester PMA, which induces the EBV lytic cycle and upregulates the production of viral proteins (25). Figs. 1, A and B, depict representative results obtained with PBMC from two healthy unrelated adult donors. In both cases, 48 h proliferation, as assessed by \(^{3}H\)thymidine incorporation, was demonstrated at levels comparable to the mitogen PHA. Maximum proliferation occurred at 72 h, although the absolute levels were not significantly higher (data not shown). To demonstrate that EBV is responsible for the strong proliferation, an EBV- Burkitt's lymphoma line, BL-2, was PMA treated for use as APC. Although allogeneic, these cells were found to be non-stimulatory or very weakly stimulatory at 48 h under identical conditions. Upon infection of the BL-2 cells with B95-8 EBV, strong proliferation was observed (Fig. 1, A and B).

The Early Activation Marker CD69 Is Preferentially Expressed on \(V_{\beta}13\) T Cells after a 4-h Stimulation with Autologous B95-8 LCL. The main characteristic of a superantigen is that it elicits a TCR \(V_{\beta}\)-restricted response, resulting in a selective alteration of the T cell repertoire by either amplifying or deleting T cell clones that express the specific \(V_{\beta}\) genes. If indeed the stimulation with autologous LCL is caused by the presence of a superantigen, we would expect to selectively activate T cells of a particular TCR \(V_{\beta}\) phenotype. To test this, we developed a novel means of defining \(V_{\beta}\)-specific T cell activation; namely, we analyzed the appearance of the early activation marker CD69 (26, 27) on individual T cell \(V_{\beta}\) subsets 4 h after stimulation. Cytokines, which are potent activators of bystander T cells, are produced between 5–6 h after stimulation; thus, we deliberately chose an earlier time point to minimize the induction of nonspecific T cell proliferation. Moreover, the absence of proliferation at this early time point guarantees detection of a polyclonal response, excluding the readout of an oligoclonal expansion. This obviates the need for sequencing the VDJ region of numerous clones to establish polyclonality.

PBMC from the same two donors depicted in Fig. 1 were stimulated for 4 h with autologous B95-8 LCL or with PHA. The cells were then double stained with a PE-conjugated anti-CD69 mAb and a panel of FITC-labeled anti-\(V_{\beta}\) mAbs. The stimulated cells were analyzed by flow cytometry to assess the percentage of each \(V_{\beta}\) subset that expressed CD69. As can be seen in Fig. 2 A, stimulation with PHA led in both donors to a significant increase in CD69 + cells in the \(V_{\beta}13\) T cell subset. As expected, the percentage of CD69 + cells after stimulation with the mitogen PHA did not vary significantly between the \(V_{\beta}\) subsets and was not significantly different from the overall percentage of CD69 + cells in unfractionated PBMC. These results suggest that EBV induces in B cells the expression of a protein that is preferentially recognized by polyclonal \(V_{\beta}13\) T cells, implicating the presence of a superantigen. Similar results were obtained after stimulation with autologous IM-1 LCL, and with a third donor using autologous LCL as well (data not shown). After longer stimulation with PHA or LCL (>4 h), CD69 was expressed on a greater percentage of T cells (data not shown), probably because of activation of bystander T cells by the ensuing production of cytokines. To ensure that the appearance of CD69 on particular \(V_{\beta}\)
subsets is truly indicative of superantigen activity, we analyzed the well-established superantigen response to SEB. The results obtained with PBMC of the same two donors are summarized in Fig. 2 B. The percentage of CD69+ cells after SEB stimulation was significantly increased in the Vβ12, Vβ17, and Vβ20 subsets, consistent with published reports using conventional methods of Vβ analyses (28). The level of CD69 was highest on Vβ12+ cells, the subset shown to be deleted in an in vivo study after immunization with SEB (29). On the other hand, the Vβ13 subset was not significantly elevated for CD69 staining after SEB treatment, indicating that the increased Vβ13 stimulation in Fig. 2 A was specific for EBV LCL. Thus, our assay system is sensitive for detection of Vβ-specific superantigen stimulation. It should be noted, however, that after 4 h incubation with SEB, CD69 expression was not significantly increased in the Vβ3a subset, despite the reported expansion of Vβ3 T cells after long-term SEB stimulation (28). Since our assay relies on the specificity of the Vβ mAb used for the detection of T cell subsets, it is our opinion that screening for CD69 expression is useful only as an initial test for detection of Vβ activation.

