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Nasopharyngeal carcinoma and the EBV-specific T cell response: prospects for immunotherapy

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T cells specific for Epstein–Barr virus (EBV) can effectively target the virus-transformed B lymphoproliferative lesions that arise in immunosuppressed transplant patients. This review explores the possibility of developing similar T cell-based strategies to treat an EBV-positive epithelial tumour, nasopharyngeal carcinoma (NPC), which arises in relatively immunocompetent individuals and where EBV antigen expression in the tumour is more limited.

Key words: Epstein–Barr virus / immunotherapy / nasopharyngeal carcinoma / T lymphocytes

Introduction

As our knowledge of T cell responses to Epstein–Barr virus (EBV) in healthy virus carriers has increased, so has interest in the possibility of adapting such responses to treat EBV-positive human malignancies. The last 7–8 years have seen significant progress in this area with the development of an adoptive T cell therapy for EBV-positive post-transplant lymphoproliferative disease (PTLD). The success of this approach is now prompting researchers to explore the possibility of developing T cell-based therapies for other EBV-positive tumours such as undifferentiated nasopharyngeal carcinoma (NPC) and Hodgkin's disease, which in world health terms are by far the most important EBV-associated malignancies. This review examines the chances of developing a successful T cell-based therapy for NPC by firstly summarising what is known of T cell responses to EBV antigens and analysing the reasons for success in treating PTLD. These issues are then related to our current understanding of EBV antigen expression and cellular immunity in NPC patients.

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T cell targeting of EBV antigens

CD8$^+$ T cells

CD8$^+$ cytotoxic T lymphocytes (CTLs) specific for EBV latent cycle antigens are readily reactivated *in vitro* from healthy virus carriers by co-culturing peripheral blood mononuclear cells (PBMCs) with the autologous EBV-transformed B-lymphoblastoid cell line (LCL). Within an LCL, the virus establishes a predominantly latent infection, with expression of at least nine viral proteins, six nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and -LP) and three latent membrane proteins (LMPs 1, 2A and 2B, where LMP2B represents an N-terminally truncated version of LMP2A).[1] Studying LCL-reactivated CTL lines has revealed a hierarchy of immunodominance amongst EBV latent proteins. Thus, for the majority of donors, regardless of their human leucocyte antigen (HLA) type, LCL-reactivated CTL lines are dominated by responses to one or more of the EBNA 3 family of proteins, EBNAs 3A, 3B and 3C.[2,3] Additional subdominant reactivities are detectable to LMP2 in a significant number of individuals[4,5] but only rarely to EBNA2, EBNA-LP and LMP1, and, in all of the earlier studies of this kind, never to EBNA1. Using synthetic peptides, many of the target epitopes of these CTL responses have now been defined. The choice of target epitope is strongly influenced by the donor's HLA type. Thus, HLA A11-positive individuals tend to make a strong response to one or two epitopes in EBNA3B, whereas B27-positive donors often target epitopes in EBNA3C.[6]

The apparent absence of CTL responses to EBNA1 in LCL-reactivated T cell cultures led to the important

discovery that this EBV protein, when expressed endogenously, cannot be processed and presented through the conventional HLA class I pathway. This effect is mediated by a glycine–alanine repeat (GAr) domain within the EBNA1 protein sequence that prevents proteasomal breakdown of the molecule.[7] Later work demonstrated that, in fact, $CD8^+$ CTL responses specific for EBNA1 epitopes are present *in vivo*.[8] Such responses may be induced through 'cross-priming' whereby antigen presenting cells (probably dendritic cells) take up EBNA1 protein from an extracellular source, process it in a manner unaffected by the presence of the GAr domain and present it to $CD8^+$ T cells through the HLA class I pathway. However, it should be stressed that EBNA1-specific $CD8^+$ T cells still do not see naturally infected cells and are, therefore, likely to be biologically ineffective *in vivo*.

