observed in reconstruction of rotated single particles (*17*).

The functional significance of the tunnel is still to be determined. However, it is possible that the tunnel could provide the path taken by the nascent polypeptide chain since (i) the tunnel originates at the projecting arms lying at the presumed site for protein biosynthesis (*10*) and it terminates on the other end of the particle, and (ii) it is of a diameter large enough to accommodate even the largest amino acids. Furthermore, this tunnel is long enough to accommodate and protect from proteolytic enzymes a peptide of about 40 amino acids in an extended conformation (*18–20*). Although the tunnel terminates at a location that may be compatible with that assigned by immune electron microscopy as the exit site for the growing polypeptide chain (*21*), it remains to be seen whether these two sites are identical.

Assignment of the known functional domains of the 50*S* ribosomal subunits to the various structural features of our model awaits further investigation. We hope to locate specific sites on a detailed model.

REFERENCES AND NOTES

1. H. G. Wittmann *et al.*, *FEBS Lett.* **146**, 217 (1982).
2. M. Shoham *et al.*, *ibid.* **208**, 321 (1986).
3. I. Makowski, F. Frolow, M. A. Saper, H. G. Wittmann, Y. Yonath, *J. Mol. Biol.* **193**, 819 (1987).
4. A. Yonath, H. D. Bartunik, K. S. Bartels, H. G. Wittmann, *ibid.* **177**, 201 (1984).
5. A. Yonath *et al.*, *ibid.* **187**, 633 (1986).
6. A. Yonath, M. A. Saper, H. G. Wittmann, in *Structure, Function and Genetics of Ribosomes*, B. Hardesty and G. Kramer, Eds. (Springer-Verlag, Heidelberg, 1986), pp. 112–127.
7. T. Arad, K. R. Leonard, H. G. Wittmann, A. Yonath, *EMBO J.* **3**, 127 (1984).
8. J. Piefke, T. Arad, H. S. Gewitz, A. Yonath, H. G. Wittmann, *FEBS Lett.* **209**, 104 (1986).
9. A. Yonath, *Trends Biochem. Sci.* **9**, 227 (1984).
10. H. G. Wittmann, *Annu. Rev. Biochem.* **52**, 35 (1983).
11. K. H. Nierhaus, K. Bordasch, H. E. Homann, *J. Mol. Biol.* **74**, 584 (1973).
12. B. W. Matthews, *ibid.* **33**, 491 (1968).
13. J. M. Hogle, *ibid.* **160**, 663 (1982).
14. T. Richmond, J. T. Finch, B. Rushton, D. Rhodes, A. Klug, *Nature (London)* **311**, 533 (1984).
15. R. A. Milligan and P. N. T. Unwin, *ibid.* **319**, 693 (1986).
16. M. W. Clark, K. Leonard, J. A. Lake, *Science* **216**, 999 (1982).
17. A. Verschoor, J. Frank, M. Boublik, *J. Ultrastruct. Res.* **92**, 180 (1985).
18. L. I. Malkin and A. Rich, *J. Mol. Biol.* **26**, 329 (1967).
19. G. Blobel and D. D. Sabatini, *J. Cell. Biol.* **45**, 130 (1970).
20. W. P. Smith, P. C. Tai, B. D. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5922 (1978).
21. C. Bernabeau and J. A. Lake, *ibid.* **79**, 3111 (1982).
22. L. A. Amos, R. Henderson, P. N. T. Unwin, *Progr. Biophys. Mol. Biol.* **39**, 183 (1982).
23. K. R. Leonard, P. Wingfield, T. Arad, H. Weiss, *J. Mol. Biol.* **149**, 259 (1981).
24. A. Jones, *J. Appl. Crystallogr.* **11**, 268 (1978).

25. We thank J. Piefke and T. Arad for conducting the electron microscopy experiments; J. L. Sussman for adapting the computer display programs; F. L. Hirshfeld for his interest and critical comments; B. Shaanan, H. Bosshard, M. A. Saper, and A. Levy for assistance with computer problems; and H. S. Gewitz, J. Müssig, B. Hennemann, and Y. Halfon for skillful technical assistance. Supported by NIH (GM34360), BMFT (05180 MPBO), and Minerva research grants.

