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**Genetic Restriction of HIV-1 Infection and Progression to AIDS by a Deletion Allele of the CKR5 Structural Gene**

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The chemokine receptor 5 (CKR5) protein serves as a secondary receptor on CD4+ T lymphocytes for certain strains of human immunodeficiency virus–type 1 (HIV-1). The CKR5 structural gene was mapped to human chromosome 3p21, and a 32–base pair deletion allele (CKR5Δ32) that is present at a frequency of ~0.10 in the Caucasian population of the United States. An examination of 1955 patients included among six well-characterized acquired immunodeficiency syndrome (AIDS) cohort studies revealed that 17 deletion homozygotes occurred exclusively among 612 exposed HIV-1 antibody–negative individuals (2.8 percent) and not at all in 1343 HIV-1–infected individuals. The frequency of CKR5 deletion homozygotes was significantly elevated in groups of individuals that had survived HIV-1 infection for more than 10 years, and, in some risk groups, twice as frequent as their occurrence in rapid progressors to AIDS. Survival analysis clearly shows that disease progression is slower in CKR5 deletion homozygotes than in individuals homozygous for the normal CKR5 gene. The CKR5Δ32 deletion may act as a recessive restriction gene against HIV-1 infection and may exert a dominant phenotype of delaying progression to AIDS among infected individuals.

In all well-characterized epidemics there are individuals in the population that respond differently to the infectious agent (1, 2). Although resistance to infection is the most common variable phenotype, variation in disease outcomes has also been observed. Epidemiologic studies have shown that inherited factors are involved in the risk of mortality from infectious agents (3, 4). The HIV-1 epidemic presents a critical challenge for the application of current genetic techniques to the study of host genetic variation for infection and susceptibility to infection. This problem is confounded in the studies of HIV-1 by the rapid rate of evolution of the virus (5–7). However, a number of groups have shown that specific alleles of the human lymphocyte antigen (HLA) locus are associated with different rates of progression from infection to AIDS diagnosis (8). Yet little evidence for non-HLA loci regulating HIV-1 infection or AIDS progression has been reported, although it does seem likely that other host genetic factors would play a role in AIDS epidemiology (8, 9).

The recent demonstration that the chemokines RANTES, MIP-1α, and MIP-1β act as natural suppressors of HIV-1 infection (10) has focused attention on the role of these chemokines during HIV-1 infection and clinical pathogenesis. Feng et al.
Table 1. AIDS cohorts studied. [A list of researchers and their affiliations for HGDS and MHCS are given in (52).]

| Study name                              | Acronym | Risk group                           | Enrollment | Participants | Sites* | Principal investigators | Reference |
|-----------------------------------------|---------|--------------------------------------|------------|--------------|--------|------------------------|-----------|
| Hemophilia Growth and Development Study | HGDS    | Hemophilia                           | 1989–1990  | 333          | 14     | E. D. Gomperts, M. W. Hilgarter, W. K. Hoots, S. M. Donfield | (31)      |
| Multicenter Hemophilia Cohort Study     | MHCS    | Hemophiliacs                         | 1985–1990  | 2472         | 10     | J. J. Goedert           | (32)      |
| DC Gay Cohort Study                     | DCG     | Homosexual men and intravenous drug users* | 1982       | 307          | 3      | J. J. Goedert, R. J. Biggar | (33)      |
| Multicenter AIDS Cohort Study           | MACS    | Homosexual                           | 1984       | 5000         | 4      | A. Munoz, J. P. Phair, R. Detels, C. R. Rinaldo Jr., A. J. Saah | (34)      |
| San Francisco City Clinic Cohort        | SFCC    | Homosexual                           | 1978–1980  | 6704         | 1      | S. P. Buchbinder        | (35)      |
| AIDS Link to the Intraavenous Experience| ALIVE   | Intraavenous drug users               | 1988–1990  | 2958         | 1      | D. Vlahov              | (36)      |

