An immunocompetent patient with a recurrence-free Epstein-Barr virus positive plasmacytoma possesses robust Epstein-Barr virus specific T-cell responses

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Supplemental: Extended materials and methods

Histology and immunohistochemistry

Immunohistochemical double staining was performed on formalin-fixed, paraffin-embedded tissue sections with the following antibodies and dilutions used: anti-IRF4 (1:50, Dako), anti-CD8 (1:50, Dako), anti-LMP1 (1:100, Santa Cruz) and anti-MHC-I (directed against beta 2 microglobulin, 1:500, Fitzgerald Industries International). For double staining of CD8 and EBER, in-situ hybridization was performed on formalin-fixed, paraffin-embedded tissue sections with the Leica Bond ISH kit, according to the manufacturer's instructions for detection of EBER and immunohistochemical staining of CD8 with anti-CD8 (1:50, Dako).

IFNγ ELISPOT assay

ELISPOT plates were activated by adding 70% ethanol for less than 2 minutes and washed using sterile deionized water. ELISPOT plates were coated with an anti-human IFNγ capture antibody (Mabtech 3420-2-1000, clone 1-D1K) diluted 1:100 in phosphate buffered saline (PBS) and incubated overnight at 4°C. The following day, the antibody solution was removed and blocked with R10 (RPMI 1640 supplemented with L-glutamine, 10% heat inactivated fetal calf serum (FCS), and 100 U/mL penicillin + 100 µg/mL streptomycin) for more than 30 minutes at room temperature. Peripheral blood mononuclear cells (PBMCs) were plated at 150,000 cells/well, and EBV peptides were added to examine immune specificities as indicated in Figure 3. These included EBNA1 (aa 400-641) peptides or pools thereof, the EBNA3A

peptide aa 379-387, an EBNA3A-C derived peptide pool, an LMP1 (aa 179-386) peptide pool and an control HIV p17 peptide pool (sequence details in Supplemental Figure 1). Cells were incubated at 37°C overnight. After the incubation, cells were removed and the ELISPOT plate incubated for 2 hours with a biotinylated detection antibody (Mabtech clone 7-B6-1) at a 1:1000 dilution in PBS + 0.5% FCS. Following several washes in PBS, Streptavidin-ALP (Mabtech 3310-10) was added at a dilution of 1:2000 in PBS + 0.5% FCS for 1 hour. The plate was washed extensively in PBS and the substrate solution BCIP/NBT-plus was added to wells following filtration. The plate was developed until spots were visible. Spot development was stopped by extensive washes with water and the plate was dried overnight and read the following day.

Patient consent

Written informed consent was obtained from patients according to institutional guidelines for this study and for anonymous scientific publication purposes.

Supplemental Figure 1

EBNA1 all: all peptides below (final concentration 1 µM)

EBNA1 Pool 1 (final concentration 1 µM):
EBNA1 (400-414) PGRRPFFHPVGEADY
EBNA1 (405-418) FFHPVGEADYFEYH
EBNA1 (409-422) VGEADYFEYHQEGG
EBNA1 (413-429) DYFEYHQEGGPDGEPDV
EBNA1 (420-434) EGGPDGEPDVPPGAI
EBNA1 (425-439) GEPDVPPGAIEQGPA
EBNA1 (430-451) PPGAIEQGPADDPGEGPSTGPR
EBNA1 (435-451) EQGPADDPGEGPSTGPR
EBNA1 (442-458 PGEGPSTGPRGQGDGGR
EBNA1 (449-461) GPRGQGDGGRRKK

EBNA1 Pool 2 (final concentration 1 µM):
EBNA1 (452-465) GQGDGGRRKKGGWF
EBNA1 (456-469 GGRRKKGGWFGKHR
EBNA1 (460-474) KKGGWFGKHRGQGGS
EBNA1 (465-478) FGKHRGQGGSNPKF
EBNA1 (469-482) RGQGGSNPKFENIA
EBNA1 (473-487) GSNPKFENIAEGLRA
EBNA1 (478-491) FENIAEGLRALLAR
EBNA1 (482-496) AEGLRALLARSHVER
EBNA1 (487-503) ALLARSHVERTTDEGTW
EBNA1 (494-508) VERTTDEGTWVAGVF

