104.39]. The disease-free survival plot indicated that HIV-1 seroincident women were significantly more likely to develop abnormal $CD4^+$ counts over time than HIV-2 seroincident women (Fig. 3; log-rank test, $P < 0.01$ and Gehan's Wilcoxon test, $P < 0.01$). Furthermore, we looked at seroincident women who were routinely skin tested over the period of the study and who had an initially positive PPD result. In those PPD skin test–positive seroincident women with multiple PPD skin test results, 6 of 17 HIV-1 seroincident women became anergic, whereas only 2 of 20 HIV-2 seroincident women became anergic over the study period (Fisher exact test, $P = 0.05$). The reduced likelihood for immune compromise over time will be important in monitoring HIV-2–infected outpatients, in staging this human immunodeficiency virus infection, and perhaps in evaluating therapeutic decisions and responses.

Because of the range of disease progression rates and the extreme rarity of outpatient natural history studies in an African setting for either HIV-1 or HIV-2 (*18*), it is important to report cohort follow-up data for both the seroincident and seroprevalent women. HIV-1–related disease development was greater in seroincident women than seroprevalent women. Four of five HIV-1–related AIDS cases developed in the seroincident group. This most likely represents a selection bias as a result of a "survivor effect" in those women enrolling in the seroprevalent group. Alternatively, it may also represent the movement of HIV-1 viral subtypes with more rapid disease outcome into the region, but this explanation remains to be investigated. Overall, the minimal loss to follow-up and the presence of HIV-1 seropositive subjects as a comparison group in the same cohort in this study allowed for a more relevant examination of the rate of disease development with HIV-2 infection.

We have previously described distinct risk determinants for HIV-2 and HIV-1 infection in both prevalent and incident infection in this cohort (*9*, *10*). Further, mathematical modeling of the HIV-1 and HIV-2 seroincidence data has suggested a five- to ninefold difference in the infectivity of HIV-2 compared with HIV-1 per sexual act (*19*). Perinatal transmission studies have also shown a 15- to 20-fold difference in the rate of HIV-2 transmission compared with HIV-1 transmission (*20*). In this study, we have shown that HIV-2– and HIV-1–associated AIDS and CDC IV disease incidence rates are distinct, and the disease-free survival time for HIV-2 is significantly longer when compared with HIV-1. This disease outcome study supports the hypothesis that these two related HIV viruses have different biologic behaviors. One determinant of these differences may be a lower viral burden in HIV-2 infection, on the basis of polymerase chain reaction and viral isolation studies (*21*). Numerous viral and host etiologies for a reduced virulence have also been proposed (*22*). Further investigations into the basic biologic differences that may explain the distinct rates of disease development with HIV-2 versus HIV-1 may help us understand the pathogenic mechanisms of this class of retroviruses.

