



of Cre-expressing mice that express this enzyme in a strictly  $\beta$  cell-specific manner will be essential. Indeed, considering that efforts are currently underway to generate conditional alleles for virtually all genes, the Cre-loxP strategy will be a cornerstone technology for understanding energy homeostasis in the mouse. However, certain lines of mice, including the B6.Cg-Tg(Ins2-Cre)25Mgn/J animals we generated nearly ten years ago (9) and that were used by Covey et al., need to be supplemented by more robust models that are free of some of the flaws that have been so aptly demonstrated here and elsewhere.

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## Role for HLA in susceptibility to infectious mononucleosis

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**Factors involved in determining whether infectious mononucleosis occurs after primary EBV infection may include age, dose of virus received, and various genetic markers. A study by McAulay and colleagues reported in this issue of the JCI shows that the presence of certain HLA class I alleles correlates with the incidence and severity of infectious mononucleosis (see the related article beginning on page 3042). These same HLA alleles are also risk factors for EBV-associated Hodgkin lymphoma (HL), supporting recent epidemiology that indicates that a history of infectious mononucleosis predisposes to HL. Recent studies suggest that an EBV vaccine might help to prevent infectious mononucleosis, and further development of this should now be considered.**

**Nonstandard abbreviations used:** EBNA, Epstein-Barr nuclear antigen; HL, Hodgkin lymphoma; IM, infectious mononucleosis; LMP, latent viral protein.

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Over 90% of the world's population become infected by EBV in their lifetime (1), and EBV is one of the best understood herpesviruses at the molecular level, but there are still some remarkable gaps in our knowledge about the details of natural EBV infection. Primary EBV infection

was shown many years ago to be the main cause of infectious mononucleosis (IM). EBV mainly infects B lymphocytes, and the current model of in vivo infection (1–3) suggests that the purpose of the various Epstein-Barr nuclear antigen (EBNA) and latent viral protein (LMP) genes in EBV is to ensure survival of infected B cells so that they can transit into the long-lived memory B cell population, in which the virus is thought to persist. In this respect, the viral strategy is analogous to that of other herpesviruses, which are characterized by persistence in a latent state in a certain cell type for the lifetime of the infected host. Replicating EBV can be found in antibody-producing plasma cells (4), which result from the end-stage of B cell differentiation, indicating that reactivation of EBV and



the production of new infectious viruses to complete the life cycle seem to occur in response to the activation of memory B cells in response to antigen.

The symptoms of IM are caused by the immune response to the infection (5). Although EBV infects mainly B cells and can cause their proliferation, the excessive number of lymphocytes observed, which account for mononucleosis, are mostly T cells. The specificity of these T cells is largely directed toward EBV proteins produced in the infected B cells. Cytokines produced during the chaotic immune response occurring in IM produce the characteristic fever, malaise, and other inflammatory symptoms. The disease subsides as the immune response adjusts, eventually to become more like that of a normal “silently” infected person. The EBV then persists in a state in which there is very little viral gene expression so that it can escape immune surveillance. However, we still don’t know why only about 30% of adults develop IM following EBV infection whereas the majority seroconvert without noticeable symptoms.

Most people are infected with EBV as young children and have no noticeable symptoms; it is only in developed countries with Western hygiene standards that primary infection is sometimes delayed until adolescence or adulthood, at which time IM may develop. A classic explanation for this observation has been that the immune systems of adults can respond differently from those of young children (1), but alternative theories propose that development of IM is mainly a consequence of the viral dose received at the time of infection or that there might be a genetic predisposition in some people to developing IM in response to EBV infection (1). Since the virus is transmitted in saliva, it is easy to imagine that the dose of virus received when a baby shares a spoon with its mother or is kissed by a parent might be less than that received by a teenager kissing in a nightclub.

The great majority of cases of IM resolve naturally and, like people infected asymptotically, these individuals then carry EBV for the rest of their lives, although it is now clear that some physiological differences exist after IM resolution. Expression of the IL-15 receptor on all peripheral T and NK cells is lost in acute IM and it can remain undetectable for many years (6). Also, a history of clinically diagnosed IM substantially increases the risk of developing EBV-positive Hodgkin lymphoma (HL)

(7–9). Thus, IM may not be the minor disease that we usually tend to consider it. In approximately 30% of all cases of HL, EBV is present within malignant Reed-Sternberg cells, and some of these cells strongly express transforming viral LMPs; therefore it seems clear that the virus is actively contributing to some cases of HL (10). EBV is also etiologically linked to various other types of cancer (e.g., nasopharyngeal carcinoma, the African form of Burkitt lymphoma, some transplant- and AIDS-related lymphomas, and a small fraction of gastric carcinomas; ref. 1), but these cancers do not have any known link to a history of IM.

### Genetic predisposition to IM

In this issue of the *JCI*, McAulay and colleagues examine the relationship between natural variation in genetic markers in the HLA class I locus, which influences many aspects of the immune response, and the frequency of IM (11). Previous reports had demonstrated an association between certain HLA class I alleles and EBV-positive HL (12, 13). In the current study, McAulay et al. found that these same alleles were significantly more frequent in IM patients than in asymptomatic EBV-seropositive or EBV-seronegative individuals. The individuals with IM and possessing these associated HLA alleles also had lower lymphocyte but higher neutrophil counts, higher EBV loads, and milder IM symptoms than individuals not carrying these alleles (11).