EBNA1 Pool 3 (final concentration 1 µM):
EBNA1 (499-510) DEGTWVAGVFVY
EBNA1 (501-514) GTWVAGVFVYGGSK
EBNA1 (505-518) AGVFVYGGSKTSLY
EBNA1 (509-522) VYGGSKTSLYNLRR
EBNA1 (513-527) SKTSLYNLRRGTALA
EBNA1 (519-532) NLRRGTALAIPQCR
EBNA1 (523-539) GTALAIPQCRLTPL
EBNA1 (527-541) AIPQCRLTPLSRLPF
EBNA1 (532-544 RLTPLSRLPFGMA
EBNA1 (535-548) PLSRLPFGMAPGPG

EBNA1 Pool 4 (final concentration 1 µM):
EBNA1 (539-554) LPFGMAPGPGPQPGPL
EBNA1 (545-559) PGPGPQPGPLRESIV
EBNA1 (549-563) PQPGPLRESIVCYFM
EBNA1 (554-569) LRESIVCYFMVFL
EBNA1 (557-571) SIVCYFMVFLQTHIF
EBNA1 (562-576) FMVFLQTHIFAEVLK
EBNA1 (566-580) LQTHIFAEVLKDAIK
EBNA1 (571-584) FAEVLKDAIKDLVM
EBNA1 (575-588) LKDAIKDLVMTKPA
EBNA1 (579-593) IKDLVMTKPAPTCNI

EBNA1 Pool 5 (final concentration 1 µM):
EBNA1 (584-599) MTKPAPTCNIRVTV
EBNA1 (588-600) APTCNIRVTVCSF
EBNA1 (591-604) CNIRVTVCSFDDGV
EBNA1 (595-609) VTVCSFDDGVDLPPW
EBNA1 (600-614) FDDGVDLPPWFPPMV
EBNA1 (605-619) DLPPWFPPMVEGAAA
EBNA1 (610-624 FPPMVEGAAAEGDDG
EBNA1 (615-629) EGAAAEGDDGDDGDE
EBNA1 (620-634) EGDDGDDGDEGGDGD
EBNA1 (625-639) DDGDEGGDGDEGEEG
EBNA1 (630-641) GGDGDEGEEGQE

EBNA3A (379-387) (final concentration 1 µM):
EBNA3A (379-387) RPPIFIRRL HLA-B7 EBV

EBNA3A-3C pool (final concentration 1 µM):
EBNA3A (325-333) FLRGRAYGL HLA-B8 EBV
EBNA3A (379-387) RPPIFIRRL HLA-B7 EBV
EBNA3A (458-466) YPLHEQHGM HLA-B35 EBV

EBNA3A (603-611) RLRAEAQVK HLA-A3 EBV
EBNA3B (416-424) IVTDFSVIK HLA-A11 EBV
EBNA3C (258-266) RRIYDLIEL HLA-B27 EBV
EBNA3C (281-290) EENLLDFVRF HLA-B44 EBV

LMP1 (final concentration 1 µg/mL/peptide):
LMP1 C-terminal peptide pool spanning aa 179-386
LMP1 (15mer) EMLWRLGATIWQLLA
LMP1 (15mer) LGATIWQLLAFFLAF
LMP1 (15mer) WQLLAFFLAFFLDLI
LMP1 (15mer) FFLAFFLDLILLIIA
LMP1 (13mer) FLDLILLIIALYL
LMP1 (15mer) LILLIIALYLQQNWW
LMP1 (14mer) IALYLQQNWWTLLV
LMP1 (15mer) LQQNWWTLLVDLLWL
LMP1 (15mer) WTLLVDLLWLLLFLA
LMP1 (15mer) DLLWLLLFLAILI
LMP1 (15mer) LLFLAILIWMYYH
LMP1 (14mer) ILIWMYYHGQRH
LMP1 (15mer) IWMYYHGQRHSDEHH
LMP1 (15mer) HGQRHSDEHHHDDSL
LMP1 (12mer) SDEHHHDDSLPH
LMP1 (14mer) EHHHDDSLPHPQQA
LMP1 (16mer) DDSLPHPQQATDDSGH
LMP1 (15mer) PQQATDDSGHESDSN
LMP1 (15mer) DDSGHESDSNSNEGR
LMP1 (15mer) ESDSNSNEGRHHLLV
LMP1 (13mer) SNEGRHHLLVSGA
LMP1 (16mer) GRHHLLVSGAGDGPPL
LMP1 (15mer) VSGAGDGPPLCSQNL
LMP1 (14mer) DGPPLCSQNLGAPG
LMP1 (14mer) LCSQNLGAPGGGPD
LMP1 (14mer) NLGAPGGGPDNGPQ
LMP1 (15mer) GAPGGGPDNGPQDPD
LMP1 (15mer) GPDNGPQDPDNTDDN
LMP1 (14mer) PQDPDNTDDNGPQD
LMP1 (15mer) DNTDDNGPQDPDNTD
LMP1 (15mer) NGPQDPDNTDDNGPH
LMP1 (13mer) PDNTDDNGPHDPL
LMP1 (15mer) TDDNGPHDPLPQDPD
LMP1 (15mer) PHDPLPQDPDNTDDN
LMP1 (14mer) PQDPDNTDDNGPQD
LMP1 (15mer) DNTDDNGPQDPDNTD
LMP1 (14mer) NGPQDPDNTDDNGP
LMP1 (13mer) DPDNTDDNGPHDP
LMP1 (15mer) NTDDNGPHDPLPHSP
LMP1 (16mer) GPHDPLPHSPSDSAGN
LMP1 (15mer) PLPHSPSDSAGNDGG
LMP1 (14mer) PSDSAGNDGGPPQL
LMP1 (14mer) AGNDGGPPQLTEEV
LMP1 (13mer) GGPPQLTEEVENK
LMP1 (15mer) PQLTEEVENKGGDQG
LMP1 (14mer) EVENKGGDQGPPLM
LMP1 (17mer) KGGDQGPPLMTDGGGGH
LMP1 (12mer) PLMTDGGGGHSH
LMP1 (14mer) MTDGGGGHSHDSGH
LMP1 (16mer) GGGHSHDSGHGGGDPH
LMP1 (14mer) DSGHGGGDPHLP
LMP1 (14mer) GGGDPHLPTLLL
LMP1 (15mer) PHLPTLLLGSSGS
LMP1 (15mer) TLLLGSSGSGGDD
LMP1 (14mer) LGSSGSGGDDDDPH
LMP1 (15mer) GSGGDDDDPHGPVQL
LMP1 (14mer) DDDPHGPVQLSYYD

