LTR in stable chromatin configuration and from the transient template by use of enzyme activity from the CAT gene and luciferase gene. Calcium phosphate–mediated transient transfections with pLTRLuc were carried out as described (*19, 24*). The cells were incubated without hormone (control) or with dexamethasone (dex) for 4 hours before the preparation of extracts. CAT and luciferase assays were performed by standard procedures as described (*19, 24*).
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LCMV-Specific, Class II–Restricted Cytotoxic T Cells in β_2-Microglobulin–Deficient Mice

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Intracranial infection of normal mice with lymphocytic choriomeningitis virus (LCMV) causes meningitis and death mediated by $CD8^+$ major histocompatibility complex (MHC) class I–restricted cytotoxic T lymphocytes (CTLs). β_2-Microglobulin–deficient mice ($\beta_2M^{-/-}$) do not express functional MHC class I proteins and do not produce significant numbers of $CD8^+$ T cells. When $\beta_2M^{-/-}$ mice were infected with LCMV, many died from LCMV disease and produced a specific response to LCMV mediated by $CD4^+$ CTLs that were class II–restricted. In these mice, $CD4^+$ CTLs may compensate for the lack of $CD8^+$ CTLs.

LYMPHOCYTIC CHORIOMENINGITIS virus–induced choriomeningitis in normal mice is mediated solely by $CD8^+$ cytotoxic T cells (*1*). Because $\beta_2M^{-/-}$ mice lack this cell population, we expected that they would be resistant to choriomeningitis, as are immunosuppressed or newborn normal mice (*2–6*). The $\beta_2M^{-/-}$ mice are derived from a C57BL/6 (B6) × 129/Ola cross and are *H-2*^{b}. We challenged B6, (B6 × 129/J)F_1, and $\beta_2M^{-/-}$ mice with LCMV (7). All three mouse strains shown (and 129/J mice) succumbed to LCMV disease (Fig. 1). The $\beta_2M^{-/-}$ mice survived longer [median survival time, 14 days; B6, 8 days; (B6 × 129/J)F_1, 7 days], with some surviving more than 3 weeks. The mean titer of LCMV isolated from the brains of $\beta_2M^{-/-}$ mice on day 6 after infection was 1.3×10^6 plaque-forming units (PFU) per gram of tissue ($n = 3$). Intracranial infection was confirmed by the presence of an antibody in all mice tested to date (16 out of 16). Histological examination of infected $\beta_2M^{-/-}$ and 129/J mice revealed similar leptomeningeal infiltrates of mononuclear cells (*8*).

Spleen cells from B6 mice infected with LCMV were cytotoxic when assayed on LCMV-infected targets matched for class I genes (Fig. 2A), including L cells (*H-2*^{k}) transfected with the *D*^{b} gene (L-D^b) and MC57 cells (*H-2*^{b}, class I^+, class II^-) (*9*). In contrast, when $\beta_2M^{-/-}$ mice were infected with LCMV, neither MC57 nor L-D^b targets were killed. Thus, there was no class I–restricted, LCMV-specific cytotoxicity. When $\beta_2M^{-/-}$ cells were tested with a class II^+ B cell lymphoma, CHB2 (*H-2*^{b}), we detected LCMV-specific CTLs (Fig. 2B). This killing was H-2–restricted: the *H-2*^{a} lymphoma CH12.LX was not killed. The B6 effector cells could also lyse CHB2 targets. To investigate the possibility that there was some killing restricted by non–β_2-associated MHC class I heavy chains of $\beta_2M^{-/-}$ mice (*3*), we prepared a fibroblast cell line from $\beta_2M^{-/-}$ embryos. Spleen cells from infected $\beta_2M^{-/-}$ mice did not lyse LCMV-infected $\beta_2M^{-/-}$ fibroblasts, which rules out an appreciable cytotoxic response restricted by the $\beta_2M^{-/-}$ class I heavy chains alone (*10*). To confirm that the killing of CHB2 target cells was restricted by class II molecules, we attempted to inhibit CTLs from $\beta_2M^{-/-}$ mice with monoclonal antibodies (MAbs) to class I or class II (Fig. 3A) (*11*). Only MAbs to class II were effective at inhibiting the killing of LCMV-infected CHB2 cells by $\beta_2M^{-/-}$ CTLs.

