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25. This experiment also supports our conclusion that we are observing the activity of a single FtsK complex. If multiple complexes were bound to the DNA, we would expect to see by chance occasional reeling in from above and below the observed particle.
26. It is difficult to measure a conventional stall force, because in almost all cases, the DNA loop releases before the motor velocity falls to zero.
27. Because processive motion is observed against a force of ~63 pN, we can place an upper limit of 1.6 nm (100 pN nm/63 pN) on the step size of the motor. This upper bound was calculated by assuming that a single ATP molecule is hydrolyzed per step and by recognizing that the efficiency must be less than 100%.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/307/5709/586/DC1
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7 September 2004; accepted 1 December 2004
10.1126/science.1104885

Restoration of Tolerance in Lupus by Targeted Inhibitory Receptor Expression

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Lupus, a multigenic autoimmune condition in which a breakdown of tolerance results in the development of autoantibodies, leads to a variety of pathologic outcomes. Despite the heterogeneity of factors influencing disease susceptibility, we demonstrate that the partial restoration of inhibitory Fc receptor (FcγRIIB) levels on B cells in lupus-prone mouse strains is sufficient to restore tolerance and prevent autoimmunity. FcγRIIB regulates a common B cell checkpoint in genetically diverse lupus-prone mouse strains, and modest changes in its expression can result in either tolerance or autoimmunity. Therefore, increasing FcγRIIB levels on B cells may be an effective way to treat autoimmune diseases.

The ability of the immune system to distinguish self from nonself is central to its ability to protect against pathogens and, at the same time, maintain nonresponsiveness to self. This property is established at discrete checkpoints both during development and in the adult. To date, several early developmental checkpoint mechanisms have been identified. These include the deletion of autoreactive lymphocytes during early development of the immune system (*1*–*3*); anergy, which converts autoreactive cells to a state that precludes them from becoming activated (*4*, *5*); and editing, a mechanism for modifying autoantibodies that renders them nonautoreactive (*6*, *7*). Although these developmental checkpoints purge the immune repertoire of autoreactive cells, the processes of central tolerance remain incomplete, allowing self-reactive cells that express antigen receptors to escape into the periphery (*8*, *9*). In addition, mechanisms that enhance antibody diversity, such as somatic mutation, can generate potentially autoreactive antigen receptors in the adult (*10*). Thus, checkpoints that operate in the periphery of mature individuals are critical for maintaining tolerance and for establishing tolerance to self-antigens that only appear after maturity. Less is known about these peripheral checkpoints, although a principal element has emerged whereby the balance between stimulatory and inhibitory signals regulates the activation and expansion of lymphoid cells. Inhibitory signaling, in particular, is a critical feature of peripheral tolerance, providing a means for establishing thresholds for stimulation and for active deletion of autoreactive cells from the peripheral repertoire. Perturbations in inhibitory signaling pathways have been shown to be genetically associated with autoimmunity (*11*, *12*).

Genetic studies have associated a large number of loci and candidate genes, in addition to inhibitory signaling pathways, with susceptibility to the development of autoimmune diseases (*11*, *13*). In the context of multifactorial and multigenic diseases such as lupus, it is possible that single overriding factors may ultimately dictate whether the disease progresses or not. The selection and proliferation of immunoglobulin G (IgG)–producing B cells represents one such overriding peripheral checkpoint that is under the potential control of inhibitory signaling pathways.

