The human T cell immune response to Epstein-Barr virus

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ABSTRACT **The Epstein-Barr virus (EBV) is a gamma-herpes virus which establishes latent, life-long infection in more than 95% of the human adult population. Despite its growth transforming capacity, most carriers control EBV associated malignacies efficiently and remain free of EBV+ tumors. Though EBV is controlled by a potent immune response, this virus uses latency to persist** ***in vivo.*** **This review summarizes work which has been done to characterize T cell responses to EBV. The CD8 T cell responses are rather well characterized and have been shown by several groups to be highly focused towards early lytic antigens. Much less is known about CD4 T cell epitopes, due to the small size of the CD4 compartment. However, recent data indicate a control of lytic and latent cycles of EBV by specific CD4+ T cells. A clear understanding of the T cell response to EBV is important with a view to developing immunotherapies for the virus and its related malignancies.**

KEY WORDS: *Epstein-Barr virus, T cell response, EBV-associated tumor*

Introduction

In a way, our interest in immune response to the Epstein-Barr virus (EBV) started with Nicole Le Douarin when, with one of us (E. H), she investigated cell lineage and tissue interactions in the developing bursa of Fabricius through interspecific chimeras. The distinct morphological differences between the nuclei of the quail and those of the chick were used to determine the origin of cells in differentiated tissues (Le Douarin, 1973). By grafting bursal rudiments of quail into chick and inversely, it was possible to demonstrate that the whole hematopoietic population of the bursa of Fabricius is derived from blood-borne extrinsic precursor cells. The inflow of hemopoietic stem cells takes place between day 7 and 15 in chick embryo. Neither endoderm nor mesoderm of the bursal rudiment is capable of differentiating into lymphoid cells (Le Douarin *et al.*, 1975). Combination of quail bursal endoderm with chick bursal mesenchyme showed that the reticular cells of the follicles are the only endodermal derivatives of the bursa. The mesenchymal bursal component gives rise to the interfollicular connective cells (Houssaint *et al.*, 1976).

A few years later, we became ''true'' immunologists and we are presently involved in the analysis of T cell responses against EBV. Before describing these responses, we will recall how this common herpesvirus establishes a latent infection in most individuals. Despite a relatively begnin course in most carriers, EBV has growth-transforming capabilities and is causatively linked to three B-cell malignant diseases: endemic Burkitt's lymphoma, Hodgkin's lymphoma and post-transplant lymphoproliferative diseases (PTLD). Therefore, it appears important to identify the mechanisms whereby healthy EBV carriers avoid development of EBV-associated malignancies.

Infection of humans with EBV

Epstein-Barr virus is a gammaherpes virus that infects more than 90% of the human population (see Rickinson and Moss, 1997, for a review). Primary infection often occurs during childhood and is generally asymptomatic (Fleisher *et al.*, 1979). However, adolescents and young adults who experience primary EBV infection frequently present with acute infection mononucleosis (Henle and Henle, 1979). There are two distinct stages of EBV cycle. In latent infection, the genome is maintained at a constant copy number and a limited number of regions of the genome are expressed. In the other stage of infection (lytic cycle), viral DNA is amplified and a variety of viral antigens (lytic antigens) are produced prior to virion maturation (Kieff, 1996). EBV has two major target tissues *in vivo*, B lymphocytes and squamous pharyngeal epithelium. The virus is transmitted orally and, at the time of primary infection, replicates in cells within the oropharynx, leading to the release of infectious virions at high titers into throat washings (Sixbey *et al.*, 1983). At the same time, EBV establishes

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a latent infection of the B lymphocyte pool that results in lifelong persistence. Entry of EBV into B cells is initiated by an interaction between the major viral envelope glycoprotein gp350 and the B-lineage-associated C3d complement receptor CR2. Infection of B lymphocytes with EBV results in persistent latent infection and immortalization of the cells to perpetual proliferation. Following primary infection, a lifelong virus carrier state is established whereby the virus is harbored within a small number of B cells in which most if not all viral latent protein expression is down-regulated (Tierney *et al.*, 1994). In healthy adults, from one to fifty B cells per million in the circulation are infected with EBV and the number of latently infected cells within a person remains stable over years (Babcock *et al.*, 1998). Throughout life, intermittent reactivation of such latently infected B cells into lytic cycle, at mucosal sites probably, underlies the low level shedding of infectious virus detectable in throat washings of asymptomatic virus carriers.