We were concerned that there might not be enough class II–expressing cells in $\beta_2M^{-/-}$ brains to serve as targets for the class II–restricted CTLs. Although there are class II^+ cells in normal brains, class II expression is low (*12*). We performed protein immunoblots to determine if up-regulation of class II was important in producing targets for these class II–restricted CTLs in $\beta_2M^{-/-}$ brains. Class II Ab^b ($A_\beta{}^b$) chains were substantially induced in both B6 and $\beta_2M^{-/-}$ brains after infection with the virus (*13*) (Fig. 3B). The infection induced Ab^b expression in B6 brains at a faster rate than in $\beta_2M^{-/-}$ brains, although the maximum expression was similar for both. Thus, it seems likely that there are sufficient targets for class II–restricted CTLs in LCMV-infected $\beta_2M^{-/-}$ brains, although this experiment did not show which cells (either resident or hematogenous) in the infected brains express the Ab^b molecules.

To determine the CD4CD8 phenotype of CTLs in $\beta_2M^{-/-}$ mice, we depleted immune $\beta_2M^{-/-}$ and B6 spleen cells of $CD4^+$ or $CD8^+$ cells by using appropriate MAbs and complement. We tested the remaining cells for residual cytotoxicity using LCMV-in-

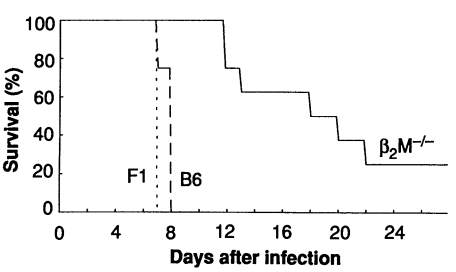

Fig. 1. Survival of $\beta_2M^{-/-}$ mice compared with B6 mice after intracranial infection with LCMV. $\beta_2M^{-/-}$ mice (solid line, $n = 8$), B6 (long dashes, $n = 4$), F1 [(B6 × 129/J)F_1] (short dashes, $n = 4$).

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fected CHB2 targets (*14*). Only anti-CD8 + complement (C) could remove activity from B6 effector cells (Fig. 4A), whereas only anti-CD4 + C removed activity from $\beta_2M^{-/-}$ cells (Fig. 4B). Thus, the B6 effectors are largely $CD4^-CD8^+$, and the effectors from $\beta_2M^{-/-}$ mice are $CD4^+CD8^-$.

$CD4^+$ cytotoxic T cell clones have been described in influenza- and vesicular stomatitis virus–infected mice and influenza-, measles-, and herpes simplex–infected humans (*15*). In these reports, however, $CD4^+$ cells were only a small fraction of the responding cells. We found that CD4 depletion had little effect on LCMV-specific CTLs from normal B6 mice, which is in agreement with studies that have shown that only class I–restricted T cells are important in the cytotoxic response to LCMV in normal mice (*1*). We show that in the absence of a significant class I–restricted, $CD8^+$ CTL response, $CD4^+$ CTLs are generated. In one of two $\beta_2M^{-/-}$ mice tested 15 days after infection, the brain contained no detectable virus, which demonstrates the ability of these mice to clear the infection. We believe the T cell response probably leads to the lethal LCMV disease observed in $\beta_2M^{-/-}$ mice, albeit with a different time course: Eichelberger *et al.* (*16*) have reported that $\beta_2M^{-/-}$ mice infected with influenza virus can clear that infection but did not test for class II–restricted CTLs in that system.

It is possible to present endogenous peptide antigens in association with class II as well as class I (*17*). Our data are consistent with the presentation of endogenous viral peptides bound to class II, although it is possible that the antigen is shed virus that is then endocytosed and presented by the conventional class II pathway. The increased killing of uninfected CHB2 cells by $\beta_2M^{-/-}$ CTLs could also be due to the uptake and processing of viral antigen from infected spleen cells.

There were two other possible mechanisms for cellular cytotoxicity in $\beta_2M^{-/-}$ mice. First, the small number of class I molecules expressed by $\beta_2M^{-/-}$ could have selected T cells in the thymus specific for the $\beta_2M^{-/-}$ class I heavy chains, but because we detected no evidence of any CTL able to recognize LCMV-infected $\beta_2M^{-/-}$ fibroblasts, we ruled out that possibility. Alternatively, natural killer (NK) cells could have produced some of the cytotoxic activity observed. This activity is sensitive to anti-CD4, which is not expressed on NK cells (*18*) and can be inhibited by MAbs to class II, unlike NK activity. Although NK activity as measured on YAC cells was detected (*19*), no NK activity was detected with the *H-2*^{a} lymphoma cell line CH12.LX (Fig. 2B). These results suggest that NK activity is not the cause of the killing of CHB2 cells.

The absence of class II proteins in bare lymphocyte syndrome in humans has profound immunological consequences resulting in severe immunodeficiency (*20*), as does the loss of $CD4^+$ cells in human immunodeficiency virus infection (*21*). Our data suggest that $CD4^+$ T cells are able to perform the role of cytotoxic $CD8^+$ cells. Because of the one-way redundancy, we speculate that although the $CD8^+$ class I system may be more specialized and recently evolved for dealing with intracellular agents, the primordial system is class II.