12 September 1986; accepted 20 February 1987

Allelic Exclusion in Transgenic Mice That Express the Membrane Form of Immunoglobulin μ

MICHEL C. NUSSENZWEIG, ALBERT C. SHAW, ERIC SINN, DAVID B. DANNER, KEVIN L. HOLMES, HERBERT C. MORSE III, PHILIP LEDER

Antibody-producing cells display a special form of regulation whereby each cell produces immunoglobulin from only one of its two sets of antibody genes. This phenomenon, called allelic exclusion, is thought to be mediated by the product of one heavy chain allele restricting the expression of the other. Heavy chains are synthesized in two molecular forms, secreted and membrane bound. In order to determine whether it is specifically the membrane-bound form of the immunoglobulin M (IgM) heavy chain (μ) that mediates this regulation, transgenic mice were created that carry a human μ chain gene altered so that it can only direct the synthesis of the membrane-bound protein. The membrane-bound form of the human μ chain was made by most of the B cells in these animals as measured by assays of messenger RNA and surface immunoglobulins. Further, the many B cells that express the human gene do not express endogenous mouse IgM, and the few B cells that express endogenous mouse μ do not express the transgene. Thus, the membrane-bound form of the μ chain is sufficient to mediate allelic exclusion. In addition, the molecular structures recognized for this purpose are conserved between human and mouse systems.

REGULATION OF IMMUNOGLOBULIN gene expression involves genomic rearrangements at a minimum of two loci that bring regulatory and coding sequences into proximity (*1*). In addition, differential cleavage and polyadenylation of messenger RNA (mRNA) precursors generate either membrane or secreted forms of immunoglobulin heavy chains (*2–4*). Continued genomic rearrangements and splicing further generate a series of heavy chain classes (*5*). Although these changes often involve both parental sets of antibody genes, only one eventually directs the synthesis of a functional immunoglobulin in a given cell. This mechanism, allelic exclusion, is central to the process of clonal selection (*6*).

There is persuasive evidence that allelic exclusion at the light chain loci is mediated by intact immunoglobulin molecules (*7, 8*). In addition, experiments with transgenic mice suggest that expression of endogenous heavy chain alleles can be influenced by an introduced murine μ chain transgene (*9–11*). Evidence thus far indicates that the human γ-1 gene (*12*) and a version of the mouse μ gene that gives rise to a secreted form of the mouse μ chain (*11*) do not mediate this process. Since immunoglobulin M (IgM) heavy chains are produced in two forms, membrane-bound and secreted, it is reasonable to suggest that such a regulatory signal is mediated by a membrane-bound molecule (*11*) because the secreted form is sequestered in the vacuolar system. To test this theory we created transgenic mice (*13*) that carry a rearranged human μ gene altered to delete the cleavage and polyadenylation signals necessary to synthesize the secreted form of the μ heavy chain (*3*). The mutant gene (Fig. 1A) is capable of producing only the membrane-bound form of the molecule in transfected tissue culture cells.

Expression of the human transgene and the endogenous mouse μ mRNAs was assessed by ribonuclease protection experiments with probes that are species specific and can distinguish between the mRNAs encoding membrane-bound and secreted forms of the μ chain (Fig. 1). Spleen cells derived from two transgenic lines, TG SA and TG SD, were used for this analysis. The two lines expressed the membrane-bound form of the human gene but differed in the amount of expression (Fig. 1B). Densitometric analysis of data from these experiments shows that spleen cells from mice of the TG SA line produce 25 to 35

M. C. Nussenzweig, A. C. Shaw, E. Sinn, P. Leder, Department of Genetics, Harvard Medical School, and Howard Hughes Medical Institute, Boston, MA 02115.
D. B. Danner, Laboratory of Molecular Genetics, National Institute on Aging, Baltimore, MD 21224.
K. L. Holmes and H. C. Morse III, Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

times as much human μ mRNA as spleen cells from the TG SD line. As expected, the form of mRNA corresponding to the secreted human μ chain was not detected in either line. In addition, the protected fragments from the transgenic lines were of the same size as those protected by authentic membrane-bound μ mRNA derived from the human Ly-65 Burkitt-lymphoma B-cell line (Fig. 1B). The finding that removal of the cleavage and polyadenylation signals renders the μ gene incapable of making the secreted form of the mRNA in vivo extends previous observations (*3*) on the regulation of the switch from the membrane-bound to the secreted form in primary lymphocytes.

Since the μ gene with associated enhancer and other regulatory signals should be expressed only in cells of the B-cell lineage, we assessed the specificity of expression of the μ transgene in a variety of organs derived from the TG SA line (Fig. 1D). Transgene mRNA was observed in the spleen and, unexpectedly, in the brain, but not in the striated muscle, salivary gland, kidney, liver, or testis. The aberrant expression of the transgene in the brain could not be accounted for by lymphoid cell contamination as assessed by the absence of mouse κ light chain mRNA in the same preparation.