Many of the characteristics of lytic and latent infection *in vivo* can be studied *in vitro* with B-LCLs (B lymphoblastoid cell lines). These cell lines are established by exogenous EBV infection of B lymphocytes. Of the nearly 100 viral genes that are expressed during replication, only ten are expressed in latently infected B cells *in vitro* : two types of non translated RNA, six nuclear proteins (EBNA1, 2, 3A, 3B, 3C and EBNA-LP) and two membrane proteins (LMP1, LMP2) (Kieff, 1996). By markedly limiting viral gene expression during latency, EBV reduces the number of viral proteins that permit the recognition of infected cells by T cell effectors. Lytic cycle can be induced *in vitro* by different technical approaches, e.g. with phorbol esters. In contrast to the latent phase of infection, when only a limited number of proteins are expressed, activation of the EBV lytic cycle is characterized by the appearance of as many as 80 virus-specific RNA species (see Kieff, 1996, for a review). On the basis of their time of appearance post-induction, these transcripts are designated as immediate early, early or late. The early antigens are composed of a series of polypeptides, such as BZLF1, a replicon activator, BMRF1 and BMLF1, which are both transactivators, BALF5, a DNA polymerase, BORF2 and BARF1, ribonucleotide reductases, BXLF1, a thymidine kinase and BHRF1, a cytoplasmic antigen. EBV late antigens are mostly structural viral proteins which are required for successful packaging of the viral DNA and formation of the virion, among them: the viral capsid antigen, the membrane antigen complex that consists of at least three major EBV-induced glycoproteins, gp350, gp110 and gp85.

EBV is controlled by a potent immune response

Host immune responses are thought to be of central importance both in limiting the primary infection and in controlling the lifelong virus carrier state. Under normal circonstances and in healthy individuals, EBV infections are not life-threatening and are generally effectively controlled by the immune system through the action of antigen-specific T lymphocytes (see Rickinson and Moss, 1997, for a review). In fact, infection of humans with EBV results in both humoral and cellular immunity to the virus. Although the finding of antibodies directed against viral structural proteins and the EBV nuclear antigens is important for the diagnosis of infection, the cellular immune response is more important for the control of EBV infection. In fact, the high frequency of EBV-associated lymphoproliferative diseases or lymphomas in immunocompromised individuals strongly supports a role for anti-EBV T cells in containing infection (Yao *et al.*, 1996). Both CD8^{+} and CD4^{+} T lymphocytes can discriminate EBV-infected or EBV-transformed B cells and, as a consequence, are able to inhibit their growth. EBV-specific T lymphocytes recognize antigens as molecular complexes formed by viral peptide epitopes with molecules of the Major Histocompatibility Complex (MHC), which are expressed on the surface of infected or transformed B lymphocytes. Although EBV-specific CD8^{+} T cell responses have been well documented, EBV-specific CD4^{+} T cell responses have been less well studied.

CD8 T cell response to EBV

Technical approaches to assess CD8 T cell responses to EBV

CD8 T cells recognize peptides, derived from endogenous proteins, associated with MHC class I molecules. In the past few years, novel methods for measuring CD8 T cell responses have been applied to a frequency analysis of CD8 T cell responses, namely (i) ELISPOT, which quantitates effector CD8 T cells, based on cytokine release (ii) intracellular cytokine staining and (iii) staining with fluorescent recombinant major histocompatibility complex (MHC) class I-peptide tetramers, which have allowed direct detection and sorting of CD8 T cells reactive against a defined peptide-MHC class I complex (Altman *et al.*, 1996).

We followed a different approach described in Figure 1, where the screening of anti-EBV responses was based on a transient COS transfection assay allowing a rapid semi-quantitative estimation of CD8^{+} T cell responses against a large number of HLA/viral protein combinations within polyclonal T cell lines (Scotet *et al.*, 1999). In order to perform a comprehensive analysis of the antigen specificity of EBV-reactive CD8 T cells, we prepared or gathered expression vectors coding for each of the latent EBV proteins or some lytic EBV proteins (BZLF1, BRLF1, BMLF1, BMRF1, BHRF1, BHLF1, BALF2, BALF4, BCRF1 and BLLF1), as well as expression vectors encoding about forty HLA class I molecules. Briefly, COS cells were cotransfected with expression vectors coding for one viral protein and for one of the donor's HLA alleles as described by Boon *et al.* (Brichard *et al.*, 1993). Transfected COS cells were screened 48 h after transfection for their ability to stimulate the release of the cytokine Tumor Necrosis Factor (TNF) by CTL, using either clones or polyclonal T cell lines (Espevik and Nissen-Meyer, 1986, Scotet *et al.*, 1996, Scotet *et al.*, 1999).