REFERENCES AND NOTES

1. F. Barin *et al.*, *Lancet* **ii**, 1387 (1985).
2. F. Clavel *et al.*, *N. Engl. J. Med.* **316**, 1180 (1987); F. Brun-Vezinet *et al.*, *Lancet* **i**, 128 (1987); F. Denis *et al.*, *ibid.*, p. 408.
3. A. Bryceson *et al.*, *Lancet* **i**, 221 (1988); M. Kawamura *et al.*, *ibid.* **i**, 385 (1989).
4. I. Romieu, R. Marlink, P. Kanki, S. M'Boup, M. Essex, *J. Acquired Immune Defic. Syndr.* **3**, 220 (1990); *Wkly. Epidemiol. Rec.* **67**, 9 (1992).
5. R. G. Marlink *et al.*, *AIDS Res. Hum. Retroviruses* **4**, 137 (1988).
6. D. C. Mabey *et al.*, *Br. Med. J.* **296**, 83 (1988); K. M. De Cock *et al.*, *Lancet* **ii**, 408 (1989); I. M. Lisse *et al.*, *AIDS* **4**, 1263 (1990); A. Nauclér *et al.*, *ibid.* **5**, 301 (1990); B. M. Le Guenno *et al.*, *J. Acquired Immune Defic. Syndr.* **4**, 421 (1991).
7. J. Pepin *et al.*, *AIDS* **5**, 1165 (1991); K. M. De Cock *et al.*, *Br. Med. J.* **302**, 496 (1991); A. Wilkins *et al.*, *AIDS* **7**, 1119 (1993).
8. A. Nauclér *et al.*, *Scand. J. Infect. Dis.* **24**, 725 (1991); H. Whittle *et al.*, *AIDS* **6**, 685 (1992).
9. P. J. Kanki *et al.*, *Am. J. Epidemiol.* **136**, 895 (1992).
10. P. Kanki *et al.*, *Lancet* **343**, 943 (1994).
11. *Wkly. Epidemiol. Rec.* **65**, 281 (1990).
12. M. Zuber, K. P. Samuel, J. A. Lautenberger, P. J. Kanki, T. S. Papas, *AIDS Res. Hum. Retroviruses* **6**, 525 (1990); K. Samuel, A. Seth, M. Zweig, S. Showalter, T. Papas, *Gene* **64**, 121 (1988).
13. *Morbid. Mortal. Wkly. Rep.* **36** (suppl. 1), 1S (1987).
14. E. L. Kaplan and P. Meier, *J. Am. Stat. Assoc.* **53**, 457 (1958).
15. A. R. Lifson, G. W. Rutherford, H. W. Jaffe, *J. Infect. Dis.* **158**, 1360 (1988); A. R. Moss and P. Bacchetti, *AIDS* **3**, 55 (1989); A. Munoz *et al.*, *Am. J. Epidemiol.* **130**, 530 (1989); R. J. Biggar and the International Registry of Seroconverters, *AIDS* **4**, 1059 (1990).
16. *Morbid. Mortal. Wkly. Rep.* **35**, 334 (1986).
17. H. A. Kahn and C. T. Sempos, in *Statistical Methods in Epidemiology*, H. A. Kahn and C. T. Sempos, Eds. (Oxford Univ. Press, New York, 1989), pp. 206–225.
18. B. N'Galy *et al.*, *N. Eng. J. Med.* **319**, 1123 (1988).
19. C. Donnelly *et al.*, *Bull. Math. Biol.* **55**, 731 (1992).
20. S. Matheron *et al.*, *Lancet* **335**, 1103 (1990); G. Morgan *et al.*, *AIDS* **4**, 879 (1990); A. J. Poulsen *et al.*, *J. Acquired Immune Defic. Syndr.* **5**, 25 (1992); P. A. Andreasson, F. Dias, A. Nauclér, S. Anderson, G. Biberfeld, *AIDS* **7**, 989 (1993).
21. B. Korber *et al.*, paper presented at the IVth International Conference on AIDS and Associated Cancers in Africa, Marseille, France, 18 to 20 October 1989 (abstr. 329); F. Simon *et al.*, *AIDS* **7**, 1411 (1993).
22. R. Marlink, in *AIDS in Africa*, M. Essex, S. Mboup, P. J. Kanki, M. R. Kalengayi, Eds. (Raven, New York, 1994), pp. 47–65.
23. K. Diem and C. Lentner, Eds., *Scientific Tables* (Ciba-Geigy Limited, Basel, ed. 7, 1970), pp. 107–108.

24. Supported in part by grant DAMD 17-87-7072 from the U.S. Army and grants AI 30795 and CA 39805 from the National Institutes of Health.

1 April 1994; accepted 18 July 1994

Analysis of Sequence Transfers Resembling Gene Conversion in a Mouse Antibody Transgene

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The role of gene conversion in murine immunoglobulin gene diversification is unclear. An antibody gene construct designed to provide the homologous donor and acceptor sequences required for conversion mechanisms was produced and used to generate transgenic mice. When these transgenic mice were immunized, DNA sequence transfers between tandem transgene VDJ regions were detectable and resembled gene conversion events. There is a strong link between these conversion-like sequence transfers and transgene somatic hypermutation, suggesting that both processes might occur at the same stage of B cell differentiation.