Lymphocyte-specific expression has been documented for mouse μ and human γ-1 genes in transgenic mice, and it has been postulated that the immunoglobulin heavy chain enhancer is important in determining this tissue specificity (*11, 12, 14*). The sequences shared by human γ-1 and μ heavy chain genes include the heavy chain enhancer. Our findings are thus consistent with a role for the enhancer in determining tissue specificity. On the other hand, aberrant expression in the brain is puzzling since it is seen in both of our transgenic strains, TG SA and TG SD, and is therefore likely to be a property of the human μ gene construct rather than a function of the site of transgene integration. In this respect, it is reminiscent of the T4 antigen (which is the human immunodeficiency virus receptor), and the Thy-1 antigen; both of these antigens are members of the immunoglobulin supergene family expressed in the nervous system and in lymphoid cells (*15–17*).

One way of assessing the effect of expression of the human μ transgene is to measure the amount of endogenous mouse heavy chain mRNA synthesized by the splenic B cells of the transgenic animals. The relative quantities of mouse μ mRNA in the spleen cells of the two transgenic strains and matched wild-type controls were compared with β_2-microglobulin mRNA as an internal standard. Mouse μ mRNA [as measured by the ribonuclease (RNase) protection of a μ probe] in spleens of the high transgene-expressing strain, TG SA, was 1/10 to 1/20 the amount in controls (Fig. 1C and Table 1). There was little, if any, reduction in endogenous IgM production in spleen cells derived from the low-expressing strain, TG SD. Thus, expression of the human transgene resulted in decreased synthesis of endogenous mouse μ mRNA, and the effect appeared to be dose-dependent.

To distinguish allelic exclusion at the level of the individual cell from uniformly decreased expression of endogenous heavy chain genes in the total B-cell population, spleen cells were assayed for production of human and mouse surface immunoglobulins by means of two-color fluorescence on a fluorescence-activated cell sorter (FACS). This analysis allowed the correlation of immunoglobulin protein expression with mRNA data. Spleen cells were reacted with biotin-labeled monoclonal antibody to hu-