The CD8 T cell response is highly focused towards early lytic antigens

Responses to EBV were previously studied by rechallenging peripheral blood lymphocytes (PBL) *in vitro* with cells of the autologous EBV-transformed B lymphoblastoid cell line (B-LCL), expressing the full range of virus latent proteins (EBNA1, 2, 3A, 3B, 3C and LP and the two latent membrane proteins LMP1 and LMP2). This led to the conclusion that the dominant responses almost always mapped to epitopes derived from EBNA3A, 3B, or 3C, sometimes accompanied by subdominant responses to one of the other latent proteins, but apparently never to EBNA1 (Murray *et al.*, 1992, Khanna *et al.*, 1995). These observations led to the discovery that endogenously expressed EBNA1 could not be presented to the CD8 T cells because an internal glycine-alanine repeat domain protects the protein from a key step in the MHC class I processing, namely proteasomal degradation to peptides (Levitskaya *et al.*, 1995, Levitskaya *et al.*, 1997).

During the last five years, we performed an extensive analysis of the CD8 T cell response in healthy donors or in patients. This led us to a conclusion quite different compared to previous studies, as we found that the response is highly focused against proteins generated during the early lytic cycle. Our analysis of CD8 T cell responses to EBV started rather fortuitly with the observation that CD8 T cell clones, originating from the synovial fluid from a patient suffering from rheumatoid arthritis, recognized, in term of proliferation the autologous B-LCL. As the B-LCL had been transformed *in vitro* by EBV, it was reasonable to think that these CD8 T cell clones recognized EBV epitopes. In fact, a link between EBV and arthritis had long been suspected. To test the hypothesis that the synovial-derived T cell clones could recognize EBV determinants, we prepared a cDNA library from the B-LCL generated from the B lymphocytes of an arthritic patient. With a first CD8 T cell clone, that was restricted through HLA-A2, we screened this cDNA library, using the COS assay and we could thus demonstrate that this clone recognized the early lytic EBV protein BMLF1, in the HLA-A2 context. We then extended our analysis and we showed that most CD8 T cell clones in the joint fluid from a patient suffering from rheumatoid arthritis recognized either BZLF1 or BMLF1, two transactivating proteins produced early in the lytic cycle of EBV (Scotet *et al.*, 1996).

To assess the generality of this observation and its relevance to autoimmunity, we performed an extensive screening of CD8 T cell responses to EBV within polyclonal T cell lines derived from the synovial fluid from a large number of patients suffering from various forms of chronic arthritis or other inflammatory diseases (n=32) and we compared these responses to those observed among PBL of healthy virus carriers (n=10) or kidney recipients (n=16). For each donor, the amount of TNF released by the polyclonal T cell line was assessed following incubation with COS cells cotransfected with DNA coding for some of the patient HLA class I alleles and with cDNA coding for one EBV protein (18 EBV cDNAs were included in the assay).

Responses against one or several EBV proteins were detected in about 2/3 of the samples analysed. In fact, in accordance with others (Murray *et al.*, 1992, Khanna *et al.*, 1995), we found that most individuals had CTL responses to more than one EBV antigen (Scotet *et al.*, 1999). Irrespective of the physiopathological status of the donor, these responses were mainly directed against the immediate early or early lytic proteins: BZLF1, BRLF1 and BMLF1. For example, a response to BZLF1 was found for 60% of the donors. Moreover, the high amounts of TNF produced by the CD8 T cells responding to BZLF1 suggested a high frequency of responding cells. This was later on corroborated by tetramer analysis, that showed that as many as 15% of synovial CD8 T cells reacted against a single BZLF1 epitope in some samples (Tan *et al.*, 2000). Weaker responses to some latent EBV proteins, namely EBNA3A, 3B, 3C and LMP2, were observed in 10 to 20% donors. The fact that we did not observe any anti-EBV response in 1/3 of donors does not necessarily mean that these donors were not responding to EBV. In fact, we only tested responses in the context of HLA class I molecules for which cDNA were available and in several instances, the HLA subtypes were not precisely identified, so that in some cases we used inappropriate HLA cDNA.

Two main conclusions could be drawn from this extensive analysis. With regard to anti-EBV CD8 T cell responses, our data showed that they are highly focused against early lytic antigens. The COS transfection assay is not sensitive enough to detect sub-dominant responses but it allows a rapid identification of the major epitopes. Concerning a possible link between EBV and rheumatoid arthritis, we have shown that the presence of EBV-specific T cells within inflamed lesions of patients suffering from autoimmune diseases is a general phenomenon. In fact, we observed an enrichment for EBV-specific CD8 T cells in all the inflammatory sites that we examined and these responses showed the same specificity patterns as those of memory responses occurring in long term virus carriers. Enrichment in CD8 T cells responding to EBV in the inflammatory sites rather reflects an unspecific trapping of activated T cells under chronic inflammation than an initiation of the autoimmune process by the virus.