Our previous work demonstrated that the expression of the inhibitory Fc receptor FcγRIIB was required for the maintenance of tolerance (*14*). C57BL/6 mice that are deficient in this receptor develop spontaneous lupus-like autoimmunity and progress to fulminate glomerulonephritis and premature mortality (*14*). Studies of bone marrow transfer into recombinase-activating gene (RAG)–deficient mice suggested that FcγRIIB deficiency in the B cell compartment is most likely responsible for the loss of tolerance seen in these mice. In support of this idea, several strains of mice that develop spontaneous autoimmune disease, such as NZB, NOD, BXSB, and MRL/lpr, have also been shown to express reduced levels of FcγRIIB on activated or germinal-center B cells. This reduced expression results from a polymorphism in the promoter of this gene (*15*–*18*). These results suggest that the absolute level of FcγRIIB expressed on some B cells may regulate the ability of these cells to maintain tolerance and that relatively small changes in the expression of this inhibitory receptor may permit the survival and expansion of autoreactive cells. To test this hypothesis, we developed retroviral vectors that are capable of expressing FcγRIIB upon transduction of bone marrow cells, which can restore the wild-type level of FcγRIIB to B cells derived from autoimmune-prone strains. Bone marrow was derived from the autoimmune-susceptible strains NZM 2410, BXSB, and B6.*Fcgr2b*$^{-/-}$ and transduced with either FcγRIIB-expressing retrovirus or parental (mock) virus lacking FcγRIIB. The bone marrow of irradiated recipients was reconstituted with autologous retroviral-transduced bone marrow, and the mice were followed for the development of autoimmunity and autoimmune disease. Mice that received autologous bone marrow transduced with the parent virus developed autoimmune disease and had reduced viability comparable to that of unmanipulated autoimmune-prone strains (Fig. 1A). In contrast, mice that received autologous bone marrow transduced with FcγRIIB-expressing retrovirus showed improved survival.

The basis for this protection was investigated by examination of the immune status of

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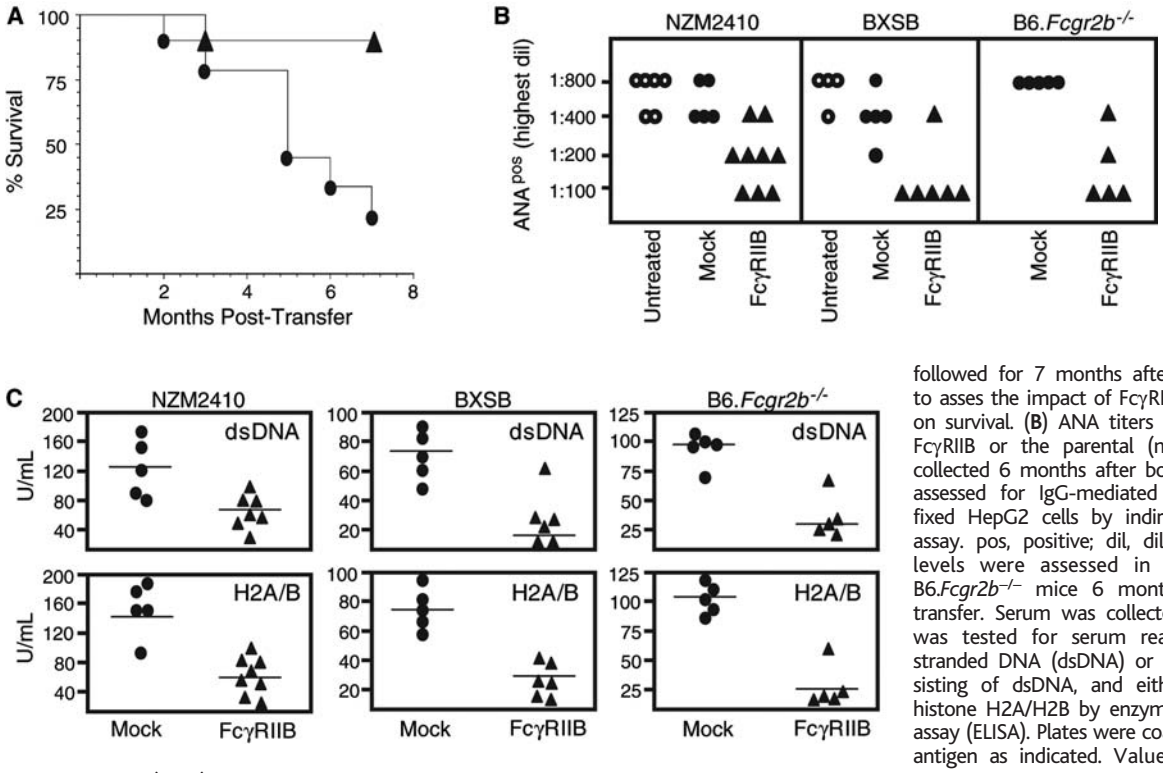