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(11) identified a chemokine receptor, fusin (12–14), as an HIV-1 entry cofactor (or co-receptor) with CD4 (15) for T cell lineotropic strains. Several groups then showed that the cysteine-cysteine (C-C)-linked chemokine receptor CKR5 (also called CC-CKR5, CCR5, and designated with gene symbol CMKBR5) (16), which serves as the principal cellular receptor for RANTES, MIP-1α, and MIP-1β, is an efficient co-receptor for macrophage-tropic isolates of HIV-1 (17–22). The closely related CKR2B and CKR3 molecules can also act as co-receptors for some HIV-1 strains (20, 21). In addition, Dragic et al. (17) observed that CD4+ T cells from some HIV-1-exposed individuals who have remained uninfected are relatively resistant to infection, suggesting that a defect in co-receptors or their expression may protect some individuals from infection.

The C-C or β chemokine receptors belong to the heterotrimeric GTP-binding protein (G protein)--coupled receptor superfamily (22–24). The C-C chemokine receptors consist of seven transmembrane domains and typically contain no introns (12–14, 16). The genes for the C-C chemokines are linked in a cluster on chromosome 17 (24). In contrast, the genes for the receptors are dispersed in the genome as single genes or in small clusters of genes that have related function and sequence (24).

To genetically map the locus encoding fusin and the CKR5 locus, we used gene-specific polymerase chain reaction (PCR) primers designed from the sequences of the genes (13, 16) to screen a panel of 90 radiation hybrid (RH) DNA samples (25). The RH panel is designed to retain small segments of the human genome in different combinations so that the map location of new markers is implicated by their concordant occurrence in the panel with previously mapped markers (Fig. 1A) (25). The distribution of RH results indicates that fusin maps to chromosome 2q21, proximal to the

![Fig. 1. Mapping of CKR genes. (A) The location of the gene encoding fusin is shown on the map of chromosome 2 in relation to the interleukin-1α (IL1A) gene and the closely related interleukin-8 receptor A and B (IL1RA, IL1RB) and their pseudogenes (IL1RBP). (B) The CKR5 gene was also typed in this RH panel and is located in the same chromosome band as the IL1RB gene. (C) The CKR2B, C-C chemokine receptor 2B, is located in the same chromosome band as the IL1RB gene. (D) The CKR1, C-C chemokine receptor 1, is located in the same chromosome band as the IL1RB gene.](http://www.sciencemag.org/content/images/1857/0223f1.png)
related interleukin-8 receptor (IL8RA, IL8RB) genes, and distal to the IL-1 and IL-1 receptor (IL1, IL1R) gene cluster (12). CKR5 maps to chromosome 3p21, very close to the CKR1 gene, which we also physically mapped in this analysis (Fig. 1B). CKR2B has been previously shown to be 18 kb from CKR5 (16) and can therefore also be assigned to 3p21. These gene-mapping assignments provide additional evidence for the occurrence of chemokine receptor genes in small clusters in different regions of the human genome.

We identified alterations in the CKR5 gene by amplifying portions of the entire coding region, digesting the fragments with Hind I, and resolving the fragments by a combined single-stranded conformation polymorphism–heteroduplex analysis approach (26, 27). DNA from more than 600 individuals (healthy controls, HIV-1 at-risk seronegatives, HIV-1–infected, non-AIDS, and AIDS patients) was typed by this procedure, and eight molecular genetic variants were identified. One of the alterations occurred in ~10% of the individuals, and this allele was sequenced. This variant contains a 32–base pair (bp) deletion (CKR5A32) that causes a frame shift at amino acid 185. After this manuscript was submitted, Samson et al. (28) and Liu et al. (29) also described the same CKR5 deletion mutation. The CKR5A32 allele is nonfunctional both as a chemokine receptor and an HIV-1 co-receptor.

The other seven variants we found were all rare and were observed in ≤1% of the individuals studied (30).