Concurrently to our study, different groups reevaluated CD8 T cell responses to EBV by using novel methods of estimating frequencies of antigen-specific T cells. These included assays to
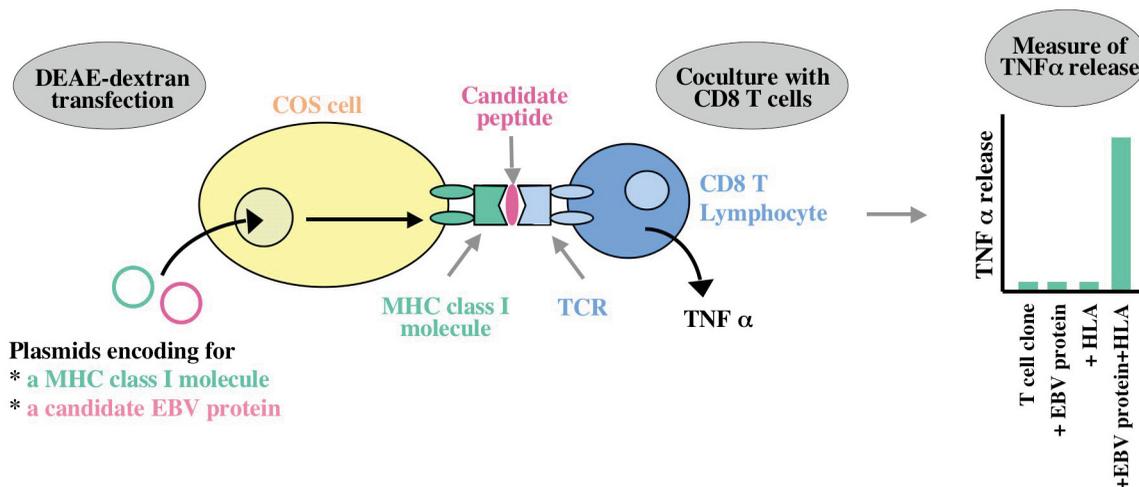

Fig. 1. Rationale of the COS transfection assay used to detect anti-EBV T cell responses of CD8 T cell clones. *In a first step, COS cells were co-transfected with plasmids encoding an appropriate MHC class I molecule and an EBV protein. After 48 h hours, the CD8 T cell clone was added to transfected COS cells and its activation state was evaluated on the basis of TNF-α production. TNF production was observed only when the T cell clone recognized onto COS cells the appropriate EBV protein plus the relevant MHC class I molecule.*

detect IFN-γ release from epitope-specific CD8^{+} T cells following peptide stimulation (Lalvani *et al.*, 1997) and the use of tetrameric MHC-peptide complexes to directly stain T cells of the appropriate specificity (Altman *et al.*, 1996). Results obtained using these techniques have led to a reevaluation of the nature of both primary and memory responses to EBV virus (Steven *et al.*, 1997, Callan *et al.*, 1998, Tan *et al.*, 1999). This revealed a surprisingly high frequency of circulating CD8 T cells specific for individual EBV proteins in healthy seropositive carriers (Tan *et al.*, 1999). During acute infection up to 40% of the total CD8^{+} cells in the blood are directed against a single lytic EBV protein epitope, while up to 2% of the CD8^{+} cells are directed against a single latent protein epitope (Callan *et al.*, 1998).

Immunodominant EBV epitopes recognized by CD8 T cells

All along this study, we screened for anti-EBV responses in 35 EBV-reactive CD8 T cell lines against a large set of 18 EBV proteins in the context of 19 HLA alleles (Saulquin *et al.*, 2000). Such a large scale analysis revealed an unexpectedly focalized response against few EBV protein/HLA combinations (nearly half of them were derived from BZLF1). Once we had determined the EBV proteins that were recognized by EBV-specific CD8^{+} T cell clones, we defined the precise EBV epitope (8 to 11 amino-acid sequence) that activated these clones by using a library of overlapping EBV peptides. To this end, we studied the responses of polyclonal T cell lines and/or T cell clones against sets of 23-residue peptides spanning the whole viral proteins. The sequence of the minimal peptide was then deduced from their consensus binding capacities to the relevant HLA molecule (Rammensee *et al.*, 1995). As expected from earlier studies (see Rickinson and Moss, 1997, for a review), responses elicited by the above combinations were in almost all cases directed against a single peptidic antigen. Many of these epitopes were frequently recognized by distinct individuals sharing the corresponding HLA allele (Table I). For instance BZLF1/B8 and BZLF1/B35 combinations were recognized by all the EBV-reactive cell lines

bearing the appropriate HLA and BMLF1/A2, EBNA3B/A11, BZLF1/B8, EBNA3A/B27 were recognized by a large proportion of the cell lines bearing the corresponding HLA. HLA-B allele-restricted CTL epitopes appear to be more dominant than HLA-A allele-restricted epitopes. As suggested by others (Khanna *et al.*, 1995), HLA-B alleles may be in general more efficient than HLA-A alleles in their loading and transport of viral peptides.