CTL responses to proteins expressed during the EBV lytic cycle primarily target proteins expressed during the immediate early and early stages of EBV replication.[9] Within the blood of infectious mononucleosis patients one can detect remarkable expansions of EBV lytic cycle-specific T cells. Thus, in some patients, almost half of all $CD8^+$ T cells in the peripheral blood are specific for a single EBV lytic cycle epitope.[10] These responses are retained in long-lived T cell memory, and although they are scaled down, in numerical terms, they frequently still dominate over responses to EBV latent antigens.[10,11]

$CD4^+$ T cells

Persistence of an effective $CD8^+$ T cell response apparently requires a concurrent antigen-specific $CD4^+$ helper T cell response.[12] $CD4^+$ T cells can also mediate tumour rejection independently of $CD8^+$ CTLs[13] and if the malignant cells are HLA class II-positive $CD4^+$ effectors, may mediate a direct cytolytic effect.[14] Until recently, however, very little was known of $CD4^+$ T cell responses to EBV, but in the last 2 years, studies on healthy virus carriers have detected $CD4^+$ responses to several EBV latent proteins.[15,16] Once again, a hierarchy of immunodominance amongst these proteins is emerging, with EBNA1 and possibly EBNA3C representing the major target antigens. Responses to these two proteins are detectable in the majority of healthy virus carriers and multiple target epitopes have been defined. Responses to LMP1 and LMP2 are detected less frequently, with fewer epitopes defined.[16] These responses were detected by gamma-interferon (γIFN) release, indicating that they are predominantly of a Th_1 phenotype, although there are conflicting reports on the Th phenotype of EBNA1-specific responses.[17] Another interesting aspect of EBNA1-specific $CD4^+$ T cells reported in some[15] but not all[16,18] studies, is their ability to lyse LCLs where the target antigen is apparently processed from an endogenous (rather than exogenous) source.

Adoptive T cell therapy for PTLD

T cell-immunosuppressed individuals such as transplant recipients are at greatly increased risk of developing EBV-positive immunoblastic B cell lymphomas (sometimes referred to as PTLD). Furthermore, these tumours often regress following a reduction in immunosuppression. These observations prompted attempts to treat EBV-positive PTLD by infusing donor-derived EBV-specific T cells. The first of these studies was reported in 1994, where five bone marrow transplant (BMT) recipients who had developed EBV-positive PTLD were treated with unselected populations of donor-derived lymphocytes.[19] In all cases there was evidence of tumour regression, albeit at the expense of severe graft-*versus*-host disease (GvHD). The following year, Rooney *et al.*[20,21] described a modified approach where donor lymphocytes were first enriched for EBV-specific T cells by *in vitro* culture following LCL stimulation. These EBV-specific T cell lines were used as prophylactic treatment for BMT recipients considered at high risk of developing PTLD. None of 39 treated individuals developed PTLD compared with 7/61 in the control group (although a formal randomised trial is yet to be conducted). Where patients carried a high EBV load in the blood prior to treatment, these levels dropped significantly within 2–3 weeks after the infusion. Furthermore, no adverse events were reported. Genetic marking of the T cells demonstrated that they not only expanded *in vivo*, but in some cases persisted for up to 5 years post-infusion. Rooney *et al.*[21] have also used adoptive transfer of EBV-specific T cell lines to treat pre-existing PTLD, and in 2/3 cases patients responded fully. The only exception, was a patient who received infusions of T cells that mainly targeted two epitopes in EBNA3B. The patient died with progressive disease 24 days after the first infusion. Further analysis revealed that prior to treatment, the patient carried more than one EBV strain, including a virus expressing a mutated EBNA3B protein lacking the two target epitopes. After T cell infusions, only the virus with the mutated EBNA3B could be detected, indicating that the infused T cells selected a resistant strain *in vivo*.[22]

Adoptive transfer of EBV-specific T cells can also mediate regression of PTLD occurring in solid organ transplant recipients.[23,24] It should be noted, however, that regression of bulky PTLD in response to T cell therapy has been associated in several cases with severe and life-threatening local inflammation and tissue damage.[21,24] Nevertheless, these clinical studies represent significant progress in the development of T cell-based therapies for human malignancy.