**Murine T Cell Hybridomas Expressing Human Vβ13.1, Vβ13.2, and Vβ13.6 Produce IL-2 in Response to B95-8 and IM-I LCL.** The human Vβ13 family contains eight members, Vβ13.1–13.8, comprising a large portion of the overall T cell repertoire (GenBank/EMBL/DDBJ accession No. L36092). The anti-Vβ13 mAb we have used, H131, was originally developed against the human BV13.1 gene product, expressed in a murine T cell hybridoma as a chimeric TCR with the murine Vα and CD3 chains (18). The H131 mAb stains a relatively large percentage of peripheral blood cells from each donor tested (3–4.5%). Because the BV13 family members are highly homologous, it is difficult to rule out mAb cross-reactivity between the subfamilies.

To confirm the finding that LCL preferentially activate Vβ13 T cells, we tested murine T cell hybridomas expressing human Vβ13.1, Vβ13.2, and Vβ13.6 chains, respectively (18, 20). In addition, T cell hybrids expressing human Vβ2 (22), Vβ3, Vβ8, Vβ9, and Vβ17.1 were included in these experiments. These hybrids lack endogenous murine Vβ genes, although the constant region and CD3 complex are of murine origin. The hVβ2 and hVβ3 hybrids express human Vα as well, while all of the other hybrids express murine Vα chains. As can be seen in Fig. 3 A, the three Vβ13+ T cell hybrids produced IL-2 after stimulation with either PMA-treated B95-8 or IM-I–derived LCL, but were only very weakly stimulated by latently infected LCL. On the other hand, the hVβ8 T hybrid was unreactive to the LCL, regardless of PMA treatment. Fig. 3 B shows that

**Figure 2.** The early activation marker CD69 is preferentially expressed on Vβ13 T cells after a 4-h stimulation with autologous B95-8 LCL, and on Vβ12 T cells after SEB stimulation. Autologous PMA-induced B95-8 LCL were cultured for 4 h with freshly isolated PBMC from two donors, as described in Materials and Methods. As controls, PBMC from each donor were simultaneously stimulated with PHA (A). Stimulated cells were immediately double stained with PE-conjugated anti-CD69 mAb and a panel of FITC-conjugated anti-Vβ mAb. Analysis by flow cytometry determined the percentage of each Vβ subset that became CD69+. As controls, PHA. (B) In a separate experiment, PBMC from the same two donors were stimulated with SEB for 4 h and stained as described in A; again, stimulation was compared to PHA. The graphs depict the percentage of cells from each Vβ family that were CD69+ after stimulation with SEB ( ), compared to PHA ( ).
Figure 3. Stimulation of murine T cell hybridomas expressing human VB313.1, 13.2, and 13.6 in response to lyrically infected B95-8 and IM-1 LCL, and lyrically infected EBV+, but not EBV-, Burkitt’s lymphomas. (A) Murine T cell hybridomas expressing hVB313.1, hVB313.2, hVB313.6, or hVB8 were cultured for 24–36 h with uninduced and PMA-treated B95-8 or IM-1 LCL. As a positive control, T cell hybrids were stimulated by TCR cross-linkage. Cell lysates were monitored for IL-2 production by assaying HT-2 cell growth by [3H]thymidine incorporation. Results were assessed in quadruplicate, and are expressed as the average cpm/well with the SD. HT-2 cell dose response to rIL-2 is depicted on the right vertical axis. IM-1, no PMA; IM-1 + PMA; B95-8, no PMA; B95-8 + PMA; anti-CD3. (B) A full panel of murine T cell hybrids expressing human BV genes were stimulated with PMA-treated B95-8 and IM-1 LCL. The results are depicted as the percentage of maximal IL-2 production, based on TCR cross-linkage. IM-1; B95-8. (C) The hVB313 T cell hybrids and the hVB8 hybrid were stimulated with EBV BL-2 and BL-41 cells, as well as B95-8-infected BL-2 and BL-41 cells. All APC were PMA treated. The results are depicted as the percentage of maximal IL-2 production, based on TCR cross-linkage. BL-2, no EBV; BL-2 + B95-8; BL-41, no EBV; BL-41 + B95-8.