CD4 T cell response to EBV

Much less is known about the CD4^{+} T cell responses to EBV, although there is an increasing awareness of their key role in 1) supporting high affinity antibody production 2) initiating and particularly, maintaining CTL numbers and function and 3) performing direct effector activity. Analysis of CD4 T cell responses has been greatly hampered by the small size of the CD4 compartment. No CD4 T cell expansions have been detected, even during the early stages of EBV acute infection (Maini *et al.*, 2000).

Strategies for the identification of CD4 T cell responses to EBV

CD4^{+} T cells recognize a peptide bound to MHC class II molecules on the cell surface of antigen presenting cells. The formation of MHC class II-peptide complexes on the cell surface is a complicated, multistep, process that favors presentation of antigens derived from exogenous proteins. This process occurs in antigen presenting cells. Therefore, the COS transfection assay is not applicable to characterize CD4 T cell epitopes as COS cells do not have the machinary required for MHC class II processing. Several technical approaches have been used by different groups to characterize CD4 T cell responses to EBV. Autologous B-LCL or dendritic cells were infected with recombinant vaccinia virus (rVV) encoding individual lytic or latent protein. Alternatively, the EBV protein, particularly EBNA1, was delivered to dendritic cells as recombinant protein. The response of CD4 T cell clones or T cell lines was then evaluated on the basis of cytokine production (IFN-γ or TNF-α). Once the EBV protein
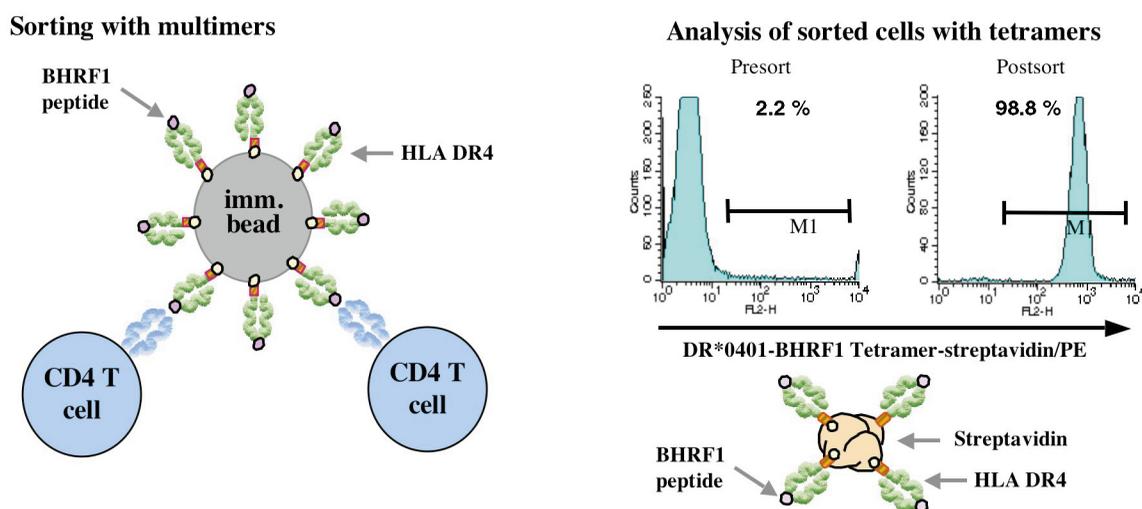