There are three basic requirements for a T cell-based therapy to be effective in treating cancer. Firstly, the tumour must express antigens that are appropriately processed and presented to T cells. Secondly, it must be possible to generate a T cell response to these antigens (either *in vitro* for adoptive transfer or directly *in vivo*). Thirdly, these T cells must home to and function appropriately at the tumour site. Given these requirements, it is possible to see why infusing EBV-specific T cells appears to be an effective treatment for PTLD. Thus, in many cases of PTLD the full panel of EBV latent proteins are expressed including the immunodominant EBNA3 family. Antigen processing and presentation pathways within the tumour appear to be intact with high level surface expression of HLA class I and II molecules, as well as expression of the co-stimulatory molecules CD80 and CD86, and adhesion molecules LFA-1 and ICAM-1.[25] It is relatively easy to reactivate the appropriate T cells *in vitro* because not only are some of the target antigens immunodominant across a wide range of HLA backgrounds, but in the context of BMT recipients, they can be generated from an immunocompetent bone marrow donor. Finally, it is likely that infusing T cells into a BMT recipient with a regenerating haemopoietic system aids the expansion and persistence of the effector cells (as does the continued presence of EBV antigens within the host). Therefore, in many ways PTLD is the ideal setting for a T cell-based therapy. What then are the prospects for treating another EBV-positive human malignancy, namely NPC?

NPC

EBV antigens expressed in NPC

When exploring the possibility of a T cell-based therapy for NPC, one of the most important considerations is that *EBV* gene expression is more restricted in this tumour. Thus, EBNA1 protein is regularly expressed in undifferentiated NPC but no other EBNAs are detectable.[26–28] Nevertheless, LMP1 protein is detectable in up to 65% of cases.[26] LMP2A and 2B mRNA transcripts are also readily detected in NPC,[29] but expression of the protein has not yet been demonstrated using current reagents. Transcripts from the BamH1 A region of the EBV genome are highly expressed in most forms of EBV infection, including NPC,[30] but it is still unclear whether these ever encode a protein product. Transcripts from some EBV lytic cycle genes have also been detected in NPC, but immunohistochemical studies have either failed to detect lytic cycle protein expression or identified only rare BZLF1-positive cells.[31] However, recent work indicates that the BARF1 protein may be expressed in this tumour.[32]

The absence of the EBNA3 proteins and possibly also the lytic cycle proteins is of particular significance since they are immunodominant targets for $CD8^+$ CTL responses. Furthermore, EBNA1 is presumably not processed through the HLA class I pathway because it is protected from proteasomal degradation. However, LMPs 1 and 2 are both known targets for $CD8^+$ CTLs. Responses detected in healthy virus carriers indicate that LMP1 is poorly immunogenic,[33] thus, the most likely target antigen for a $CD8^+$ CTL-based therapy is LMP2. Clearly, therefore, it is important to clarify at what level, if at all, the LMP2 protein is expressed in NPC. (In this context it is interesting to note that NPC patients are unique in possessing high levels of serum antibodies to LMP2.[34]) Recent work using the Elispot assay and synthetic peptides representing three putative protein sequences encoded within the BamH1 A region (BARF0, RPMS1 and A73) have so far failed to detect specific responses in healthy virus carriers (C. W. Tsang, G. Taylor, A. B. Rickinson and S. P. Lee, unpublished data). Weak CTL responses have been reported to an A2-restricted peptide potentially encoded by the *BARF0* gene. However, it is unclear if these truly reflect EBV-induced responses as they did not recognise naturally infected cells; furthermore, the encoding region is deleted from the majority of BARF0 transcripts.[35] As yet there are no reports of T cell responses to BARF1.