Figure 4. The anti-HLA DR mAb D1-12 blocks the 48-h proliferation of PBMC to autologous, lyrically infected EBV LCL. Proliferation assay was performed as in Fig. 1; however, in some of the wells, LCL were pre-incubated with the mAb D1-12 or an isotypic control mAb 116-13.1, which remained in the cultures throughout the assay. Autologous B95-8 or IM-1 LCL were used as APC at a stimulator/responder ratio of 1:10. D1-12 added to PHA-treated cells did not affect stimulation.
cell proliferation, while mitogen-induced stimulation was unaffected. Preincubation with an isotopic control antibody, 116-13.1, did not block 48-h T cell proliferation.

**T Cell Proliferation to EBV-transformed B Cells Is Not Caused by a Memory Response.** Since the vast majority of adults are EBV+, the massive proliferation induced by EBV-transformed LCL could be caused by a recall antigen response. To test this possibility, we used CBMC as responders in this assay because newborns are EBV- and cannot have memory T cells. As can be seen in Fig. 5, CBMC vigorously proliferated at 48 h to allogeneic B95-8 and IM-1 LCL after PMA induction. Weak stimulation was observed in response to uninduced LCL. While the magnitude and the early kinetics of the response pointed to the action of a superantigen rather than an alloresponse, the Burkitt lymphoma lines actually confirmed that the stimulation was caused by EBV. Strong proliferation was detected only after PMA treatment of B95-8-infected BL-2 and BL-41 cells. PMA treated EBV- BL cells were weakly stimulatory, as would be expected from the alloresponse, which is not optimal at 48 h.

It should be mentioned that the LCL in all of our assays induce higher levels of T cell stimulation than do the B95-8-infected Burkitt’s lymphoma lines. In Fig. 5, although the proliferation levels induced by all of the EBV+ PMA-treated cells are comparable, the stimulator/responder ratios are 1:3 and 1:10 for the LCL, but 1:1 and 1:3 for the Burkitt's lymphomas. The difference in stimulation could be caused by several factors. LCL express higher levels than do most Burkitt’s lymphoma lines of different cellular adhesion molecules, such as CD23, which possibly enhance T cell activation. In addition, since PMA treatment is necessary for optimal stimulation, we have surmised that the activation is most likely caused by an EBV lytic gene. LCL are easily induced into the lytic cycle with phorbol ester (2). Even in uninduced LCL, a small number of cells is present which spontaneously undergo lytic cycle viral replication. This number is significantly reduced in EBV+ Burkitt’s lymphoma lines (2). Spontaneous lytic cycle gene expression, in combination with the alloresponse, could account for the weak stimulation induced by non-PMA-treated LCL of the CBMC in Fig. 5, as well as of the T cell hybrids in Fig. 3 A.

**Discussion**

Taken together, the results presented here demonstrate the expression of a superantigen in EBV-transformed LCL and EBV+ Burkitt’s lymphomas that have been induced into the lytic cycle. The early activation of a large proportion of nonimmune T cells, combined with the fact that the stimulation is HLA-DR dependent, but not restricted, and the specific activation of VB13 T cells, all support this conclusion. Furthermore, the response of the CBMC to EBV+, but not EBV- Burkitt's lymphoma cells, exclude the possibility of an anamnestic recall antigen response because CBMC are by definition EBV-.