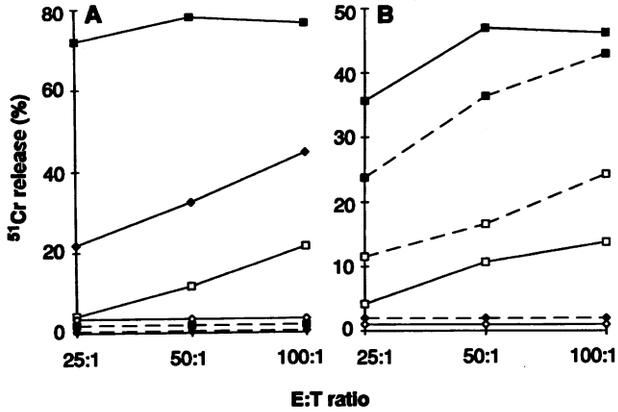

Fig. 2. Cytotoxic activity of spleen cells from LCMV-infected $\beta_2M^{-/-}$ mice (dashed lines) and B6 mice (solid lines). (**A**) Target cells expressing class I molecules. L-D^b [L929 fibroblast cell line (*H-2*^{k}) transfected with H-2D^b] infected with LCMV (solid squares) and uninfected (open squares); MC57 cells (*H-2*^{b}) infected with LCMV (solid diamonds) and uninfected (open diamonds). Pooled SEM was 6.0%. (**B**) CHB2 target cells (*H-2*^{b}) expressing class I and class II molecules are derived from a lymphoma that occurred in B10.129(21M) mice (*22*). Infected with LCMV, solid squares; uninfected, open squares. CH12.LX is a lymphoma expressing H-2^a (K^k, D^d class I and A^k, E^k class II molecules). Infected, solid diamonds; uninfected, open diamonds. Pooled SEM was 5.3%. Representative results from one of three similar experiments are shown. E:T ratio, effector:target ratio.

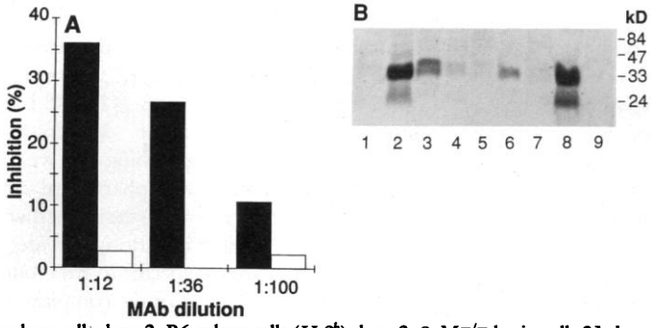

Fig. 3. (**A**) Inhibition of the cytotoxic activity of $\beta_2M^{-/-}$ mouse splenic lymphocytes for CHB2 target cells by MAb to class II (dark bars) but not to class I (light bars). Pooled SEM was 4.7%. Effector:target ratio was 50:1. (**B**) Protein immunoblot showing increased class II expression in brains of LCMV-infected $\beta_2M^{-/-}$ and B6 mice. Lane 1, B10.K (*H-2*^{k}) spleen cells; lane 2, B6 spleen cells (*H-2*^{b}); lane 3, $\beta_2M^{-/-}$ brain cells 21 days after infection with LCMV; lane 4, $\beta_2M^{-/-}$ brain cells 6 days after infection with LCMV; lane 5, $\beta_2M^{-/-}$ brain cells from uninfected mice; lane 6, B6 brain cells 6 days after infection with LCMV; lane 7, B6 brain cells from uninfected mice; lane 8, CHB2 cell line (*H-2*^{b}); and lane 9, CH12.LX cell line (*H-2*^{a}).

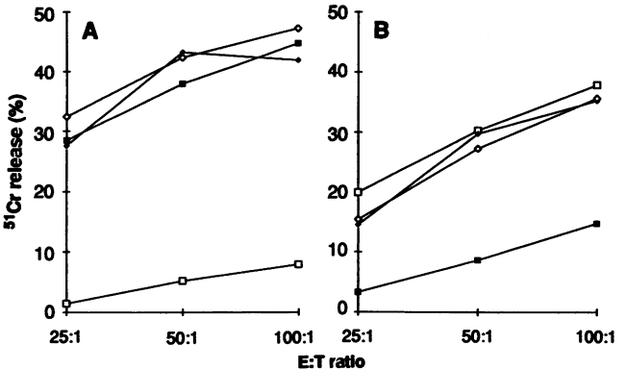

Fig. 4. Depletion of effector cells by anti-CD8 or anti-CD4 MAb and complement. (**A**) B6 effector cells. Pooled SEM was 7.1%. (**B**) $\beta_2M^{-/-}$ effector cells. Pooled SEM was 4.9%. Media alone (open diamonds); media + complement (solid diamonds); anti-CD4 + complement (solid squares); anti-CD8 + complement (open squares). E:T ratio, effector:target ratio.