Additional forms of genetic predisposition to IM have been described previously. Natural variation in expression of cytokines or their receptors can alter cytokine responses, and individuals that produce low levels of IL-10 have been associated with susceptibility to EBV infection (14, 15). Also, variation in the IL-1 complex has been linked to EBV seronegativity (16). The new data presented in this issue by McAulay et al. (11) clearly show a tendency for certain HLA alleles to be linked to IM and indicate that genetic variation in T cell responses influences the outcome of primary EBV infection and the level of viral persistence. Since HLA class I determines the efficiency of the presentation of viral peptides to T cells, it is easy to envisage how this genetic variation might affect the immune response to EBV infection although the exact details of how these particular alleles affect immune response to EBV are not yet known. A suboptimal T cell response to virus during IM could result in a higher level of viral persistence in

B cells, thus increasing the chance of EBV infection of these cells and subsequent survival of abnormal B cells that have malignant potential. The fact that the same HLA class I alleles reported here to influence the frequency of IM have also been linked to EBV-positive HL suggests a genetic basis for the increased risk of EBV-positive HL reported in individuals that have suffered from IM (12, 13). A mechanism by which EBV could contribute to the development of HL has been indicated by recent results demonstrating that the EBV LMP2A protein allows survival of EBV-infected germinal center B cells that have otherwise deleterious somatic hypermutation of their immunoglobulin genes (3) and a report of the high frequency of such “crippled” immunoglobulin genes present in EBV-positive HL (17).

It is possible that naturally occurring EBV strain differences might also play a role in determining whether IM arises upon primary infection. There are 2 EBV types (types 1 and 2), which are distinguished by substantial variations in the sequence of the *EBNA2* gene. A previous report from this same research group provided some evidence that type 1 EBV strains are linked to IM (18); however, another investigation (19) concluded that EBV type 1 and type 2 incidence in IM patients was broadly in line with infection prevalence in the general population. This second study also examined additional strain variation and concluded that the multiple strains detectable within many EBV carriers were probably acquired at the time of initial infection, implying that infection protects against subsequent viral challenge. This latter point is significant for future vaccine design.

### An EBV vaccine to prevent IM

Experience with other herpesvirus vaccines indicates that it is unlikely that primary EBV infection could be completely prevented, and the lack of a convenient animal model for EBV (it only infects humans and a few closely related primates) has meant that the most useful information has come from a limited number of phase I/II clinical trials in humans. The obvious immunogen is the EBV gp350 protein, which is a major target for antibodies that neutralize EBV infection, and almost all studies have focused on this protein. The data reported to date suggest that a gp350 vaccine administered to a population of EBV-negative volunteers (ages 18–37) who were then monitored for IM and serologi-



cally assessed for EBV infection for 3 years had little effect on the frequency of silent seroconversion but greatly reduced the frequency of IM (20). This is an important result because it suggests that a vaccine to prevent IM might actually work and also because it points the way to the most sensible vaccine strategy, namely to try to prevent IM but not the normal silent infection with EBV. One can easily envisage how a partially effective gp350 vaccine might prevent IM but not silent EBV infection – if a high viral dose proves to be required for the development of IM, the immune response induced by the vaccine might be able to neutralize most of this virus (preventing IM) but not completely protect against infection. There is therefore an increasing case for further efforts to develop an EBV vaccine that could be given to EBV-seronegative teenagers or adults to try to prevent the development of IM.

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# Overstaying their welcome: defective CX3CR1 microglia eyed in macular degeneration

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**Age-related macular degeneration (AMD), the most common cause of blindness in the elderly, is characterized by degeneration of the macula and can lead to loss of fine color vision. Alterations in inflammatory and immune system pathways, which arise from genetic differences, predispose individuals to AMD. Yet the mechanism of disease progression with respect to inflammation is not fully understood. In this issue of the JCI, the study by Combadière and colleagues shows that CX3C chemokine receptor 1-deficient (CX3CR1-deficient) mice have abnormal microglia that accumulate beneath the retina and contribute to the progression of AMD (see the related article beginning on page 2920).**

**Nonstandard abbreviations used:** AMD, age-related macular degeneration; Ccl2, CC chemokine ligand 2; Ccr2, CC chemokine receptor 2; CX3CL1, CX3C chemokine ligand 1; CX3CR1, CX3C chemokine receptor 1; RPE, retinal pigment epithelium.

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Age-related macular degeneration (AMD), a degenerative disease of the retina, is the most common cause of visual impairment in the elderly in the developed world (1). The prevalence of AMD is expected to increase as the population ages. AMD is characterized by degeneration of the macula, an area in the central retina with the highest concen-

tration of cones that is responsible for high-acuity and color vision (Figure 1). Damage to the macula can cause profound loss of fine vision (Figure 1A). Genetic factors identified for AMD risk involve regulation of the inflammatory, complement, and chemokine pathways, including the fractalkine receptor CX3C chemokine receptor 1 (CX3CR1) (2). CX3CR1 is expressed in microglia, the resident macrophages in the CNS, and the retina (3, 4) and mediates migration and adhesion of these cells in response to CX3C chemokine ligand 1 (CX3CL1) (5).

In this issue of the *JCI*, a new study by Combadière et al. (6) confirmed that in humans, the CX3CR1 M280 allele increases the risk of human AMD and that microglia isolated from these individuals migrate defectively. The authors also showed that in