SUPPLEMENTAL INFORMATION

Extended Experimental Procedures can be found with this article on line.

EXTENDED EXPERIMENTAL PROCEDURES

Cell Lines

Nalm6, a human pre B-cell line from a patient with acute lymphoblastic leukemia, was a gift of Arnold Freedman (Dana-Farber Cancer Institute, Boston MA). K562, derived from a patient with chronic myeloid leukemia in blast crisis, Raji, an EBV+ Burkitt lymphoma (BL) and B958 a marmoset B-LCL were purchased from ATCC (Manassas, VA). The Akata BL cell line was a gift of Dr. Kenzo Takada (Aichi Cancer Center Research Institute, Nagoya, Japan). All other BL cell lines, both EBV- and EBV+ (Table S1) were gifts of Drs. Beverly Blazar (Wellesley College, retired) and Dr. Fred Wang (Brigham and Women's Hospital, Boston, MA). BJAB clones that express individual EBV genes were a gift of Dr. Fred Wang. The EBV producer B-LCL line of marmoset origin B958, in which virus was genetically modified to express enhanced green fluorescent protein (EGFP), EBfaV-GFP, was a gift of Dr. Richard Longnecker (Northwestern University, Chicago IL). The Tet-BZLF1 B958 cell line in which the immediate early gene BZLF1 was placed under an inducible promoter was a gift of Professor Tatsuya Tsurumi (Aichi Cancer Research Center, Nagoya, Japan). The EBV+ BL line Akata-GFP was a gift of Dr. Lindsey Hutt-Fletcher (Louisiana State University, Shreveport. Louisiana). Dr. Jack Strominger (DFCI, Boston, MA) provided the EBV+ human LCLs listed in Table S1, P3HR1 (BL line) and the marmoset line B958. All cell lines were maintained in RPMI-1640 medium supplemented with heat inactivated 10% FBS, 40mM Hepes, pH 7.4, 1mM sodium pyruvate, non-essential amino acids, 100 units/ml of penicillin and 100μg/ml of streptomycin (Mediatech Inc).

Construction of pCI-puroCD21

The puromycin resistance gene from the vector pPUR (Clontech) was excised with *Pvu* II and *Bam* HI sites and replaced the excised neomycin resistance cassette of pCI-neo. A full length CD21 cDNA encoding 16 SCRs then was excised from pH3MCD21 (Prota et al., 2002), with *No*t I and *Sp*e I and cloned into the polylinker of pCI-puro. Fidelity was confirmed by DNA sequence analysis.

Generation of Recombinant Cell Lines

Nalm6 and K562 cell lines were washed in PBS and seeded (5 x 10^5 cells/well) in six-well plates using Opti-MEM reduced serum media (Invitrogen) and transduced with 2 μg of plasmid DNA from the respective plasmid constructs using Lipofectamine™ (Life Technologies) in accordance with the directions of the manufacturer. Plasmids pCI-neo, pCI-neoCD35, CD35 ED truncation mutants (LHRs A-D and SCRs29-30), pCI-puro, and pCI-puroCD21 were individually transfected into Nalm-6, K562 or K562+ cells (see below). Transfectants were selected with the appropriate antibiotic (Geneticin or puromycin (Life Technologies)). Five independent clones of Nalm6, K562, K562+ cells that contained each of the described constructs and survived antibiotic selection were chosen for further study. The cells were expanded and analysed by FC to document surface expression and IB to verify the molecular weight of the respective recombinant proteins. C' receptor expressing cells were sorted five additional times to further isolate relevant cell populations and then used for experiments. Cells were maintained in selection and re-analyzed to verify stable expression before individual experiments.

To express HLA II in human K562 cells (denoted K562+), the plasmid pZeoCIITA containing CIITA, a transcriptional activator of the HLA II promoter, gift of Dr. Jeremy Boss (Emory University School of Medicine, Atlanta, GA), was transfected as described above. Forty-eight hrs after transfection, cells were incubated with media containing Zeocin (Life Technologies) to select for cells expressing HLA II. Five clones of K562+ cells that survived antibiotic selection were amplified and analysed by FC for expression of HLA II. Positive cells were sorted five times and then transfected with additional plasmids (e.g. pCI-puro, pCI-puroCD21, pCI-neo, pCI-neoCD35, truncated forms of CD35 in pCIneo or combinations of the respective plasmids as described in Results. K562+ cells were selected with both Zeocin in combination with Geneticin and/or puromycin. Five clones of K562+ cells that survived antibiotic selection were expanded and analysed by FC and IB to document expression of CD35, CD21 and HLA II.

Flow Cytometry

Cells, 1 x 10^5 - 10^7 depending on the experiment, were washed three times in PBS containing doubly heat inactivated 2% FBS and incubated with relevant Abs directly conjugated with fluorochrome on ice for 30 min. In some experiments unconjugated Ab was used in the first step, washed twice in PBS and then a fluorochrome conjugated secondary Ab was incubated with the cells for an additional 30 min on ice before two final cold washes. Ab concentrations were ten fold in excess of saturation based on prior titration by cytometry using appropriate

positive and negative control cell lines. In some experiments cells were pre-incubated in 10mM sodium azide for 30 min and stained as above at the indicated temperature. Abs used for these experiments and their applications are listed in Table 2. Cytometric analysis was carried out on a FACScan or LRSII benchtop flow cytometer (B-D) and analyzed using CellQuest ProVersion 4.0.1 software (B-D) and/or FlowJo Cytometry analysis software (Tree Star Inc, Ashland, OR). A minimum of ten thousand events was recorded for each experiment.