Lymphoblastoid B cell lines were established for over 1900 participants who are members of six well-characterized long-term cohorts of hemophilic, homosexual male, and intravenous drug user risk groups (Table 1) (31–36). Genomic DNA from 156 cell lines derived from Caucasian participants from the DCG and MACS studies was screened by using 170 defined and mapped polymorphic loci (37), including candidate genes (for example, CD4, chemokine SCYA1, HLA-DQA1, TCRA, TCRB, and CKR5), for distortion of allele and genotype frequency among HIV-1–infected versus HIV-1 antibody–negative individuals at risk for exposure to HIV-1. Loci were selected on the basis of available polymorphisms, potential involvement in retroviral infection or pathogenesis, and their genetic location, producing an average 20-centimorgan interval of markers (37, 38). The significance level estimated with a G test (39) for each of the 170 loci for the occurrence of genotypic association between HIV-1–infected versus HIV-1 antibody–negative individuals is presented in Fig. 2. With the exception of CKR5, none of the loci tested displayed a significant distortion of genotype frequencies among the infected versus uninfected individuals. The genotypic distribution of the two common alleles of CKR5 [normal or wild type (+) and CKR5A32 deletion] in 738 Caucasian homosexual men displays a highly significant ($P = 2.0 \times 10^{-5}$) departure from genotypic equilibrium when frequencies among HIV-1–infected versus uninfected individuals are examined. Although these 170 markers can only a portion of the genome for HIV restriction loci, the absence of any association at any of the loci suggests that there is not a major effect on HIV-1 infection from genes close to (within 1 cM of) the test markers. The list includes the HIV receptor (CD4), a β chemokine (SCYA1), and several immune function genes. These markers provide negative controls for the result observed with CKR5.

To examine further the role of the CKR5A32 allele in HIV-1 infection, we determined the distribution of alleles and genotypes with genomic DNA from 1955 individuals from the six cohorts listed in Table 1. Each of these studies represents a long-term epidemiological project designed to characterize important variables in HIV-1 infection and disease progression. All cohort participants are in high-risk groups for HIV-1 infection; namely, hemophiliacs potentially exposed to contaminated clotting factor before HIV-1 screening and viral inactivation, sexually active homosexual men, and intravenous drug users. Participants include HIV-1–exposed seronegative individuals, HIV-1–infected AIDS patients, and HIV-1–infected individuals

Fig. 2. Genotypic markers 37 and HIV-1 infection [G test (39)]. The significance value of the genotype association for each marker is plotted in physical order along each chromosome. The dotted line corresponds to significance at the 1% level for individual tests. The right arrow corresponds to an experiment-wide 1% significance level, estimated with the Bonferroni procedure for multiple samplings [see (40)].
studied for various periods who have not progressed to clinical AIDS. Among Caucasian participants in AIDS cohorts, the allele frequency of CKR5Δ32 was 0.115 (n = 1250), whereas the frequency among Caucasians not part of an HIV-1 high-risk group was 0.080 (n = 143). The frequency among African Americans was lower (0.017; n = 620) (40).

There was no significant difference in the allele frequencies of CKR5 between HIV-1–infected and HIV-1 antibody–negative individuals in any of the cohorts (41); however, a dramatic difference became apparent when the CKR5 genotype distribution was examined (Table 2). There were 17 homozygotes for CKR5Δ32 found among the individuals tested, all of which were HIV-1 antibody–negative individuals with a high risk for HIV-1 exposure. The association of the Δ32/Δ32 genotype with HIV-1–negative status is highly significant overall (G = 35.0, P = 2.5 × 10^-8; Table 2), as well as in each of three individual cohorts (MACS, SFCC, and MHCS, Table 2) (42). Hence, the CKR5Δ32 allele appears to confer a recessive phenotype that is associated with resistance to HIV-1 infection and antibody production.

Although we did not find individuals homozygous for CKR5Δ32 among 1343 HIV-1–infected patients, there was a sufficient number of heterozygous (+/Δ32) infected patients (n = 195) for us to examine the cohorts for an association between CKR5 genotype and different rates of progression to AIDS. The homosexual cohorts, but not the hemophilia cohorts, showed greater than twice the percentage of heterozygotes among long-term nonprogressors compared with rapid progressors (Fig. 3A) (43).