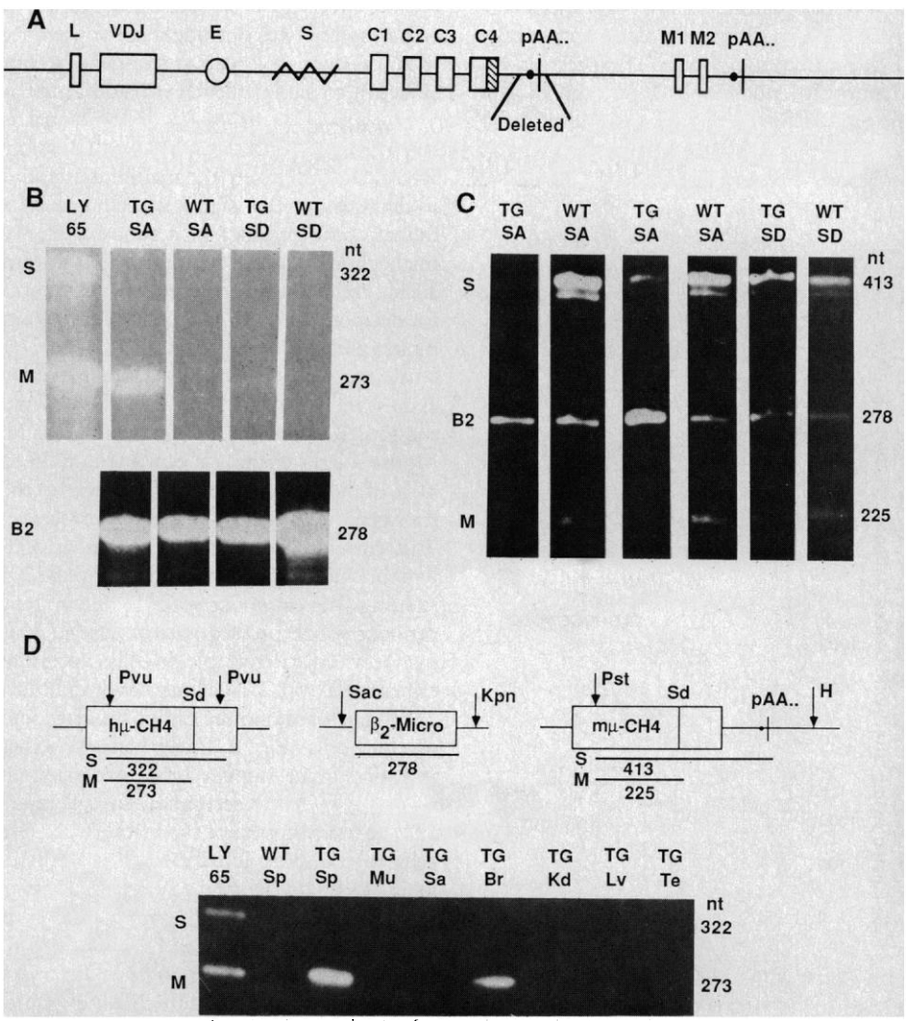

Fig. 1. RNase protection experiments in transgenic (TG) CD1 × C57BL6 mice and their wild-type (WT) littermates. Ten micrograms of total RNA was annealed with the appropriate antisense RNA at 53°C overnight. Single-stranded RNA was digested away with a mixture of RNase A and RNase T1 (*19*). Protected fragments were visualized by autoradiography of sequencing gels made of 5% acrylamide and 7*M* urea. (**A**) Human μ transgene. (**B**) Human μ expression in spleens of TG SA and TG SD animals and wild-type littermates. The Burkitt-lymphoma cell line (Ly-65) (*20*) was included to demonstrate the size of authentic secreted and membrane human IgM. (**C**) Mouse IgM and β_2-microglobulin expression in spleen RNA from TG SA and TG SD animals and their wild-type littermates. Mouse μ and β_2-microglobulin probes were hybridized with spleen RNA in the same reaction mixture. (**D**) Human μ expression in tissues of the TG SA line. The structures of the RNase protection probes used in (B) to (D) and the expected protected subfragments in nucleotides are diagrammed (*21*). Symbols: (A) L, leader exon; VDJ, recombined variable region; E, heavy chain enhancer; S, switch region; C1 through C4, constant region exons; (●), hexanucleotide AATAAA; pAA. ., polyadenylation/cleavage site (vertical line 3′ of the closed circle); M1 and M2, exons for cell membrane integration function. (B and C) TG, transgenic; WT, wild type; S, protected by secreted μ mRNA; M, protected by membrane μ mRNA; B2, β_2-microglobulin exon II; nt, nucleotide. (D) Pvu, Pvu II site; Sd, splice donor; hμ-CH4, human μ constant region exon 4; Sac, Sac I site; Kpn, Kpn I site; Pst, Pst I site; mμ-CH4, mouse μ constant region exon 4; H, Hind III site; Sp, spleen; Mu, muscle; Sa, salivary gland; Br, brain; Kd, kidney; Lv, liver; Te, testes.

man μ chains (*18*) and Texas red–labeled avidin. A panel of fluorescein-labeled antibodies specific for either mouse μ or cell lineage antigens was used as second reagents (Fig. 2). Five TG SA transgenic mice assayed expressed human μ on the surface of 25 to 35% of their spleen cells. Matched wild-type littermates served as controls. The portion of spleen cells bearing mouse μ was reduced from 50% in the normal littermates to 5 to 15% in the transgenic animals. Coexpression of transgenic and endogenous μ was not detected either by the FACS analysis or on direct microscopic inspection (Fig. 2). Immunoglobulin D expression was identical to endogenous IgM expression in the TG SA line. Thus, at the level of detection of the cell sorter, approximately 10,000 molecules per cell, the transgene induces