Gene conversion is an important mechanism for the generation of antibody diversity in chickens and rabbits (*1*–*3*). However, despite many similarities in the organization of chicken, rabbit, and mouse immunoglobulin (Ig) genes, there is currently little evidence that conversion plays any role in murine antibody diversification (*4*–*6*). We have designed a transgene construct (VVC_μ, Fig. 1) that contains tandem homologous heavy chain VDJ segments [variable (V), diversity (D), and joining (J)] to simplify the identification of gene conversion events during mouse B cell development.

The VVC_μ transgene construct is based on the previously described ARS_μ transgene (*7*) which encodes a μ heavy chain that has a VDJ region derived from the R16.7 hybridoma. The ARS_μ heavy chain (H chain) can participate in an antibody response to the phenylarsonate (ARS) hapten and is reactive with the rat antiidiotypic monoclonal antibody (mAb), AD8 (*7*). In VVC_μ, a second 900–base pair VDJ region is inserted 1.5 kb upstream of the R16.7

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VDJ region present in ARS_μ (Fig. 1). This second VDJ region was isolated from the previously described 2B4 hybridoma (*8*) and is highly homologous to the R16.7 VDJ region. However, there are 17 nucleotide differences in the coding region between the two VDJ segments, and the H chain encoded by the 2B4 VDJ is not reactive with AD8 (*8*). In VVC_μ, the upstream 2B4 VDJ does not have a promoter region; thus, H chains expressed from the VVC_μ transgene would be expected to derive from the downstream R16.7 VDJ segment. To compare the affinities of the R16.7 and 2B4 VDJ regions for binding to the ARS hapten, we analyzed affinity-purified R16.7 and 2B4 mAbs by fluorescence-quenching assays (*9*). The measured affinities of the R16.7 and 2B4 mAbs were $3.8 \times 10^6\ M^{-1}$ and $7.5 \times 10^6\ M^{-1}$, respectively.

We produced two transgenic mouse lines, $VVC_\mu1$ and $VVC_\mu5$, using the VVC_μ construct (*10*). As judged by serum levels of AD8-reactive antibodies, both before and after immunization with ARS–keyhole limpet hemocyanin, expression of the VVC_μ transgene is indistinguishable from that reported for the ARS_μ transgene (*7*). In particular, as previously described for ARS_μ (*7*, *8*), the VVC_μ transgene is capable of isotype switching after immunization.

To analyze diversification of the VVC_μ transgene during an immune response, we produced hybridomas from an immunized $VVC_\mu5$ transgenic mouse and obtained 31 hybrids secreting IgG mAb to ARS. The expressed H chain VDJ regions in a random sample from this panel were sequenced (Table 1). Among 18 hybridomas, 9 showed expression of the R16.7 VDJ region with no mutations, 4 showed expression of the R16.7 VDJ with single mutations, and 5 showed expression of a transgene VDJ having more than 10 mutations with respect to the R16.7 VDJ. As shown in Fig. 2, in four of the five most heavily mutated hybridomas, the expressed VDJ sequences show mutation patterns that indicate sequence transfers within the transgene from the 2B4 VDJ region to the R16.7 VDJ region. This is most apparent in the CDR2 regions. Taking advantage of a Hind III site polymorphism between the CDR2 regions of the R16.7 and 2B4 VDJ regions, we used Southern blots to determine that in kidney cells from $VVC_\mu5$ mice, there are no transgene copies that have the 2B4 CDR2 region inserted into the R16.7 VDJ segment. Thus, the sequence transfers found in the hybridomas have occurred somatically during B cell differentiation. In addition, the shared mutation patterns in the hybridomas are unlikely to be due to antigenic selection of individual somatic mutations because mutated transgene copies derived from ARS5 mice show only a limited number of shared

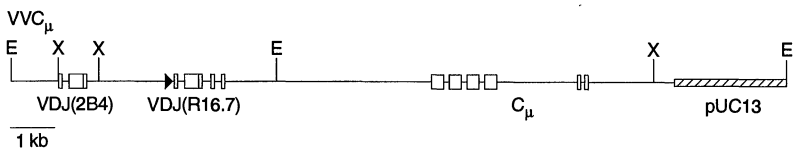

Fig. 1. The VVCμ transgene. In the mouse genomic DNA segment, exons are shown as open rectangles and introns as lines. The pUC13 vector sequences are indicated by cross-hatching. The Ig promoter is depicted as a solid arrow (not to scale) and the R16.7 and 2B4 VDJ regions are labeled. Restriction enzyme sites are shown for Eco RI (E) and Xho I (X).

mutations in the CDR2 region (*7*, *8*, *11*). For brevity, we designate the observed transfer of sequence information between the tandem VVC_μ VDJ regions as transgene conversion, although we do not yet know the mechanism of transfer.