HIV p17 pool (final concentration 1 µg/mL):

HIV- 1 Con B Gag (15- mer) MGARASVLSGGELDR
HIV- 1 Con B Gag (15- mer) ASVLSGGELDRWEKI
HIV- 1 Con B Gag (15- mer) SGGELDRWEKIRLRP
HIV- 1 Con B Gag (15- mer) LDRWEKIRLRPGGKK
HIV- 1 Con B Gag (15- mer) EKIRLRPGGKKKYKL
HIV- 1 Con B Gag (15- mer) LRPGGKKKYKLKHIV
HIV- 1 Con B Gag (15- mer) GKKKYKLKHIVWASR

HIV- 1 Con B Gag (15- mer) YKLKHIVWASRELER
HIV- 1 Con B Gag (15- mer) HIVWASRELERFAVN
HIV- 1 Con B Gag (15- mer) ASRELERFAVNPGLL
HIV- 1 Con B Gag (15- mer) LERFAVNPGLLETSE
HIV- 1 Con B Gag (15- mer) AVNPGLLETSEGCRQ
HIV- 1 Con B Gag (15- mer) GLLETSEGCRQILGQ
HIV- 1 Con B Gag (15- mer) TSEGCRQILGQLQPS
HIV- 1 Con B Gag (15- mer) CRQILGQLQPSLQTG
HIV- 1 Con B Gag (15- mer) LGQLQPSLQTGSEEL
HIV- 1 Con B Gag (15- mer) QPSLQTGSEELRSLY
HIV- 1 Con B Gag (15- mer) QTGSEELRSLYNTVA
HIV- 1 Con B Gag (15- mer) EELRSLYNTVATLYC
HIV- 1 Con B Gag (15- mer) SLYNTVATLYCVHQR
HIV- 1 Con B Gag (15- mer) TVATLYCVHQRIEVK
HIV- 1 Con B Gag (15- mer) LYCVHQRIEVKDTKE
HIV- 1 Con B Gag (15- mer) HQRIEVKDTKEALEK
HIV- 1 Con B Gag (15- mer) EVKDTKEALEKIEEE
HIV- 1 Con B Gag (15- mer) TKEALEKIEEEQNKS
HIV- 1 Con B Gag (15- mer) LEKIEEEQNKSKKKA
HIV- 1 Con B Gag (15- mer) EEEQNKSKKKAQQAA
HIV- 1 Con B Gag (15- mer) NKSKKKAQQAAADTG
HIV- 1 Con B Gag (15- mer) KKAQQAAADTGNSSQ
HIV- 1 Con B Gag (15- mer) QAAADTGNSSQVSQN

Supplemental Figure 1: Peptides used in the ELISPOT assay. Peptides were used at the indicated concentrations and stored at -80°C in DMSO.