Fig. 1. Effect of FcγRIIB retroviral-mediated bone marrow transduction on spontaneous autoimmune disease in NZM2410 and BXSB mice. (**A**) Survival curve for NZM2410 mice transduced with the parental (mock) virus (solid circles) or the FcγRIIB-expressing retrovirus (solid diamonds). Mice were followed for 7 months after bone marrow transfer to asses the impact of FcγRIIB retroviral transduction on survival. (**B**) ANA titers in mice transduced with FcγRIIB or the parental (mock) virus. Serum was collected 6 months after bone marrow transfer and assessed for IgG-mediated ANA reactivity against fixed HepG2 cells by indirect immunofluorescence assay. pos, positive; dil, dilution. (**C**) Autoantibody levels were assessed in NZM2410, BXSB, and B6.*Fcgr2b*$^{-/-}$ mice 6 months after bone marrow transfer. Serum was collected and a 1:100 dilution was tested for serum reactivity against double-stranded DNA (dsDNA) or chromatin subunits consisting of dsDNA, and either histone H1 (*19*) or histone H2A/H2B by enzyme-linked immunosorbent assay (ELISA). Plates were coated with 100 ng of each antigen as indicated. Values represent the mean optical density (O.D.) for duplicate wells normalized against autoimmune serum with high levels of reactivity against both dsDNA and chromatin components.

these bone marrow–recipient animals. The mice that received FcγRIIB retroviral-transduced bone marrow exhibited reduced levels of serum antinuclear antibodies (ANAs), antibodies to DNA, or antibodies to chromatin (Fig. 1, B and C), when compared with mice that received autologous bone marrow transduced with the parent retrovirus. This reduction of ANAs, antibodies to DNA, and antibodies to chromatin accounted for the lack of immune complex deposition in the kidneys of FcγRIIB retroviral-transduced NZM or BXSB recipients (Fig. 2, A and B) and thus explained the absence of renal disease in these mice (Fig. 2C). Renal function in NZM or BXSB mice whose marrow was reconstituted with marrow that was transduced with the FcγRIIB retroviral constructs was comparable to that of wild-type mice, with the majority showing little to no urine protein. In contrast, the majority of control or mock-treated mice exhibited a marked reduction in their kidney function associated with severe proteinuria (>100 mg/deciliter) (Fig. 2C). Histological examination showed that the kidneys of FcγRIIB retroviral-transduced mice resembled those of healthy mice (Fig. 3), and untreated or mock-transduced NZM2410 and BXSB mice exhibited substantial renal pathology with proliferative glomerulonephritis, tubulointerstitial inflammation, and pronounced glomerular sclerosis (Fig. 3). Similarly, FcγRIIB retroviral transduction significantly reduced vasculitis and lung inflammation in NZM