Using the hybridomas described above, we developed enzyme-linked immunosorbant assays (ELISAs) and polymerase chain reaction (PCR) assays that were useful tools for screening further hybridomas for transgene conversion. We found that the hybridomas exhibiting transgene conversion secreted mAbs that were not reactive with AD8. In addition, we developed a complementary DNA (cDNA) PCR assay that we found to be specific for mRNAs exhibiting transgene conversion. This cDNA-PCR assay takes advantage of a primer (5′-CCGAATTCTGATCAGTGTCCTCTCCACACTC-3′) that hybridizes to a sequence located within the 5′ untranslated region of the R16.7 VDJ segment but that is not present upstream of the 2B4 VDJ region in VVC_μ, and a primer (5′-CCGAATTCTATAAAAGCTTCCAGTACTTT-3′) specific for the CDR2 region of the 2B4 VDJ segment. To characterize additional transgene conversion events, we screened the remaining 13 $VVC_\mu5$ hybridomas using these ELISA and PCR assays. From this screen, we identified four candidate hybrids; sequence analyses subsequently confirmed transgene conversion events in each (Fig. 2).

We also generated panels of hybridomas from two immunized VVCμ1 transgenic mice. Among these, we detected four candidate hybridomas on the basis of ELISA and PCR assays; subsequent sequence analyses showed transgene conversion in each (Fig. 2). Our results demonstrate that transgene conversion events occur repeatedly in immunized transgenic mice from two separate transgenic lines. Approximately 10 to 20% of IgG-producing hybrids derived from these immunized mice showed the transgene conversion phenomenon (Table 1). Because the R16.7 and 2B4 mAbs have similar affinities for ARS, we suspect that the relatively frequent occurrence of hybridomas exhibiting transgene conversion is not due to antigenic selection.

Comparison of the hybridoma sequences with those of the R16.7 and 2B4 VDJ regions also showed numerous nucleotide differences that were not accounted for by transgene conversion (solid circles in Fig. 2). These appear to be due to somatic hypermutation as previously reported for the

Table 1. Sequence analysis (*14*) of hybridomas secreting anti-ARS IgG mAb produced from $VVC_\mu5$ transgenic mice. The portion of the VDJ region of the γ mRNA sequenced in each hybrid and the number of mutations observed in each hybridoma sequence relative to the R16.7 VDJ sequence are indicated. Insertion, ins.

Hybridoma	Codons sequenced		Mutations
	5′	3′	
5I61E36	−4	111	17
5I72B40	−4	121	15
5I74B40	−4	121	28+ins (9)
5I93D25	−4	121	18
5I16D37	−4	121	12
5I7C	30	70	1
5I8A	20	74	1
5I44A	20	90	1
5I71A	−5	100	1
5I18A	−2	110	0
5I19E	30	70	0
5I23A	−1	70	0
5I27A	30	92	0
5I47C	1	70	0
5I50C	1	90	0
5I56E	30	90	0
5I82A	30	70	0
5I89B	30	70	0

Table 2. Evidence suggesting multiple transgene conversions in a single B cell. Sequences from hybridomas are compared to the R16.7 and 2B4 VDJ regions. Only the informative codons that differ between R16.7 and 2B4 within the relevant region are shown; amino acid sequences at these positions are indicated.