What are the possible advantages for EBV expressing a superantigen in infected B cells? We would predict that the superantigen is required for the establishment and/or the maintenance of persistent infection. The EBV latent genes have the capacity to directly induce B cell proliferation in vitro without T cell help, rendering superantigen stimulation superfluous. However, the site of viral persistence in vivo is a resting B cell that does not express the growth-promoting latent genes (30). We suggest, therefore, that the role of superantigen-activated T cells is to provide signals necessary for the growth and survival of these latently infected B cells in vivo, possibly through CD40-CD40L interaction (see model in Fig 6). This would replenish the pool of latently infected B cells, which does not decrease over time, but remains remarkably stable for years (30). T cell regulatory signals might also induce differentiation of latently infected B cells, driving some of them to terminally differentiate, reactivate the virus, produce more superantigen, and thereby complete the cycle (Fig. 6). This model proposes that viral replication is essential for viral persistence, not through reinfection of B cells, but through superantigen driven activation of T cells.

A role for T cells influencing EBV infection was suggested from earlier studies demonstrating that the spontaneous outgrowth of LCL in vitro from EBV-seropositive PBMC is more efficient in the presence of T cells (31). This requirement for T cells is usually obscured by the seemingly converse finding that EBV-specific CTL, which arise later in culture, must be depleted because they will eliminate the newly generated LCL (32, 33). Our model fits data obtained in vivo in SCID mice transplanted with EBV-seropositive human PBMC (34-36). These mice
Infection of Resting B Cell

Site of Viral Persistence

Latent Gene Expression

Reactivation of Latent EBV

Cytokines

EBV-specific CTL

Killing

EBNA-LMPs EBERs

Infection of Resting B cell

Proliferating B Cell Latent Cycle

B Cell Differentiation

Resting B Cell

SAG-driven T Cells

T Cell Help

EBV-specific CTL

SAG

Plasma Cell Lytic Cycle

Figure 6. Role of the EBV-associated superantigen in infection, latency, and disease. After EBV infection of resting B cells, latent gene expression induces B cell proliferation. T cell help provides survival signals and signals for terminal differentiation. This enables lytic cycle viral replication and superantigen expression in the plasma cells. Activation of Vβ13 T cells initiates cytokine production and nonspecific activation of T cells, which can provide help to latently infected B cells. Primed EBV-specific CTL will kill B cells expressing the full latent gene repertoire, selecting for a population of cells that downregulate latent gene expression and assume a resting phenotype. This becomes the site of viral persistence throughout the lifetime of the host. Periodically, these cells can be pushed into viral replication by T cell signaling, while memory CTL will counteract the process, maintaining a constant EBV burden.

spontaneously developed EBV-associated B cell lymphomas only when T cells were cotransferred, although the development of EBV-specific CTL limited tumor formation in some of the mice. Transplantation of highly purified B cells never yielded lymphomas, even in the presence of cytokines known to be secreted by activated T cells (35), suggesting that a direct T cell-B cell interaction is requisite. The tumors that develop are a mixture of latently infected proliferating B cells, and terminally differentiated, lytically infected B cells that do not divide (37). We would predict that the nondividing differentiated B cells provide an essential growth component for the growing tumor, namely superantigen-activated T cells. T cell signaling would promote B cell survival, allowing full latent gene expression, resulting in perpetual B cell proliferation. Simultaneously, T cells might push a fraction of cells to terminally differentiate, growth arrest, produce virus, and more superantigen (Fig. 6). Eventually, some of the latently infected, rapidly dividing cells would accumulate mutations in cellular oncogenes such as myc, allowing them to lose the T cell dependence and become transformed. This hypothesis is in fact supported by the SCID/hu mouse model, since the tumors that arise in SCID mice after long periods of time no longer contain the fully differentiated, lytically infected cells (37).