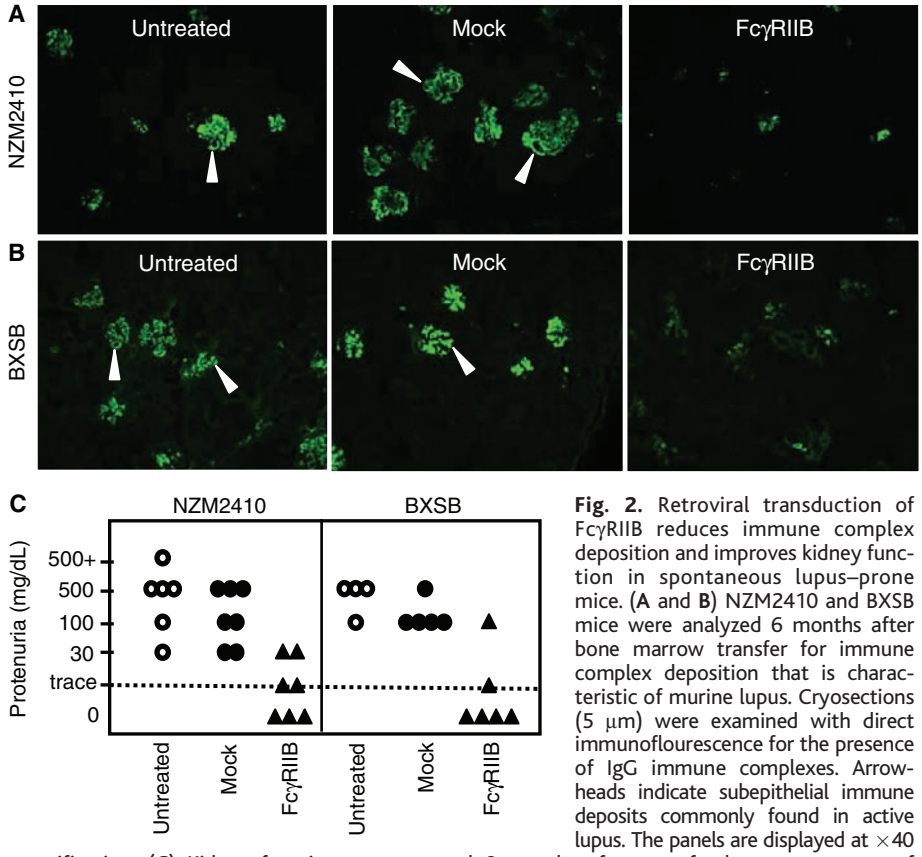


Fig. 2. Retroviral transduction of FcγRIIB reduces immune complex deposition and improves kidney function in spontaneous lupus–prone mice. (**A** and **B**) NZM2410 and BXSB mice were analyzed 6 months after bone marrow transfer for immune complex deposition that is characteristic of murine lupus. Cryosections (5 μm) were examined with direct immunoflourescence for the presence of IgG immune complexes. Arrowheads indicate subepithelial immune deposits commonly found in active lupus. The panels are displayed at ×40 magnification. (**C**) Kidney function was assessed 6 months after transfer by measurement of protein detectable in the urine of autoimmune-prone mice. Twenty-four–hour urine samples were collected and assayed for protein content as described elsewhere (*14*). The dotted horizontal line represents the normal protein levels found in mouse urine. A value of 100 mg of protein or more per deciliter is considered to be a marker of significantly altered kidney function.

2410 mice as compared with the control groups (Fig. 3).