Although there are differences in ascertainment and disease group definition between these groups (for example, there are fewer AIDS cases in the hemophilia cohorts), the frequency of the +/Δ32 genotype was not significantly different between rapid progressors and long-term nonprogressors in the hemophilia cohorts when different AIDS endpoints [for example, 1987 AIDS definition (44), CD4+ T cell counts ≤200, or death by AIDS] were used or the "middle groups" of patients with intermediate criteria were subtracted. The differential response of hemophiliacs versus homosexual men may be related to different routes of transmission, to exposure levels, or to viral load among individuals in different risk groups. Hemophilia patients received large doses of HIV-1–contaminated clotting factors by intravenous injection, whereas sexual transmission would involve HIV-1 infection of mucosal epithelium.

Survival analyses using the clinical 1992 AIDS definition (which includes HIV-1 infection plus either AIDS-defining illness or a decline of CD4+ T cell numbers below 200) (45) were performed for each cohort and for combined cohorts comparing the CKR5 genotypes (+/+ and +/Δ32). The results demonstrate that +/Δ32 heterozygotes have a delayed progression to AIDS compared with CKR5 +/+ homozygotes (χ² = 8.1, P = 0.0045) (Fig. 3, B and C, and Table 3). After correction for multiple tests, this result is still significant, and the same result is also found when the stricter 1987 AIDS definition (HIV-1 infection plus AIDS-defining pa-

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**Fig. 3** Analysis of CKR5Δ32 genotypes with reference to progression to AIDS (43) based on previously described clinical disease categories. (A) Frequency of CKR5Δ32/ + heterozygotes in HIV-1–infected patients: DCG plus MACS, seroconversion patients who progressed to clinical AIDS in ≤7 years compared with patients that did not progress to AIDS in 7 years (8); SFCC, patients that progress to AIDS or CD4+ T cell counts <200 within 10 years of seroconversion compared with patients without AIDS and CD4 counts ≥200 for 10 or more years (35); MHCS, patients with clinical AIDS in ≤10 years of estimated seroconversion compared with patients without clinical AIDS for more than 10 years after estimated seroconversion dates (32); HGDS, patients with CD4 counts ≤200 before 10 years after estimated seroconversion date compared with patients that had CD4 counts ≥200 for 10 or more years after estimated seroconversion (31). (B) Kaplan-Meier survival distribution curves demonstrating the dependence of disease progression on CKR5 genotype in seroconverters from MHCS, SFCC, and DCG (8, 33–35). A total of 309 patients with known seroconversion dates and for which long-term data were available were followed for development of AIDS [as defined in 1993 definition (49)—progression to an AIDS-defining illness, CD4 counts ≤200, or death]. The HGDS was not included because all patients were seropositive on study entry. The curves were different significantly (χ² = 8.1, 1 degree of freedom [df], P = 0.005). Relative hazard equals 0.61 in a Cox proportional hazards model. (C) Kaplan-Meier survival distribution curves demonstrating the dependence of disease progression to AIDS (1993 definition) on the CKR5 genotype, among 148 HIV-1–seropositive members of the SFCC with well-characterized dates of seroconversion who were seen for the study after 1987 (χ² = 3.9, 1 df, P = 0.05). A value of P = 0.05 would not be significant when a correction for multiple tests is applied. Relative hazard, controlling for age, was 0.59 in a Cox proportional hazards model. The endpoint was defined as the first of two consecutive CD4 counts <200 or diagnosis of AIDS, with followup censored at the beginning of April 1996. CD4 counts were ascertained at 6- to 12-month intervals from the beginning of recruitment in 1984; AIDS diagnoses have been ascertained by active local surveillance by the San Francisco Department of Public Health as well as regular matches with the Centers for Disease Control and Prevention registry of AIDS cases.
Table 2. CKR5 genotype distribution among HIV-1-seropositive and HIV-1-seronegative individuals in the same risk group. G tests (39) for departure of three observed CKR5 genotypes within HIV-1-infected versus uninfected compared with overall cohort genotypic frequencies are listed. G tests using only Caucasian individuals are given in parentheses in the G and P columns. NC, no Caucasians.