Although EBNA1 is an unlikely target for $CD8^+$ effectors, it is a dominant target for $CD4^+$ T cells. $CD4^+$ T cell responses to EBNA1 could, therefore, help to maintain an effective $CD8^+$ response at the tumour site and/or mediate tumouricidal activity, possibly through cytokine secretion and the recruitment and activation of monocytes. Furthermore, since HLA class II molecules are expressed in NPC, if direct processing of the endogenously synthesised EBNA1 protein can occur in the malignant cells, $CD4^+$ effectors with cytotoxic activity could mediate a direct

antitumour effect. LMPs 1 and 2 could also be recognised by $CD4^+$ T cells, though current evidence suggests that such responses are rarely induced *in vivo*[16] and there are as yet no *in vitro* clones available to look at LMP processing via the HLA class II pathway.

Conservation of epitope sequences in EBV strains from Chinese populations

Table 1 lists all T cell epitopes so far defined in EBNA1, LMP1 and LMP2. Most of these were identified from studies on Caucasian donors, where T cell responses were reactivated *in vitro* using an EBV strain (B95.8) derived from a Caucasian population. Yet NPC is predominantly found in Southern Chinese populations. The EBNA1 protein sequence in Chinese virus isolates shows 97.8% amino acid identity with the B95.8 sequence.[42] Recent work using synthetic peptides and the Elispot assay indicates that EBNA1 is immunodominant for $CD4^+$ T cells in healthy Chinese donors, as it is in Caucasians, with responses mainly focussed on a few areas of peptide sequence again within the C-terminal half of EBNA1. Furthermore, there is a good deal of tolerance of epitope changes in these regions. For example, an EBNA1 epitope (residues 515–528; see Table 1) encoded by Chinese isolates carries two amino acid changes compared to the B95.8 sequence (residue 524, T → I; residue 528, I → V); yet both sequences are recognised by Caucasian and Chinese donors (A. B. Rickinson, unpublished data).

The LMP1 protein in Chinese viruses shows 93.5% amino acid identity with the B95.8 sequence,[43] whereas LMP2 is more conserved (97.2%) (G. Taylor, unpublished data). Analysing virus isolates from the blood of healthy Chinese carriers and from the tumour of Chinese NPC patients, four CTL epitope sequences in LMP2 (designated according to the first three amino acids in the sequence as LLW, LTA, SSC and RRR; see Table 1) were shown to be identical to the B95.8-derived sequence. Another three LMP2 epitope sequences (CLG, TYG and IED) consistently showed one or two amino acid changes in Chinese virus isolates, but there was no evidence that these affected antigenicity.[5] Furthermore, CTLs specific for TYG and IED epitopes have been reactivated from the blood of Chinese virus carriers, indicating that these variant epitope sequences do elicit responses *in vivo*.[38]

Antigen processing and presentation in NPC

Immunohistochemical studies of NPC biopsies have demonstrated surface expression of HLA class I molecules on tumour cells in the majority of cases.[44,45] Transporters associated with antigen processing 1 and 2 (TAPs 1 and 2) deliver peptide fragments from the cytosol to the endoplasmic reticulum for association with nascent class I molecules. As such they are important components of the HLA class I processing pathway and both are expressed in NPC.[46] Functional studies of antigen processing and presentation in NPC have been confined to the analysis of rare NPC-derived cell lines. The results of these *in vitro* studies indicate that NPC cells are capable of processing and presenting endogenously synthesised protein to HLA class I-restricted CTL clones.[38,46] HLA class II molecules are also expressed in the majority of NPCs,[44,5] but as yet nothing is known of the ability of the malignant cells to process and present antigens (either exogenously or endogenously derived) to class II-restricted T cells.

NPC cells express several molecules that are important in activation of T cell responses, namely CD40, CD70, CD80 and CD86.[47] The adhesion molecules ICAM1 and LFA3 mediate conjugate formation between T lymphocytes and their target cells. ICAM1 is highly expressed on NPC cells, but only low levels of LFA3 are detectable.[48] It is yet to be determined to what extent this may affect T cell interactions with the tumour cells.