Fig. 2. Efficient sorting of BHRF1-specific CD4 T cells by using magnetic beads covered with multiple DR*0401 monomers loaded with the BHRF1$_{122-133}$ peptide. *After a 2-week culture, the frequency of BHRF1-specific cells among the sorted cells was estimated by staining with the relevant tetramer. Phycoerythrin (PE)-labeled streptavidin was used to produce fluorescent DR*0401 tetramers loaded with BHRF1 peptide. In the experiment described here, while only 2% of BHRF1-specific CD4 T cells were present within the polyclonal T cell line, nearly all the cells sorted with BHRF1 multimers were BHRF1-specific.*
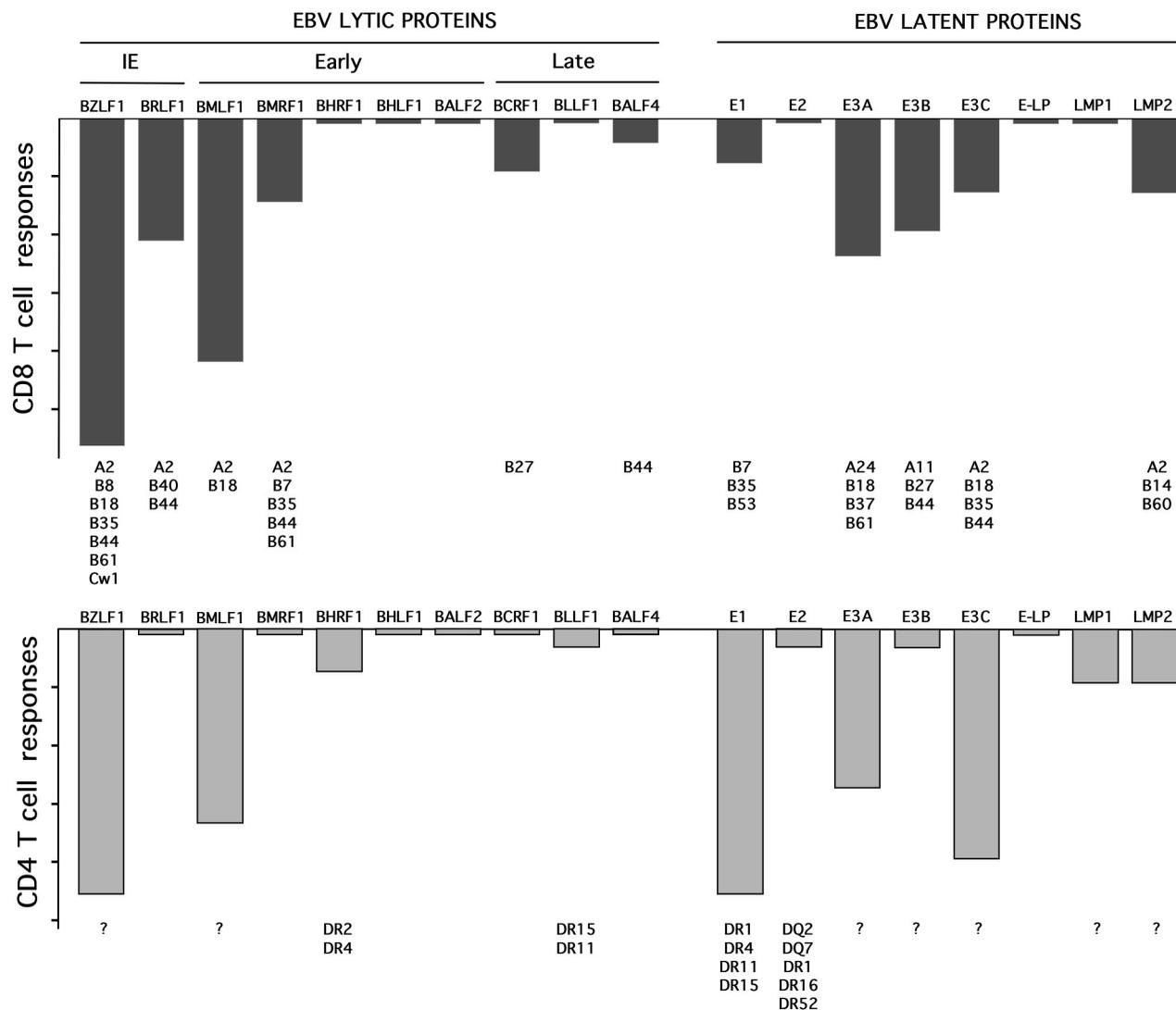
Fig. 3. Features of the CD8 and CD4 T cell responses to EBV from a compilation of data from different groups. *The length of the bar gives an idea of the dominance of the response. The HLA restriction of the responses that have been described for a given EBV protein is indicated. Abbreviations: IE, immediate early; E, early; L, late lytic protein; E1, E2, E3A, E3B, E3C and ELP represent EBNA1, 2, 3A, 3B, 3C and LP.*

identified, sets of overlapping peptides spanning different EBV proteins were used to define precisely the epitope recognized, through IFN-γ release by activated CD4 T cells. Some groups used only the second step, that is, systematic screening of overlapping synthetic peptides. To identify the EBV protein recognized by a given CD4+ T cell clone, we co-cultured this CD4 T cell clone for a few hours with the B-LCL from the same donor, hence sharing the appropriate MHC class II alleles, infected with individual recombinant vaccinia virus encoding 10 lytic and all latent EBV proteins. Activation of the clone was then evaluated both in terms of cytotoxicity and of TNF-α production assay. The precise epitope was defined in a second step by using the set of overlapping peptides covering the whole sequence of that protein.