| Cohort | Risk group | HIV-1 antibody status | Total | Number of patients (% of total) with CKR5 genotype | Statistics |
|--------|------------|-----------------------|-------|---------------------------------------------------|------------|
| DCG    | Homosexual men | Positive | 137 | +/+ (118 (86)) +/Δ32 (19 (14)) Δ32/Δ32 (0 (0)) | G (2.0 (1.6)) P (0.38 (0.44)) |
|        |            | Negative | 212 | 175 (83) 33 (16) 4 (2) | |
|        |            | Total    | 349 | 293 52 4 | |
| MAC    | Homosexual men | Positive | 265 | 201 (76) 64 (24) 0 (0) | G (9.9 (9.6)) P (0.007 (0.008)) |
|        |            | Negative | 24 | 14 (58) 6 (23) 3 (17) | |
|        |            | Total    | 289 | 215 70 3 | |
| SFCC   | Homosexual men | Positive | 150 | 110 (73) 40 (27) 0 (0) | G (9.2 (7.9)) P (0.01 (0.02)) |
|        |            | Negative | 42 | 34 (81) 5 (12) 3 (7) | |
|        |            | Total    | 193 | 145 45 3 | |
| HGDS   | Hemophiliacs | Positive | 133 | 106 (80) 27 (20) 0 (0) | G (0.62 (0.1)) P (0.73 (0.85)) |
|        |            | Negative | 104 | 87 (84) 17 (16) 0 (0) | |
|        |            | Total    | 237 | 193 44 0 | |
| MHCS   | Hemophiliacs | Positive | 192 | 156 (81) 36 (19) 0 (0) | G (8.0 (8.9)) P (0.02 (0.01)) |
|        |            | Negative | 191 | 158 (82) 26 (14) 7 (4) | |
|        |            | Total    | 383 | 314 62 7 | |
| ALIVE  | Intravenous drug users | Positive | 466 | 457 (98) 9 (2) 0 (0) | G (0.1 (NC)) P (0.77 (NC)) |
|        |            | Negative | 39 | 39 (100) 0 (0) 0 (0) | |
|        |            | Total    | 505 | 496 9 0 | |
| All    | Homosexual men and hemophiliacs | Positive | 1343 | 1148 (85) 195 (15) 0 (0) | G (35.0 (33.3)) P (2.5 × 10⁻⁶ (5.8 × 10⁻⁶)) |
|        |            | Negative | 612 | 508 (83) 87 (14) 17 (3) | |
|        |            | Total    | 1955 | 1656 282 17 | |

Table 3. Survival analysis for progression to AIDS among HIV-1-infected seroconverters of individual cohorts. The number of seroconverters is shown for each cohort, broken down into homozygous wild-type (+/+ ) and heterozygous (+/Δ32) individuals. The x² and uncorrected with 1 df P values for a difference between the +/+ and +/Δ32 survival curves were computed for each cohort using both 1987 and 1992 AIDS definitions (44, 45).