Source	Codons		
	75	89	105
	Ser	Glu	Ser
R16.7	TCC	GAG	AGC
5I61E36	--G	---	--T
5I93D25	--G	---	--T
1A38-5	--G	---	--T
2B4	--G	-.-A	--T
	Ser	Glu	Ser

ARS_{μ} transgene (*7*, *8*, *11*). Unexpectedly, all the hybridomas that exhibited transgene conversion also showed somatic hypermutation even though half of the IgG-producing anti-ARS hybrids showed no evidence of hypermutation (Table 1). This link between transgene conversion and hypermutation suggests that these processes might occur during the same stage of B cell maturation. Furthermore, the marked absence, among these hybridoma panels, of cells that exhibit transgene conversion without accompanying hypermutation supports the notion that antigenic selection does not play a major role in selectively amplifying the population of B cells that have undergone transgene conversion.

Comparisons of the expressed VDJ regions in hybridomas that exhibit transgene conversion also suggest that multiple transgene conversion events might occur in a responding B cell clone. As shown in Fig. 2 and Table 2, some hybridomas exhibit VDJ regions in which positions that match the R16.7 sequence (open circles in Fig. 2) are bounded upstream and downstream by positions that match the 2B4 sequence (Fig. 2 and Table 2). In some cases, this pattern is clearly not due to antigenic selection because the relevant nucleotide differences are all silent mutations (Table 2). One explanation for these results is that two different portions of the expressed VDJ region have undergone transgene conversion events and that these are separated by an "unconverted" segment. These findings suggest that transgene conversion might be mediated through multiple "mini-conversions" as found in chicken λ light chain genes (*1*). Interestingly, the sequences of codons 83 to 84 and 88 to 89 in the R16.7 VDJ region form a palindrome and, as sug-

A

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                -4 -2  6  7  8  10 31 33 38 45 50 51 52 53 55 56 57 58 59 60 61 62 63 64 65 66 67 69 70 71 72 74 75 77 79 80 82 84 85 88 89 93 101 105 106 110
R16.7           GT CAC CAG TCT GGA GAG AGC GGT AAA CTG TAT CTT AAT CCT AAT GTT TAT ACT AAG TAC AAT GAG AAG TTC AAG GGC AAG ACA CTG ACT GTA AAA TCC AGC GCC TAC CAG AGA GGC TCT GAG GTC TAC AGC TAC TAC
2B4             -- --- --A --- --- --- -A- --- --- --- --- --- -AA -G- A-- -GC T-- --- -G- -C- --- --- --- --- G-- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --A --- --- --T --T --T