To determine which cell populations were targeted by the retroviral transduction, we quantified levels of FcγRIIB and green fluorescent protein (GFP) by flow cytometry on lymphoid, myeloid, and dendritic cells in wild-type mice, autoimmune mice with marrow reconstituted with FcγRIIB retrovirus, and autoimmune mice with marrow reconstituted with parental retrovirus (table S1). FcγRIIB is normally expressed on B cells, myeloid cells, and dendritic cells, and is absent from T cell populations. Reconstitution of these autoimmune strains with FcγRIIB retroviral-transduced bone marrow resulted in a 50% increase in FcγRIIB expression on total B cells, which was contributed by the retrovirally encoded RIIB gene (Fig. 4). Because the retrovirally encoded RIIB gene was derived from a B6 donor and bears the allele recognized by the Ly17.2 antibody, it can be distinguished from the endogenous RIIB allele that is expressed by the NZM parent that is recognized by the Ly17.1 antibody. No change in Ly17.1 staining was seen on B220+ cells from untreated, mock-transduced, or RIIB-transduced animals (*19*). In contrast, Ly17.2 staining was noticeably enhanced in RIIB-transduced animals (Fig. 4). The efficiency of retroviral transduction in the B220+ B cell compartment for both bone marrow– and splenic-derived cells, which was determined by retroviral-encoded GFP expression, was 38% for the RIIB retrovirus and 42.5% for the parental vector alone (Fig. 4). Expression of RIIB in animals with marrow reconstituted with the RIIB-transduced bone marrow was also detected in immature T cells and a small population of myeloid cells. 20% of the immature thymocytes expressed GFP and RIIB, and 10% of the macrophages expressed these markers (figs. S1 to S3). Single positive thymocytes or single positive T cells had no detectable GFP or RIIB expression (figs. S1 and S2), and macrophages had a statistically insignificant increase in RIIB expression (fig. S3). Thus, we cannot rule out the possibility that macrophages or immature T cells play a role in the phenotype observed, although it is unlikely that they do. No overt changes in lymphoid or myeloid populations were noted in either mock- or RIIB-transduced animals (figs. S4 and S5). The basis for the apparent B cell restricted enhancement of RIIB expression may have resulted from the inactivation of the retroviral integrates in specific cellular populations (*20–24*).

These studies demonstrate that reestablishing tolerance in autoimmune mouse strains with diverse genetic backgrounds can be achieved by increasing the surface expression of the inhibitory FcγRIIB receptor on B cells. Although only 40% of the B cell compartment was transduced, the effect of increasing RIIB expression to wild-type levels on this percent of the population was sufficient to reestablish the peripheral checkpoint regulated by this inhibitory molecule and to prevent autoimmunity. These data are consistent with results obtained when the bone marrow of irradiated C57BL/6 recipient mice was reconstituted with mixed bone marrow derived from B6 and B6.*Fcgr2b*$^{-/-}$ mice. A significant reduction in ANAs was seen when 45% of the bone marrow was of wild-type origin (fig. S6).

Although the precise mechanism by which FcγRIIB expression on B cells con-

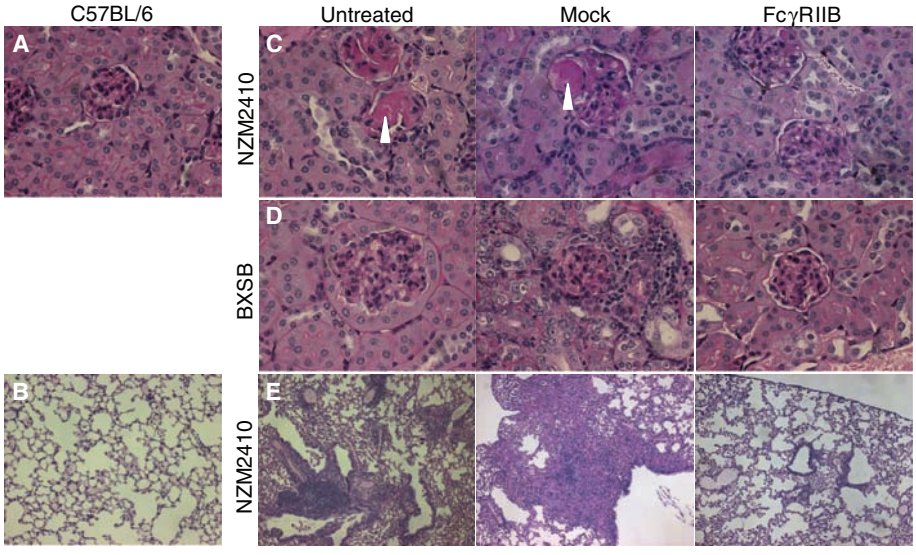
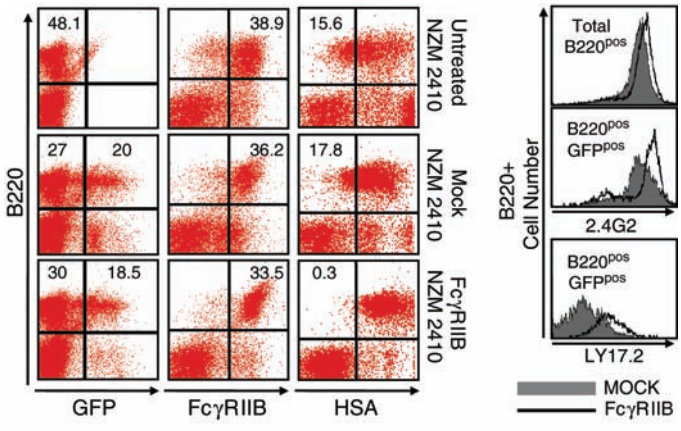