| Cohort | Genotype | Total | x² | P |
|--------|----------|-------|----|---|
|        | +/+      |       |    |   |
| MACS   | 91       | 24    | 115| 1.5| 0.22|
| SFCC   | 110      | 39    | 149| 1.2| 0.27|
| MHCS   | 78       | 17    | 95 | 0.83| 0.36|
| DCG    | 33       | 9     | 42 | 0.01| 0.93|
| All    | 312      | 89    | 401| 4.0| 0.04|
|        | Δ32/Δ32  |       |    |    |
| 1987 AIDS definition as survival outcome |
| MACS   | 91       | 24    | 115| 1.5| 0.22|
| SFCC   | 110      | 39    | 149| 3.36| 0.07|
| MHCS   | 78       | 17    | 95 | 0.83| 0.36|
| DCG    | 33       | 10    | 43 | 0.04| 0.84|
| All    | 239      | 70    | 309| 8.1| 0.005|
|        | +/+      |       |    |   |
| 1992 AIDS definition as survival outcome |
| MACS   | 91       | 24    | 115| 1.5| 0.22|
| SFCC   | 110      | 39    | 149| 3.36| 0.07|
| MHCS   | 78       | 17    | 95 | 0.83| 0.36|
| DCG    | 33       | 10    | 43 | 0.04| 0.84|
| All    | 239      | 70    | 309| 8.1| 0.005|
competing for HIV-1 binding sites on CKR5+ cells. Drugs or gene-targeting constructs that down-regulate or inactivate CKR5 may have therapeutic value. It is also possible that transplantation of bone marrow stem cells from a Δ32/Δ32 donor could have therapeutic benefit. Anti-CKR5 therapies may augment the effectiveness of other anti–HIV-1 compounds already in use.

A large difference in the frequency of the CKR5Δ32 allele was observed between Caucasians (0.11) and African Americans (0.017). If widespread screens of African racial groups affirm the absence of the CKR5Δ32 allele as has been reported in a survey of 124 Africans (29), it may be that CKR5Δ32 is a recent mutation that occurred on the Caucasian lineage subsequent to divergence from the African-Caucasian ancestor estimated to have occurred 150,000 to 200,000 years ago (48). The frequency observed in African Americans could be entirely due to admixture from Caucasian gene flow in the New World, estimated to be as high as 30% (49). The relatively high prevalence of the inactivating CKR5Δ32 allele is suggestive of an historic selective pressure among Caucasians, perhaps by another pathogenic virus or parasite, that also used the CKR5 receptor as an entry point. Such historic increases in recessive disease mutations due to epidemiologic agents have been suggested for other hereditary diseases, including sickle cell anemia, thalassemia, and lysosomal storage diseases (50).

Liu et al. demonstrated that the well-characterized EU2 and EU3 patients (29) are CKR5Δ32 homozygotes and that no functional CKR5 protein is present in the cell surface. In addition, they showed that cells from CKR5Δ32 homozygous fail to respond to MIP-1α in vitro. Samson et al. found no CKR5Δ32 homozygotes among 723 HIV-1-infected individuals and a rather low frequency for the CKR5Δ32 allele (0.054) in HIV-1-infected patients, leading them to suggest that +/Δ32 heterozygotes may be less susceptible to infection than CKR5 +/+ individuals (28). Our results (Table 2) did not reveal a difference in CKR5Δ32 allele frequency in HIV-1-infected (0.11) compared with HIV-1–negative exposed (0.12) individuals. Our data suggest that in the heterogeneous state, the CKR5Δ32 allele does not markedly affect susceptibility to infection but does postpone progression to AIDS in infected patients.

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40. The ALIVE cohort is composed of African American intravenous drug users and includes 496 homozygous (+/+) and 9 heterozygous (+/Δ32) individuals. With so few individuals with the CKR5Δ32 allele, the ALIVE cohort was not included in subsequent comparisons. Although behavioral variables are probably more relevant, the relatively low CKR5Δ32 allele frequency in African Americans may contribute to a higher rate of infection in this ethnic group among intravenous drug users [I. Vlahov et al., Am. J. Epidemiol. 144, 541 (1996)].
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CD40 Ligand-Dependent T Cell Activation: Requirement of B7-CD28 Signaling Through CD40

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The role of CD40 ligand (CD40L) in the primary activation of T cells is not clear. The cellular and humoral immune responses to adeno-associated virus (AAV) vectors in a murine model of liver-directed gene transfer were studied to define the mechanisms responsible for CD40L-dependent T cell priming. CD40L-deficient mice did not develop effective cytotoxic T cells to transduced hepatocytes, and T cell-dependent B cell responses were absent. Full reconstitution of cellular and humoral immunity was achieved in CD40L-deficient mice by administration of an activating antibody to CD40 that increased expression of B7.2 on spleen cells. Wild-type mice could be made unresponsive to vector by administration of antibodies to B7. Thus, CD40L-dependent activation of T cells occurs through signaling of CD40 in the antigen-presenting cell to enhance requisite costimulatory pathways that include B7.