5I61E36         -- --- --- C-- --- --A --- --- --- --- --- --- -AA -G- A-- -GC T-- --- -G- -C- --- --- --- --- G-- --- --- --- --- --- --- --T --G --- --G --- --- --T --- --- --- --- --- --- --- --T --- ---
5I72B40         -- --- --- C-- --- --- -A- --- --- --- --- --- -AA -G- A-- CGC T-- --- --- -C- --- --- --- --- G-- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --A --- --- --T --- ---
5I74B40         -C --- --A C-- --- --A -A- --A --- ---#--- -AA --- A-- -GG C-- --- -G- GC- --- --- --- --- G-- --- --T --- --G --- --- --- -G- --G --- -G- --- G-- --- --- --- --A A-- --T --T --T --T
5I93D25         -- --T --- --- C-- --- -A- --- --- --- --- --- -AA -G- A-- -GC TC- --- --- -C- --- --- --- --- G-- --- --- --- --- --G --- --- --- --- --G --- --- --- --- --- --- A-- --- --- --- --- --T --T ---
5I117-1                         --- --- --- --- --- --- -AA -G- A-- -GC T-- --- -G- -C- --- --- --- --- G-- --- --- --- --- --- --- --- --G --- --G --- --- --T --- --C --- --- --- --- --- --T --- ---
5I127-4                         -A- --- --- --A -GG NAA -G- A-- -GG TC- --- ATC TCA --- --- --- --- G-- --- -AT --- --- --- --- --- --- --G --- --- --- --- --- --- --- --A --- --- --- --T --T --T
5I134-1                         -A- --- --- --- --- --- -AA -G- A-- -GC TC- --- --- -C- --- --- --- --- G-- --- --- --- --- --G --- --- --- --- --G --- --- --- --- --- --- A-- --- --- --- --- --T --T ---
5I57-9                                                  -AA -G- A-- -GG TC- -C- --- -G- --- -G- --- --A G-- --- -AT --- --- --- --- A-- --- --G --- --- --- --- --- --- --- --A --- --- --- --T --T --T
1A38-5                          -A- --- --- --- -GG -AA -G- A-- --C T-C --- -GG GN- -T- --- --- --- --- --- --- -G- --- --- --- --- --C --G --- --- --- --T --- --- --- --- --- C-- --T --- ---
12620-5                         -A- --- --- --- --- A-- -G- A-- -GC T-- --- -G- -C- --- --- --- --- G-- --- -A- --- --- --- --- --T --- --G --- --- --- --- --- -A- --- --A --T --- --T --T --T
12631-6                         -AT --- -G- --- -GG -AA -G- A-- --- T-- --- CG- TC- --- --- --T --T G-- -G- -A- -G- --- T-C --- --- --- --G -A- --- --- --- G-- --- A-- --A --T --T --T --T --T
12632-4                         -AT --- -G- --- -GG NAA --- A-- --- T-- --- CG- TC- -T- --- --T --T G-- -G- -A- --- --- --- --- --- --- --G -A- --- --- --- G-- --- A-- --A --T --T --T --T --T
```

B

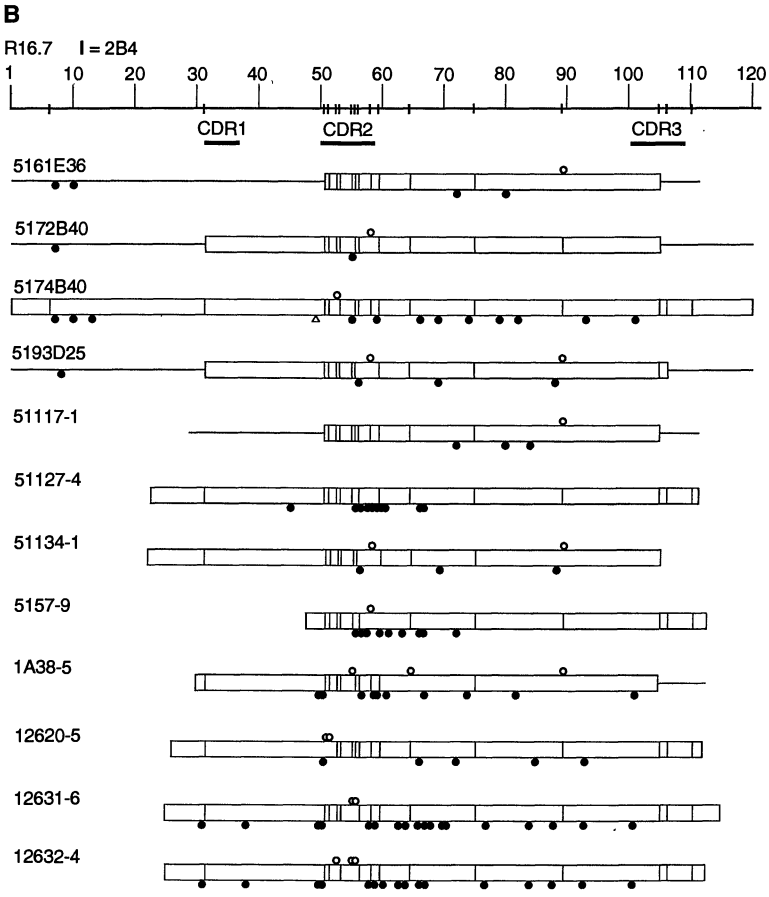

Fig. 2. Transgene conversion in hybridomas. (**A**) Sequences of the expressed VDJ regions in hybridomas that exhibit transgene conversion (*15*) are compared to the R16.7 and 2B4 VDJ sequences. Only those codons that exhibit mutations are shown. Nucleotides identical to those of R16.7 are indicated by a dash. (**B**) The sequences in (A) are diagrammed to indicate transgene conversion and hypermutation. Thin lines represent regions of identity to the R16.7 sequence; open bars indicate regions identical to the 2B4 sequence. Boundaries of the open bars cannot be precisely assigned and are arbitrarily set to the last informative nucleotide position that matches the 2B4 sequence reading outward from the CDR2 region. Within the open bars, vertical lines indicate positions matching the 2B4 sequence rather than the R16.7 sequence. Solid circles (below the bars) indicate positions that differ from both the R16.7 and 2B4 sequences; these appear to be somatic mutations. Open circles (above the bars) indicate positions that match the R16.7 sequence but are bounded by positions that match the 2B4 sequence. A nine–base pair insertion (TGGATTGGT) in 5I74B40 is indicated by a # in (A) and an open triangle in (B).

gested by previous studies (*12*), could represent a potential breakage point for promoting gene conversion.