CD4 T cell recognition of lytic and latent EBV proteins

As we had observed an enrichment for EBV-specific CD8 T cells in the joint of patients suffering from rheumatoid arthritis, we started

from several CD4 T cell clones derived from synovial fluid samples to attempt characterizing CD4 T cell responses to EBV. One of these clones recognized an epitope derived from BHRF1, a protein produced during the early stages of the EBV lytic cycle, in the HLA-DR*0401 context. We will summarize here the identification and the functional characteristics of this response. The CD4 T cell clone was activated by the autologous B-LCL infected with the rVV encoding BHRF1, as documented by TNF-α production. In contrast, the B-LCL infected by rVV encoding 16 other EBV proteins was not recognized by that clone. The epitope recognized by this clone (BHRF$_{122-133}$), was identified through screening of synthetic overlapping peptides covering the entire BHRF1 sequence and the HLA-restriction of this clone was found to be HLA-DR*0401. We then wanted to investigate whether BHRF$_{122-133}$–specific T cells could be found within polyclonal T cell lines derived either from HLA-DR*0401+ donors. Peptide-MHC class I multimeric complexes had revealed efficient tools to detect and to sort out rare specific

CD8 T cell populations. Thus we thought using a similar strategy to sort out CD4 T cells. To this end, recombinant DRA1*0101/DRB1*0401 monomers loaded with the BHRF$_{122-133}$ peptide were produced by the group of William Kwok (Novak *et al.*, 1999, Novak *et al.*, 2001) and we performed immunomagnetic sorting using streptavidin beads coated with biotinylated DRB1*0401-BHRF$_{122-133}$ monomers (hereafter referred to as multimers) (Fig. 2). After a 2-week culture under polyclonal activation, response of the sorted cells to the BHRF$_{122-133}$ peptide was tested either by staining with the tetramer or by functional assays (TNF production, cytotoxic assay). Successful sorting of BHRF1-specific cells was achieved for all T cell lines derived from DRB1*0401 individuals (be they derived from the peripheral blood of healthy virus carriers or from the synovial fluid of arthritis patients), indicating that the response to BHRF1 is not specifically linked to artritis. The expanded CD4 T cells were nearly 100% specific to BHRF1, thus underlying the efficiency of this sorting approach with DR4 multimers (Fig. 2).

Functional analysis of BHRF1-specific CD4 T cell clones demonstated their cytolytic action against DR*0401$^+$ EBV-transformed B-LCL and potent IFN-γ production, thus suggesting that this CD4 T cell population could contribute to the control of EBV replication. The consistent killing of B-LCL, with 40-80% killing efficiency, was rather surprising, as about 5% of cells within a B-LCL are expected to express lytic EBV proteins. Analysis of killing mechanism will need further investigation. These data show that it is possible to efficiently sort out and expand CD4 T cells specific for defined epitopes. Such peptide/MHC class II multimer sorting strategy could be of potential therapeutic interest for the immunotherapy of viral infections or cancers.

The CD4 T cell response to EBV was also documented by other groups. EBV specificity of some CD4$^+$ T cell clones had been previously reported (Wallace *et al.*, 1991, Khanna *et al.*, 1995, White *et al.*, 1996, Khanna *et al.*, 1997). In more recent systematic analysis, using polyclonal CD4$^+$ T cell lines, the nuclear antigen EBNA1 was found to be a main EBV latency antigen for CD4$^+$ T cells (Munz *et al.*, 2000, Leen *et al.*, 2001, Paludan *et al.*, 2002). During primary infection, it was shown that up to 2.7% of circulating effector/memory CD4$^+$ T cells are EBV-specific (Precopio *et al.*, 2003). Responses to BZLF1, BMLF1 and EBNA3A were more commonly detected than responses to EBNA1 in patients with infectious mononucleosis (Precopio *et al.*, 2003). A compilation of the CD4 T cell responses to EBV, compared to EBV-specific CD8 T cell responses, is presented in Fig. 3.

An interesting feature of some of the CD4 T clones, that have been described to respond to EBV antigens, is that they kill the autologous B-LCL with a rather high efficiency (Paludan *et al.*, 2002) or they inhibit B-LCL proliferation (Omiya *et al.*, 2002). Moreover, it has been shown that several EBV epitopes recognized in the MHC class II context are highly ''promiscuous'', as they are restricted by numerous different MHC class II alleles. This indicates that these peptides could be recognized by a large proportion of the general population. This could help designing widely applicable peptide-based vaccine to prevent and perhaps treat PTLD in transplant patients.