The role of CD40L in humoral immunity is illustrated by the multiple defects in B cell activation that characterize its genetic deficiency in mice and humans, including a failure to form germinal centers, activate memory B cells, and class switch (1). Experiments in knockout mice have implicated CD40L in the antigen-specific priming of T cells, although the precise mechanism by which this occurs is unclear (2). Enhanced susceptibility of CD40L-deficient (CD40L−/−) mice to leishmanial infection is consistent with an important role of this molecule in cellular immunity (3). The relation between CD40L and other T cell costimulatory pathways such as B7-CD28 is unclear; in models of allograft rejection, these signaling pathways appear uncoupled (4).

Adenoviral vectors are being tested as a possible approach to gene therapy, but the cytotoxic T lymphocyte (CTL) and B cell responses to the viral proteins and transgene products make this strategy less tenable (5). Activation of CD4+ T cells to input viral capsid proteins, which requires stimulation through the CD40L-CD40 and B7-CD28 pathways, is necessary for both the CD8+ T cell (that is, CTL) and B cell (that is, neutralizing antibody) effects (6–8). If one could minimize the immune response, the adenovirus becomes a viable option. We have used this well-studied immune response to further elucidate the mechanisms of CD40L-dependent activation of T cells (9).

Infusion of adenovirus missing the early (E1) genes and containing the lacZ gene into C57BL/6 mice led to transgene expression in 93% of hepatocytes in liver harvested 3 days later that diminished to undetectable levels by day 24 (Table 1). Analysis of CD4+ lymphocytes in vitro demonstrated activation of T helper (Th) cells to viral antigens of both the Th1 [that is, secretion of interferon-γ (IFN-γ) and interleukin-2 (IL-2)] and Th2 (that is, secretion of IL-4 and IL-10) subsets (Fig. 1A) (10). Chromium release assays showed the presence of CTLs to viral-infected targets in splenocytes harvested 10 days after gene transfer (Fig. 1B) (10). Infusion of vector stimulated the development of germinal centers [20.4 ± 1.5 per section (11)] and the formation of antiviral antibodies against immunoglobulin M (IgM), IgG1, and IgG2 isotypes (Fig. 2) that are neutralizing (Table 1).

Similar studies performed in mice genetically deficient in CD40L demonstrated the requirement of CD40L-CD40 interactions in the full spectrum of cellular and humoral immune responses to adenoviral vectors in mouse liver; transgene expression was stable for 24 days in CD40L−/− mice (82% of hepatocytes still express lacZ, Table 1), and activation of CTLs to viral-infected cells was markedly blunted (8) (Fig. 1B). CD4+ T cells harvested 10 days after gene transfer failed to respond to viral antigens; however, the basal secretion of cytokines was increased as compared with that observed in C57BL/6 mice (Fig. 1A). CD40L−/− mice failed to develop germinal centers [that is, no germinal centers were detected in four sections from two mice (11)] or neutralizing antibodies (Table 1). Antiviral IgM was formed, but class switch to IgG1 or IgG2a was virtually absent (Fig. 2). Several models have been suggested to explain the dependent role of CD40L on T cell priming. A critical question is whether CD40L directly transduces an activating signal to the T cell at the time of engagement with its receptor CD40, or whether the role of CD40L is indirect, effecting T cell activation through CD40-mediated signaling in the antigen-presenting cell (APC) that leads to enhanced costimulation of downstream pathways. Previous in vivo studies in the CD40L−/− mouse documented a primary defect in T cell priming to soluble antigens without clarification of the mechanisms (2), although in vitro studies have shown that wild-type but not CD40L-deficient T cells activate costimulatory activity in B cells (12). Activation of CD40L with soluble CD40 in the CD40L−/− mice partially reconstituted formation of germinal centers, although isotype switching was not observed (2); thus,

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