HLA restriction

If a T cell-based therapy for NPC is to be applicable to a significant number of patients, the target epitopes must be presented through HLA alleles present at high frequency in the patient population. In this context, LMP2-specific responses restricted through A11, A24 and B40 are of particular interest because these alleles are very common in the Southern Chinese population (A11, 56%; A24, 27%; B40, 28%).[39] HLA A2 is the restriction element for several epitopes in LMP1 and LMP2 and is carried by approximately half of all Caucasians and Chinese. However, the relative frequency of A2 subtypes within these populations is quite distinct. Thus, almost all A2-positive Caucasians carry the A*0201 subtype, whereas A*0207 is the most common subtype in Singapore Chinese.[41] It is not yet clear to what extent A2 subtype polymorphism affects the ability of donors to respond to the A2-restricted LMP epitopes. Nevertheless, at least two LMP epitopes (originally identified from studies on A*0201-positive donors) can be processed and presented in association with other A2 subtypes[5,33] (see Table 1). Turning to HLA class II-restricted responses, two epitopes in

Table 1. T cell epitopes defined in NPC-associated EBV proteins

Target protein	Epitope sequence[a]	Amino acid residues	HLA restriction	Frequency of restricting allele in Chinese population (%)[b]	Reference
$CD8^+$ T cell targets					
LMP1	YLLEMLWRL*[c]	125–133	A*0201[d]	12	33
LMP1	YLQQNWWTL	159–167	A*0201	12	33
LMP2	PYLFWLAAI	131–139	A23	<1	36
LMP2	IEDPPFNSL*	200–208	B*40011	28	5
LMP2	RRRWRRLTV	236–244	B*2704	2	37
LMP2	LLWTLVVLL	329–337	A*0201	12	5
LMP2	WTLVVLLI	331–338	B63	<1	unpub.
LMP2	SSCSSCPLSK	340–349	A11	56	5
LMP2	FLYALALLL	356–364	A*0201	12	unpub.
LMP2	TYGPVFMCL*	419–427	A24	27	5
LMP2	CLGGLLTMV*	426–434	A*0201[e]	12	4
LMP2	VMSNTLLSAW*	442–451	A25	<1	unpub.
LMP2	LLSAWILTA	447–455	A*0203	12	38
LMP2	LTAGFLIFL	453–461	A*0206	4	5
$CD4^+$ T cell targets					
EBNA1	RRPQKRPSCIGCKGT	71–85	nd[f]		16
EBNA1	RPFFHPVGEADYFEY*	403–417	nd		16
EBNA1	VPPGAIEQGPADDPGEGPST*	429–448	nd		16
EBNA1	DGGRRKKGGWFGRHR	455–469	nd		16
EBNA1	NPKFENIAEGLRALL*	475–489	DR11	19	16
EBNA1	LRALLARSHVERTTD*	485–499	nd		16
EBNA1	VYGGSKTSLYNLRRGTALAI*	509–528	nd		16
EBNA1	TSLYNLRRGTALAI*	515–528	DR1	5	16,18
EBNA1	NLRRGTALAIPQCRL*	519–533	nd		16
EBNA1	PQCRLTPLSRLPFGM*	529–543	nd		16
EBNA1	APGPGPQPGPLRESIVCYFM	544–563	nd		16
EBNA1	LRESIVCYFMVFLQTHIFAE	554–573	nd		16
EBNA1	MVFLQTHIFAEVLKD	563–577	DR15	22	16
EBNA1	VLKDAIKDLVMTKPAPTCNI	574–593	nd		16
EBNA1	RVTVCSFDDGVDLPPWFPPM*	594–613	nd		16
EBNA1 (Q/T)	DGEPDMPPGAIEQGPADDPG*	424–443	nd		16
EBNA1 (Q/T)	KTSLYNLRRGIALAIPQCRL*	514–533	nd		16
EBNA1 (Q/T)	PTCNIKATVCSFDDGVDLPP*	589–608	nd		16
LMP1	LWRLGATIWQLLAFF*	130–144	nd		16
LMP1	SGHESDSNSNEGRHH*	212–226	nd		16
LMP1	TDGGGGHSHDSGHGG[g]	340–354	nd		16
LMP2	STVVTATGLALSLLL*	149–163	nd		16
LMP2	SSYAAAQRKLLTPV*	169–182	nd		16
LMP2	VLVMLVLLILAYRRRWRRLT	224–243	nd		16
LMP2	STEFIPNLFCMLLL*	385–398	nd		16