Fig. 3. Retroviral therapy with FcγRIIB reduces kidney and lung pathology in NZM2410 and BXSB mice. Periodic acid Schiff (PAS) staining of the kidney and hematoxylin and eosin (H&E) staining of the lung were done on paraformaldehyde-fixed tissue, which was then embedded in paraffin and sectioned at 5 μm thickness. Panels showing kidney sections (**A**, **C**, and **D**) are presented at ×63 magnification and lung sections (**B** and **E**) are presented at ×10 magnification. "Untreated" panels depict mice that received unmanipulated bone marrow, and "mock" and "FcγRIIB" panels represent mice that received cognate bone marrow transduced with the parental virus or the FcγRIIB-expressing virus, respectively. Untreated and mock-treated NZM2410 mice exhibited significant proliferative glomerulonephritis associated with obliterative sclerosis, and the presence of numerous hyaline cysts is indicated by arrowheads in (C). In contrast, BXSB mice exhibited proliferative glomerulonephritis associated with significant interstitial nephritis but little observable sclerosis as shown in (D). In all cases, the mice were assessed 6 months after bone marrow transfer. C57BL/6 panels [(A) and (B)] represent normal controls for comparison.



MFI of FcγRIIB Staining On B cells

Untreated	416+/-40.6
Mock	398+/-41.2
FcγRIIB	764+/-26.4

Fig. 4. FcγRIIB retroviral transduction reduces B cell activation and increases FcγRIIB expression on B cells. Five months after bone marrow transduction, B220+ splenocytes were collected and analyzed for GFP expression and FcγRIIB expression and activation. B cells were also examined for total FcγRIIB expression as assessed by staining with either the FcγRIIB/III-specific antibody 2.4G2, the C57BL/6 FcγRIIB allele-specific Ly17.2 antibody, or the NZM FcγRIIB allele-specific Ly17.1 antibody. No changes in the numbers of B220/FcγRIIB-positive cells were seen in the transduced mice as compared with untransduced controls. However, mean fluorescent intensity (MFI) indicative of FcγRIIB staining was increased by 43% on B cells. This increase in FcγRIIB expression is caused by the expression of the retroviral-encoded Ly17.2-detected FcγRIIB gene. Panels show representative samples from groups of five or six mice.

tributes to the maintenance of tolerance is still under investigation, we have recently demonstrated that RIIB expression on B cells regulates the accumulation of autoreactive plasma cells (*25*). Thus, relatively small changes in the surface expression of this receptor appear to be critical for determining disease progression, and these changes provide a rational basis for a therapeutic approach based on manipulating the expression of this receptor to restore tolerance in cases of autoimmune disease.

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26. Supported by grants from NIH and the Alliance for Lupus Research (J.V.R.) and by a National Research Service Award (T.L.M.). We thank P. Smith and M. Patt for their technical assistance and R. Steinman, M. Nussenzweig, M. Madaio, and P. Bruhns for helpful comments.