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14. Cell-mediated cytotoxicity assays were performed as in (*9*) with LCMV-infected CHB2 target cells. Spleen cells (10^7) from LCMV-infected mice were incubated in an appropriate dilution of anti-CD4 (MAb 172-4) [R. Ceredig, J. W. Lowenthal, M. Nabholz, H. R. MacDonald, *Nature* **314**, 98 (1985)], anti-CD8 (MAb 31M) [M. Sarmiento, A. L. Glasebrook, F. W. Fitch, *J. Immunol.* **125**, 2665 (1980)], or medium alone and incubated for 15 min at 37°C. Rabbit complement (Low-Tox-M) (Cedarlane) was added at a 1:15 dilution for 45 min at 37°C. Spleen cells were washed and reconstituted to the original volume before CTL assays were done.
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Immunoglobulin A–Induced Shift of Epstein-Barr Virus Tissue Tropism

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Increased immunoglobulin A (IgA) antibodies to the Epstein-Barr virus (EBV) appear months to years before the clinical onset of nasopharyngeal carcinoma and define populations at high risk for this EBV-associated epithelial cancer common in south China. In the human HT-29 epithelial cell line, polymeric IgA (pIgA) specific for EBV promoted infection of the otherwise refractory epithelial cells. When bound to pIgA, EBV entered epithelial cells through secretory component–mediated IgA transport but no longer infected B lymphocytes. Such an immune-induced shift in EBV tissue tropism provides a paradigm for endogenous spread of EBV in the immune host that predicts infectious sequelae of epithelium.

PRIMARY EBV INFECTION IS MARKED by virus replication in oropharyngeal epithelial cells with subsequent spread of the virus to mucosal lymphocytes (*1*). Although there is debate on the tissue reservoir for EBV that sustains the lifelong carrier state (*2*), late manifestations of infection indicate that EBV is a mucosal pathogen: nasopharyngeal carcinoma and endemic Burkitt's lymphoma involve mucosal epithelium and mucosa-associated lymphoid tissue, respectively (*3*). Prominent IgA responses accompany two EBV-related lesions of human mucosal epithelium—nasopharyngeal carcinoma and oral hairy leukoplakia—that arise years after primary infection (*4–7*), but virus-specific secretory antibodies (Abs) are absent from the saliva of most asymptomatic shedders of the virus (*8*). Because IgA appears to herald EBV-associated epithelial disease, we investigated whether the process of IgA transport through epithelium might promote endogenous spread of this mucosal pathogen from B lymphocytes to epithelium in the persistently infected host.

In mucosal secretions more than 90% of IgA is polymeric, whereas in human serum the monomeric IgA (mIgA) molecule predominates (*9*). At the mucosa, plasma cells secrete pIgA into subepithelial interstitial spaces, where it specifically binds, by means of the J (joining) chain, to the secretory component (SC), a transmembrane protein expressed on basolateral surfaces of epithelial cells. This ligand-receptor complex is endocytosed by the epithelial cell and transported to the luminal surface, where the functional Ab, still bound to a portion of SC, is released (*10*). Of the three types of epithelia lining the nasopharynx, two express SC: pseudostratified columnar epithelium and intermediate epithelium (*11*). Both types cover much of the lateral nasopharyngeal wall including the fossa of Rosenmüller, where nasopharyngeal carcinoma arises (*12*). SC is also expressed on thymic and parotid epithelia, known sites of EBV pathology, as well as on tumor cells in some nasopharyngeal carcinomas (*5, 11*).

To test whether IgA enhances epithelial infection, we identified a source of pIgA specific to EBV but (unlike the local Ab) not already complexed with SC. Although serum IgA is largely monomeric, acute viral infections often induce transient serum pIgA responses (*13*). Thus, we isolated IgA from the sera of patients with acute infectious mononucleosis, using a lectin (jacalin) that selectively binds human polymeric and monomeric IgA1 (*14*). When the molecular size distribution of IgA from the jacalin eluate was analyzed by sucrose density gradient ultracentrifugation (*15*), two IgA peaks with specificity for the EBV membrane glycoprotein gp340 were detected (Fig. 1). Sucrose gradient fractions containing the faster sedimenting, polymeric form of Ab cosedimented with beef liver catalase (11.4*S*), which was used to mark the approximate position of dimeric IgA (10.2*S*) (Fig. 1). To determine if our IgA preparations could bind SC after separation, we

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