Notes: unpub., unpublished data from S. P. Lee and A. B. Rickinson.

[a] All epitope sequences are derived from the EBV strain B95.8, with the exception of three epitopes derived from a Q/T variant EBNA1 sequence.

[b] See References 39,40. The frequency of individual A2 subtypes has been extrapolated from those reported in the Singapore Chinese population.[41]

[c] Indicates that Chinese virus isolates encode an altered epitope sequence.

[d] Also presented through HLA A*0202, A*0203, A*0204, A*0206, A*6802, A*6901.

[e] Also presented through HLA A*0206, A*0207, A*0209.

[f] Not determined.

[g] Sequence deleted from Chinese isolates.

EBNA1 are presented through HLA DR11 and DR15 which are carried by 19 and 22% of the Chinese population, respectively.[40] However, the HLA restriction of many EBV-specific $CD4^+$ T cell responses is less clearly defined because there is greater promiscuity in the ability of class II alleles to present epitopes to T cells. Consequently, the frequency of individual class II alleles within the Chinese population may be less limiting in the context of immunotherapy.

Cellular immunity in NPC patients

Peripheral blood

When compared with healthy Chinese controls, the majority of NPC patients are hyporesponsive in non-specific tests for cell-mediated immunity.[49] The ability to prevent *in vitro* outgrowth of an LCL following EBV infection of PBMCs is also significantly reduced in most patients, but is nonetheless detectable.[50] A recent study of Chinese NPC patients probed the antigenic specificity of T cell clones reactivated *in vitro* following LCL stimulation of PBMCs.[38] EBV-specific CTLs were detected in 6/10 NPC patients and 14/21 healthy Chinese controls, and mostly targeted the immunodominant EBNA3 proteins. However, from 3/10 patients (and 11/21 control donors), CTLs specific for LMP2 were isolated, albeit at low frequency.

Tumour

NPC is characterised by a large cellular infiltrate comprised mainly of $CD4^+$ and $CD8^+$ T lymphocytes that is intimately associated with the tumour cells. The function of these cells is as yet unknown. It has been suggested that T cells within the tumour may promote growth of the malignant cells through the interaction of various cell surface receptor/ligand pairings and the release of cytokines,[47,51] although there is as yet no direct evidence for this. Equally, there is little evidence that the cellular infiltrate has arisen as part of an immune response against EBV antigens in the tumour. Early *in vitro* studies on freshly isolated tumour-infiltrating lymphocytes (TILs) identified cytotoxic activity towards EBV-positive target cells, however, it is not clear whether this effect was EBV specific[52] or HLA restricted.[53] More recently, HLA class I-restricted EBV-specific CTL clones were reactivated *in vitro* from 3/6 NPC biopsies using LCL reactivation. However, none were specific for tumour-associated viral proteins such as LMPs 1 and 2.[38] It remains to be determined whether LMP-specific responses are excluded from the tumour site since such responses were also undetectable in the blood of five of six of the NPC patients from whom the biopsies were obtained. Nevertheless, the fact that a weak LMP2-specific CTL response could be detected in the blood of one patient, but not in the tumour from that individual, indicates that these CTLs do not accumulate or expand at the tumour site.

Immune evasion

A number of observations suggest that T cell responses may be suppressed within the tumour microenvironment. For example, NPC cells have been reported to express Fas ligand which could result in apoptosis of activated T cells.[54] In addition, interleukin 10, a cytokine known to inhibit T cell-mediated regression of LCL outgrowth *in vitro*, is said to be expressed by the tumour cells[55] (although a recent study failed to confirm this finding[56]).