We do not yet know whether the "transgene conversion" process we have found reflects a gene conversion or DNA recombination mechanism. However, the similarity of our results with studies of gene conversion in chickens and rabbits is particularly noteworthy. Furthermore, evidence suggesting a possible VDJ gene conversion event has been reported for a mouse hybridoma cell line (*13*). The relative frequency of gene conversion events in diversifying murine Ig genes might depend greatly on the availability of highly homologous V-genes and the relative location and distance between potential donor and acceptor sequences; this could explain the absence of gene conversion in some previous studies that have addressed this question (*4*–*6*). The results from our transgenic mice raise the possibility that gene conversion might contribute significantly to the diversification of some murine antibody responses.

REFERENCES AND NOTES

1. C. A. Reynaud, V. Anquez, H. Grimal, J. C. Weill, *Cell* **48**, 379 (1987).
2. C. B. Thompson and P. E. Neiman, *ibid.*, p. 369.
3. K. Knight and R. S. Becker, *ibid.* **60**, 963 (1990).
4. N. C. Chien, R. R. Pollock, C. Desaymard, M. D. Scharff, *J. Exp. Med.* **167**, 954 (1980).
5. S. Crews, J. Griffin, H. Huang, K. Calame, L. Hood, *Cell* **25**, 59 (1981).
6. L. J. Wysocki, M. L. Gefter, M. N. Margolies, *J. Exp. Med.* **172**, 315 (1990).
7. J. Durdik *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2346 (1989).
8. R. M. Gerstein *et al.*, *Cell* **63**, 537 (1990).
9. T. L. Rothstein and M. L. Gefter, *Mol. Immunol.* **20**, 161 (1983).
10. Transgenic mice were produced with the VVC_μ transgene construct as described previously (*7*). Two transgenic lines, $VVC_\mu1$ and $VVC_\mu5$, were obtained. $VVC_\mu1$ mice have ~10 to 20 copies of the integrated transgene, whereas $VVC_\mu5$ mice have ~25 to 50 copies. In both lines, breeding and Southern blot analyses indicated that the transgene copies are integrated as a tandem array at a single chromosomal site.
11. J. Sohn, R. M. Gerstein, C. L. Hsieh, M. Lemer, E. Selsing, *J. Exp. Med.* **177**, 493 (1993).
12. U. Krawinkel, G. Zoebelein, A. L. M. Bothwell, *Nucleic Acids Res.* **14**, 3871 (1986).
13. U. Krawinkel, G. Zoebelein, M. Brüggemann, A. Radbruch, K. Rajewsky, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4997 (1983).
14. RNAs were isolated from each hybridoma and cDNA-PCR was performed with L and C_γ primers as described previously (*11*). The L primer (5′-GTGGAATTCAAACCATGGGATGGAGCTTC-3′) hybridizes to the L exons of both the 2B4 and R16.7 VDJ regions, whereas the C_γ primer (5′-GGAATTCCGGGGCCAGTGGATAGAC-3′) hybridizes to the C_H1 exons of either $C_\gamma1$, $C_\gamma2a$, or $C_\gamma2b$. For each hybridoma, PCR products from an amplification reaction were first directly sequenced with the L or C_γ primers and the CircumVent DNA sequencing kit (New England Biolabs). Sequences for the 5I61E36, 5I72B40, 5I74B40, and 5I93D25 hybridomas were subsequently confirmed by cloning PCR products from an entirely independent cDNA-PCR reaction and sequencing PCR clones with pUC sequencing primers (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′ and 5′-AGCGGATAACAATTTCACACAGGA-3′) as well as the L and C_γ primers. The sequences determined from the two independent PCR analyses were identical.
15. Sequences of the 5I61E36, 5I72B40, 5I74B40, and 5I93D25 hybridomas were determined as described in Table 1. For the remaining hybrids, cDNA-PCR products were amplified with the L and C_γ primers, PCR clones were produced and isolated, and sequences of the PCR clones were determined with pUC sequencing primers as well as L and C_γ primers. Single PCR amplifications were used in these cases because specific PCR and ELISA assays also indicated transgene conversion events in these hybridomas (see text). The 5I hybridomas were derived from an immunized $VVC_\mu5$ mouse, and the 1A and 126 hybridomas were from two immunized $VVC_\mu1$ mice. Comparing sequences from 5I61E36 with 5I117-1, 5I93D25 with 5I134-1, and 12631-6 with 12632-4 suggests these pairs might represent sister clones derived from single B cell precursors.