The other side of the equation, for healthy humans, is the cytotoxic T cell response (38). Persistent infection with EBV can thus be seen as a balance between the superantigen driven, T cell-dependent B cell growth, and the elimination of latently infected proliferating B cells by CTL (Fig. 6). The clinical symptoms of IM would arise as a result of the disruption of this balance. During IM, there is a rapid activation of both CD4+ and CD8+ T cells early after infection, presumably as a consequence of superantigen stimulation, mediated by the cytokine production of Vβ13+ T cells. A characteristically massive expansion ensues of nonspecific, atypically activated, apoptotic CD8+ T cells (39, 40). The nonspecific activation of CD8 cells possibly delays the development of virus-specific CTL. This could be a mechanism for EBV to evade a specific immune response in establishing latency.

Paradoxically, EBV infection during childhood leads to seroconversion with limited or no clinical symptoms. The following scenario provides an explanation for this apparent incongruity: In adolescents exposed for the first time to this pathogen, the mature T cell pool is poised to mount a rapid superantigen response, while the development of virus-specific CTL requires priming, and is therefore only effective after an initial lag period. CTL priming might be further delayed by the superantigen-induced activation of nonspecific T cells, resulting in the symptoms of IM. Children, on the other hand, would predictably mount a suboptimal T cell response to the EBV superantigen, as inferred from murine studies, indicating an impaired immune response to viral superantigens in immature animals (41). Thus, in children, the slower and weaker superantigen response would be balanced by the induction of EBV-specific CTL, preventing clinical manifestations. It is unclear whether IM represents an atypical immune response because the symptoms of IM are self-limiting and viral latency is established in both cases.

EBV reactivation in AIDS patients and transplant recipients often leads to the development of lymphomas and other lymphoproliferative diseases. Immunosuppression preferentially would affect the virus-specific memory CTL,
compared to the superantigen-reactive primary T cells, disturbing the balance in the favor of the latter. The loss of CTL, which control the spread of latently infected proliferating B cells, would lead to unregulated growth, favoring tumor development.

EBV has also been linked to the induction of autoimmunity, especially in Sjogren’s syndrome, which is associated with elevated levels of the virus in patients (42). Of note are reports describing a significant increase in VB13 T cells in the lesions of patients with this autoimmune disease, indicative of the possible action of an EBV-associated superantigen (43–45).

Recently, it has been suggested that the presence of a superantigen in another herpesvirus, CMV, causes enhanced HIV-1 replication in VB12 T cells, which function as a reservoir for HIV-1 in infected patients (46). It is plausible that EBV might play a similar role as CMV in AIDS, particularly since EBV infection is ubiquitous, and it has been reported that perturbations in the VB13 compartment occur in patients with HIV infection (47). In addition, a recent report describes increased HIV-1 replication as a consequence of EBV lymphoma development in SCID mice transplanted with EBV-seropositive PBMC from HIV-1 infected patients (48).

Perhaps more importantly, we predict that some of the anergy and apoptosis of T cells occurring in HIV patients might actually be caused by activation-induced cell death, resulting as an indirect consequence of EBV superantigen stimulation, e.g., the increased cytokine environment. Furthermore, we speculate that the atypical, apoptotic CD8+ T cells, which are massively expanded during IM, constitute the same T cell subset that is augmented in HIV infection (49, 50). In both diseases, elimination of CD4+ T cells occurs, possibly caused in part by the nonspecific cytotoxic activity of these activated CD8+ T cells. It is conceivable that these cells arise during HIV infection as a result of superantigen expression after EBV reactivation by HIV-1 Tat (51). In support of our postulate, a correlation was recently found between T cell apoptosis in HIV-1–infected children and an increased burden of EBV (52). Thus, interfering with the activation of VB13 T cells might have relevant clinical consequences.

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