Visualization of CD35 and CD21 Expression on Stable Transfectants.

Cells were washed three times in PBS and incubated with AlexaFluor-488 anti-CD35 and/or AlexaFluor-594 anti-CD21 mAbs (Table 2) for 30 min on ice. Stained cells were washed three times, mounted (Fluorescence Mounting Media,DakoCytomation) and imaged using an UPlanApo 60x1.42 NA objective on an Olympus BX62 microscope fitted with a cooled Hamamatsu Orca AG CCD camera. The microscope, filters and camera were controlled by iVision v. 4.0.9 (Biovision Technologies). For co-localization analyses, the microscope was fitted with Zero-Shift TRITC and FITC filters (Semrock) that provide registration at pixel-resolution between the TRITC and FITC channels. Acquired images were further processed using the co-localization modules of iVision and Volocity 4.2 (Improvision).

Deconvolution Microscopy

Z-stacks through stained cells were acquired in 0.25 μm intervals from bottom to top using sequentially green and red channels. Each volume was then separately deconvoluted using an iterative restoration algorithm (Volocity 5.0, Perkin Elmer) based on a measured point spread function that was derived from images acquired using channel-specific sub-resolution fluorescent beads (Invitrogen). The volumes were further analyzed using the co-localization module of Volocity 4.2 (Improvision). For analysis of the intensity profiles on cells, both the green and the red channel of each image were analyzed with iVision 4.0.9 and the values generated exported to Prism 4.0 (GraphPad).

Immunoblot

Cells were washed three times in PBS and lysed in Radio Immuno-Precipitation Assay Buffer (RIPA) (Boston Bioproducts) containing protease inhibitors (complete protease inhibitor cocktail, Roche Applied Science). Individual lysates were incubated on ice for 30 min and then centrifuged for 5 min at 12000 x g in a benchtop microcentrifuge model 5415 D (Eppendorf) to

remove cell debris. Concentrations of protein extracts were determined by Bradford protein assay using Coomassie Brilliant Blue (Sigma). Protein extracts were boiled for 10 min in Laemmli SDS-sample buffer (Boston Bioproducts) under non-reducing conditions. Total protein (10 μg - 30 μg in different experiments) was loaded onto a 4-12% polyacrylamide gel for separation and transferred to a PVDF membrane (Immobilon-P, Millipore) for analysis. After transfer, membranes were pre-incubated with 5% non-fat dry milk (LabScientific) for one hr to prevent non-specific binding of protein, incubated with specific primary antibodies (anti-CD35, anti-CD21, anti-HLA-DR, anti-LMP1, anti-β-actin, anti-GAPDH, see Table 2) diluted in TBST overnight at 4°C, washed thrice in TBST and incubated with appropriate HRP-conjugated secondary antibodies (Santa Cruz Biotechnology). Protein bands were visualized with an ECL kit (GE-Life Sciences) and images were obtained using a LAS-4000 luminescent image analyzer (Fujifilm). β-actin or GAPDH served as an internal loading control.

EBV Binding Assays

For direct binding assays, cells were seeded at a density of 1 x 10^6. After seeding cells were washed two times with PBS containing doubly heat inactivated 2% FBS and incubated with either wild type B958, B958-Tet-BZLF1, EBfaV-GFP, Akata, or Akata-EGFP virions. Assays were performed on ice or in some cases at 37^0C in the presence of azide. Excess virus was removed by washing twice in PBS (or PBS azide). Cells that were pre-incubated with virus and controls (no virus) were then incubated with mAb 2L10 for 1 h, washed and then re-incubated with GAM conjugated Ab (Table 2) for 30 min. After staining cells were washed two additional times and then analyzed for EBV binding by FC.

EBV Infection Assays

Direct Infection Assays Cells were seeded at a density of $1x10^6$ in triplicate and washed in PBS. Washed cells were incubated with pre-determined concentrations of the B958-EBfaV-GFP virus for 1 hr on ice or at 37°C. Cells were then washed three times in PBS and infection was allowed to proceed at 37°C in a six well plate. Expression of EGFP in infected cells was visualized as described (Speck and Longnecker, 1999) at 24, 48, 72 and 96 hr.

Receptor Blocking Assays (Infection) Cells were seeded as above and then incubated with anti-CD21 or anti-CD35 Abs or isotype/serum controls (Table S2) for one hr on ice with primary Ab (if a secondary Ab was used for 30 min each with two washes in between). At the end of the

incubation period cells were washed thrice and then incubated with B958-EBfaV-GFP for 1 hr on ice, again washed thrice and incubated at 37°C for the times indicated post-infection.

EBV Blocking Assays (Infection) Cells were seeded as described above. Purified B958-EBfaV-GFP virus was incubated in the absence of Ab or with one of the following: UPC10, 2L10, 72A1, F-2-1 alone or followed by goat F(ab)'2 anti-mouse Ig, polyclonal anti-C3, or alternatively with one of the following soluble recombinant proteins CD21 (full length), CD21 SCRs1-2, CD35(full length) for 1 hr on ice or at 37^{0}C. Each independent mixture was then added to cells for 1 hr on ice. Cells were then washed thrice with PBS and incubated at 37°C in complete RPMI until visualized as described.