Therapeutic strategies

Adoptive therapy

The first attempt to treat NPC using adoptive T cell therapy was reported last year.[57] Using essentially the same approach as that employed by Rooney *et al.* to treat PTLD, LCL-reactivated T cell lines were generated *in vitro* and used to treat four advanced cases of NPC. No adverse events occurred and infusion of the CTLs was associated with a reduction in plasma EBV levels. However, there was no evidence of tumour regression. This was not entirely unexpected since LCL reactivation of PBMCs favours the outgrowth of CTL responses to the immunodominant EBNA3 proteins rather than to viral proteins expressed in NPC.[2,3,38] Unless it proves possible to activate expression of the EBNA3 proteins within the tumour, perhaps by treating NPC patients with a demethylating agent such as 5′-azacytidine,[58] there is clearly a need for an alternative approach that selectively reactivates responses to tumour-associated EBV antigens. Several such strategies have recently been described, many involving the use of dendritic cells loaded with the appropriate EBV antigen. Antigen is delivered either as a synthetic peptide epitope[59] or expressed endogenously following infection with a recombinant viral vector[60] or transfection with RNA transcripts.[61] Alternatively, the appropriate antigenic specificity may be conferred upon the patient's T cells by transfer of T cell receptor genes.[62] Adoptive therapy might also be performed using allogeneic T cells from partially HLA-matched donors.[23] Although such effectors are unlikely to persist *in vivo*, they can be generated in advance from

immunocompetent healthy EBV carriers and stored for immediate use.

Vaccination

An alternative approach to adoptive therapy is vaccination. In a recent study, 12 advanced cases of NPC were immunised with autologous dendritic cells loaded with an LMP2 peptide epitope (SSC, TYG or IED depending on the patient's HLA type). The treatment was safe, and elicited a detectable immune response in 6/12 cases that persisted for at least 3 months. Two of these six patients experienced a partial response with shrinkage of metastatic lesions (C.-L. Lin, manuscript submitted).

Recent studies have demonstrated that plasma EBV DNA levels correlate with tumour burden.[63] This observation should greatly facilitate future clinical trials in NPC as it provides a surrogate marker for clinical response. In addition, the Elispot assay provides a sensitive and quantitative method for tracking immune responses.[11]

Concluding remarks

Several factors suggest that a T cell-based therapy may be successful in treating NPC: (i) The tumour expresses EBV proteins that are known targets for $CD8^+$ and/or $CD4^+$ T cells. (ii) T cell responses to these viral proteins are restricted through HLA alleles present at high frequency in the patient population. (iii) Antigen processing pathways within the malignant cells appear to be intact. (iv) Strategies have been developed to selectively reactivate the appropriate CTL responses. Furthermore, the fact that only weak CTL responses to tumour-associated EBV antigens can be detected in the blood of a minority of NPC patients and are so far undetectable at the tumour site provides a rationale for boosting/eliciting these responses as a therapy for NPC.

However, there is still the possibility of immune evasion by the tumour cells. Equally, there is a risk of selecting for antigen-loss variants since we know nothing of the stability of the viral genome in NPC when under selective pressure. Furthermore, can we be sure that effector T cells will home to the tumour site? As yet we know very little of the molecular mechanisms that may be important in this process (although recent studies have begun to shed light on this issue).[64] Finally, if T cells contribute to tumour growth, targeting them into the tumour may do more harm than good.

If NPC is to be successfully treated using $CD8^+$ T cells, much may depend on whether LMP2 protein is expressed in the tumour. If this antigen is not expressed, the challenge will be to induce CTL responses to LMP1 or BARF1. Alternatively, EBNA1-specific $CD4^+$ T cells may mediate antitumour function, although much has still to be learnt about $CD4^+$ T cell responses in NPC patients.

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