Supporting Online Material

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13 September 2004; accepted 30 November 2004
10.1126/science.1105160

Endogenous MHC Class II Processing of a Viral Nuclear Antigen After Autophagy

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CD4$^+$ T cells classically recognize antigens that are endocytosed and processed in lysosomes for presentation on major histocompatibility complex (MHC) class II molecules. Here, endogenous Epstein-Barr virus nuclear antigen 1 (EBNA1) was found to gain access to this pathway by autophagy. On inhibition of lysosomal acidification, EBNA1, the dominant CD4$^+$ T cell antigen of latent Epstein-Barr virus infection, slowly accumulated in cytosolic autophagosomes. In addition, inhibition of autophagy decreased recognition by EBNA1-specific CD4$^+$ T cell clones. Thus, lysosomal processing after autophagy may contribute to MHC class II–restricted surveillance of long-lived endogenous antigens including nuclear proteins relevant to disease.

The main protein degradation machineries in eukaryotic cells are the proteasome and lysosomal proteases. The immune system monitors the products of these catabolic processes for pathogenic determinants. For this purpose, peptides generated by the proteasome are presented on MHC class I products, whereas products of lysosomal degradation are displayed on MHC class II (*1*, *2*). CD8$^+$ and CD4$^+$ T cells survey these MHC class I and II complexes, respectively. Classically, CD8$^+$ T cell epitopes are of endogenous origin, synthesized in the antigen presenting cell (APC), whereas CD4$^+$ T cell epitopes are of exogenous origin, endocytosed by the APC. However, analysis of natural ligands eluted from MHC class II molecules has revealed that a large proportion of MHC II–bound peptides are derived from endogenous antigens (*3*).

In addition, endogenous MHC class II processing has been described for self- [GAD65 (*4*), complement C5 (*5*), and immunoglobulin light chain λ2 (*6*)], viral [influenza matrix protein (*7*), influenza nucleoprotein (*8*), and influenza hemagglutin (*9*)], and model antigens [hen egg lysozyme (*10*) and neomycin phosphotransferase II (*11*)]. However, these studies all involved overexpression and often ectopic expression of the antigens by transfection or infection with recombinant viral vectors. To date, the underlying endogenous MHC class II processing pathways are poorly characterized at the cell biology level.

We investigated endogenous MHC class II processing of the nuclear antigen 1 (EBNA1) of the Epstein-Barr virus (EBV). EBNA1 is the dominant EBV-latent antigen for CD4$^+$ T cells and can be detected by CD4$^+$ T cells after endogenous MHC class II processing in EBV-positive lymphoma cells (*12*). In order to address whether EBNA1 could enter MHC class II processing via lysosomal degradation, we incubated EBV-transformed lymphoblastoid cells (LCLs) with inhibitors of lysosomal acidification, ammonium chloride (Fig. 1) or chloroquine (*13*), for 2 days (*14*). On blocking of lysosomal acidification, EBNA1 accumulated in cytosolic vesicles, partially co-staining with the lysosome-resident protein LAMP1 (Fig. 1A). Subcellular fractionation confirmed that EBNA1 was enriched in microsomes on blocking of lysosomal acidification (Fig. 1B). In EBNA1 transfectants of the EBV-negative Hodgkin's lymphoma cell line L428 (L428E1PC5), EBNA1 was confined to whole-cell lysate and the nuclear fraction. After chloroquine treatment, EBNA1 could be found in the postnuclear supernatant and the high-speed pellet derived thereof. Discontinuous sucrose gradient centrifugation revealed that EBNA1 had accumulated in microsomes after chloroquine treatment. EBNA1 fractionated together with the lysosomal marker LAMP1. Accumulation of EBNA1 in microsomes after chloroquine inhibition was confirmed in the EBV-transformed B cell line LG2 (Fig. 1C). EBNA1, but not another nuclear EBV antigen and prominent CD8$^+$ T cell antigen, EBNA3A, was enriched in the microsomal fraction after

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