16. We thank J. Sharon for help with affinity measurements; T. Imanishi-Kari for help with hybridoma production; S. Pak, E. Anderson, S. Campbell, and J. Lee for assistance with the experiments; and N. Rosenberg, H. Wortis, P. Brodeur, and T. Imanishi-Kari for reading the manuscript. Supported by American Cancer Society grant IM-628.

6 May 1994; accepted 18 July 1994

Involvement of Nitric Oxide in the Elimination of a Transient Retinotectal Projection in Development

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The adult pattern of axonal connections from the eye to the brain arises during development through the refinement of a roughly ordered set of connections. In the chick visual system, refinement normally results in the loss of the ipsilateral retinotectal connections. Inhibition of nitric oxide synthesis reduced the loss of these transient connections. Because nitric oxide is expressed by tectal cells with which retinal axons connect and because reduction of nitric oxide synthesis by tectal cells resulted in a change in the connections of retinal axons, nitric oxide probably serves as a messenger from tectal cells back to retinal axons during development.

Axons from ganglion cells in the retina form synapses with neurons in the primary visual centers in a highly ordered, predictable pattern in the adult brain. Normal visual function depends on the proper pattern of axonal connections. In some species, this adult pattern arises during development by the refinement of an early, roughly ordered pattern of connections. This early projection is characterized by transient retinal connections to the inappropriate side of the brain, inappropriate brain centers, and inappropriate positions within visual centers and to the other eye. The process by which these transient projections are eliminated is incompletely understood.

The developmental refinement of the visual projection is believed to be an activity-dependent process that involves activation of *N*-methyl-D-aspartic acid type glutamate receptors (NMDA receptors) on postsynaptic neurons in the brain (*1*). If activity in the retinal axons is blocked during the refinement period, transient projections persist (*2*). Furthermore, if NMDA receptors on the postsynaptic neurons are blocked during this period, anomalous projections also persist (*3*). The involvement of postsynaptic neurons in the refinement of retinal axon connections suggests that postsynaptic neurons must, in some way, communicate with the retinal axons. The nature of this retrograde communication is unknown.

The characteristics of nitric oxide (NO) make it an appealing candidate for a retrograde messenger in the developmental refinement of connections (*4*, *5*). Nitric oxide is synthesized and released from certain brain cells upon glutamate activation of NMDA receptors (*6*). It is able to cross cell membranes by diffusion, obviating the need for vesicular release. Moreover, NO causes an increase in guanosine 3′,5′-monophosphate (cGMP) in cells that respond to NO (*6*). In turn, cGMP can open cGMP-gated Ca^{2+} channels (*7*), and changes in intracellular Ca^{2+} concentrations influence axonal growth and retraction (*8*), essential activities for modifying patterns of axonal connections. In addition, NO appears to participate in the induction of long-term potentiation (LTP) in the adult hippocampus (*9*), and the developmental refinement of connections and LTP appear to be similar processes (*5*).

The spatial and temporal pattern of expression of nitric oxide synthase (NOS), the enzyme responsible for the synthesis of NO, is consistent with the idea that NO mediates the refinement of visual projections (*10*, *11*). In avians and rodents, the major projection from the retina is to the

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