EBV virus uses latency to persist *in vivo*

Despite an efficient control of the immune system, EBV persistslifelong. How can one explain that the immune system does not succeed in eliminating the virus? The EBV persistence in memory B cells in vivo has been recently revisited by the group of Thorley-Lawson (Babcock *et al.*, 2000, Kurth *et al.*, 2000, Thorley-Lawson, 2001). In fact, EBV-infected B cells in vivo can express four different programs of gene usage depending on the location and the differentiation state of the infected B cell. One of these programs is used to produce infectious virus. The other three are all associated with latent infection, in which no infectious virus is produced: i) the growth program, also called latency III, in which all nine latent proteins are expressed ii) the default program, or latency II, in which a restricted set of three latent proteins are expressed and iii) the latency program (latency I), in which few, if any latent genes are expressed. The virus uses the latency program to persist *in vivo* in a transcriptionally quiescent state (Tierney *et al.*, 1994, Babcock *et al.*, 1998) within resting memory B cells that circulate in the peripheral blood (Babcock *et al.*, 1998, Babcock *et al.*, 1999, Thorley-Lawson, 2001). These resting B cells express none of the EBV proteins (the transcripts for LMP2 are often detected in these cells) and they exhibit a particular phenotype of resting memory cells (IgD$^-$, CD27$^+$, CD5$^-$), which ensures escape from immune surveillance. In this fashion >95% of all adults are infected benignly for life. Persistent infection is characterized by stable numbers of latently infected B cells in the blood (0.5 to 50 per million, depending on the individual) and the steady shedding of infectious virus into saliva. In this way, a balance between rare EBV-harboring B cells and EBV-specific T cells is established during persistence of the virus and the virus is continuously monitored by the immune system.

EBV-associated diseases

EBV has potent B-cell transforming activity and is causatively linked or associated with various B-cell malignant diseases: endemic Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma and post-transplant lymphoproliferative disease (PTLD). All four types of tumour have distinct cellular phenotypes and patterns of EBV gene expression, suggesting that the pathogenetic role of the virus might be different in each case. EBV-transformed cells exhibit one of three latency types distinguished from each other by the panel of expressed EBV antigens (Kieff, 1996). In Burkitt's lymphoma, only EBNA1 is expressed (latency I). In nasopharyngeal carcinoma (latency II), LMP1 and LMP2, as well as EBNA1, are expressed. Only in latency III immunoblastic lymphomas, are the nine latent proteins expressed, particularly the highly immunogenic EBNA3A, B and C.

As documented in part 2, EBV-specific CD8$^+$ and CD4$^+$ T cell responses are preferentially directed towards the early lytic proteins and, to a lesser extent, towards the nuclear antigens, EBNA3A, EBNA3B and EBNA3C, which are not expressed in most EBV-associated malignancies. EBNA1 appears as an optimal EBV-specific antigen because it must be expressed in all proliferating EBV-infected cells to maintain the viral episome. Therefore, all EBV-induced tumors express this foreign antigen. The problem is that EBNA1 is invisible to CD8$^+$ cytotoxic T lymphocytes because its Gly/Ala repeat domains prevents proteasome-dependent processing for presentation on MHC class I (Levitskaya *et al.*, 1995, 1997). Therefore, many EBV-associated malignancies do not seem to provide good targets for the dominant EBV-specific

CD8 T cell response. The recent finding that CD4^{+} T cells consistently respond to EBNA1 suggests that EBNA1-specific CD4^{+} T cells provide resistance to the development of Burkitt's lymphoma, Hodgkin's and other EBV-associated malignancies. Likewise, these new data suggest that EBNA1 be tested as an antigen to prevent and treat such malignancies.

The question is different for the posttransplant lymphoproliferative disorders (PTLD). In immunosuppressed individuals, such as transplant patients, primary EBV infection usually results in PTLD that often progress into B cell lymphomas£(Hsieh *et al.*, 1999, Paya *et al.*, 1999, Hopwood and Crawford, 2000). Current prophylactic and therapeutic approaches for PTLD and lymphomas are far from optimal. The reduction of immunosuppressive therapy facilitates the immune-mediated viral elimination, but with a high risk of organ rejection and the use of antiviral agents is of unclear effectiveness (Paya *et al.*, 1999). Results obtained using anti-CD20 mAb (CD20 is a differenciation marker of B lymphocytes) therapy appear promising, but have the disadvantage of long term B cell depletion and the occurrence of CD20-negative relapses (Faye *et al.*, 2001, Zilz *et al.*, 2001, Verschuuren *et al.*, 2002). One of the most effective ways to prevent or treat PTLD and B cell lymphomas is via adoptive immunotherapy using EBV-specific T lymphocytes (Heslop and Rooney, 1997, Rooney *et al.*, 1998), but this approach is labor intensive, costly and not widely available. An alternative approach would be to use synthetic peptides, representing EBV CD8 or CD4 T cell epitopes, to stimulate *in vivo* responding T lymphocytes. Such a vaccine is not yet available.

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