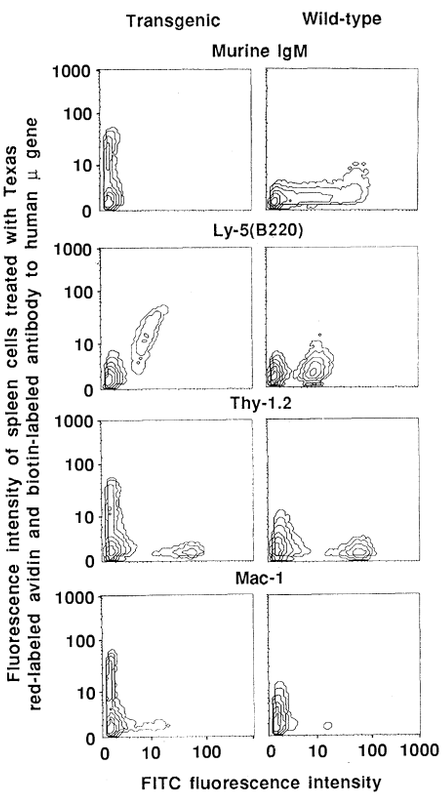

Fig. 2. Analysis by FACS of human IgM expression on spleen cells of transgenic mice and their wild-type littermates. Spleen cells were prepared and stained for simultaneous two-color FACS analysis on a FACS II as previously described (*22*). Spleen cells were stained with biotin-labeled monoclonal antibody to human μ (*18*), developed with Texas red–labeled avidin, and with fluorescein-labeled (FITC) monoclonal antibodies to mouse IgM (*23*), Ly-5(B220) (*24*), Thy-1.2 (*25*), and Mac-1 (*26*). In this experiment, 6% of the cells were positive for mouse μ and 26% for human μ. Simultaneous two-color analyses are presented as contour plots on a logarithmic scale with increasing levels of green (FITC) fluorescence on the *x*-axis and red fluorescence on the *y*-axis. Single-color FACS studies confirmed the specificity of the reagents and lack of any cross-reactivity.

allelic exclusion in the TG SA line. An identical analysis of the spleen cells of the TG SD line that produces <4% of the human mRNA found in the TG SA line failed to detect human μ protein. This lack of expression could be due to absence of cell surface protein or the presence of amounts of authentic protein below the level of detection of the cell sorter.

Since allelic exclusion is only observed in the transgenic line that expresses high levels of human IgM and since the TG SD line has normal numbers of mouse IgM-bearing B cells in spleen, we conclude that the presence of a productively rearranged gene is not sufficient to signal allelic exclusion at the μ heavy chain locus. The exclusion signal is mediated by a further product, possibly RNA but more likely the membrane-bound μ chain. Since this signal is mediated by a human gene product in a mouse cell, we further conclude that the signals that govern allelic exclusion and the cellular apparatus for deciphering them are conserved between mouse and human systems.

All the cells that express human μ also express the Ly-5(B220) antigen (Fig. 2) and the ThB B-cell surface markers as well as Ia. Mouse κ light chain was expressed on 35 to 40% of the lymphocytes in the spleens of the transgenic animals by immunofluorescence. This corresponds to the total number of B lymphocytes as determined by Ly-5(B220) staining. We can infer that the human gene product is coexpressed with mouse κ. Human μ was not detectable on cells expressing either the Thy-1.2 T-cell marker or the Mac-1 antigen of mononuclear phagocytes and myeloid cells (Fig. 2, lower half). Whether lack of surface human IgM expression in these cell types is secondary to cell type–specific transcription or the inability of these cells to transport μ to the cell surface remains to be determined.

Transgenic mice carrying intact murine μ genes have been produced by other investigators (*10, 11, 14*). Although allelic exclusion was not determined by direct examination of normal B cells in these animals, analysis of hybridomas and Abelson virus–transformed cell lines was instructive (*9, 10*). Only 1 of 29 (*9*) and 1 of 19 (*10*) hybridomas analyzed retained both heavy chain alleles in germline configuration. Some cell lines produced only transgenic μ chains and showed DJ rearrangements as well as complete VDJ rearrangements. Other cell lines derived from the same animals produced both transgenic as well as endogenous μ chains in an apparent violation of allelic exclusion. This exception and the heterogeneity of rearrangement events at the heavy chain locus could be explained by the production of secreted μ chains since the secreted form of IgM may not be able to signal allelic exclusion (*11*). The gene we introduced into our transgenic animals is capable of producing only the membrane form of the μ chain.

Table 1. Relative quantity of mouse μ chain mRNA protected by mμ-CH4 probe in spleen cells of mice of the TG SA and TG SD lines and their wild-type littermates. Data shown represent individual mice from one litter of TG SA and of TG SD. The quantity of mouse IgM mRNA protected was determined with a Beckman laser densitometer on the film from the experiment shown in Fig. 1C. The data from individual lanes was normalized to a β_2-microglobulin level of one. Symbols: TG, transgenic; WT, wild type; s, secreted form; m, membrane-bound form.

IgM	Relative quantity of mouse μ mRNA					
	TG SA	WT SA	TG SA	WT SA	TG SD	WT SD
s	0.36	8.03	0.24	3.84	2.3	3.7
m	0.18	1.1	0.07	0.98	0.67	1.5

Our findings suggest that expression of the membrane form of the μ chain is sufficient to signal allelic exclusion in the absence of secreted immunoglobulin. That allelic exclusion is induced by membrane μ implies that an immunoglobulin molecule can signal the genome through two pathways. One pathway signals B-cell maturation and depends on light chain expression and antigen binding. The other pathway must be antigen-independent since it occurs before light chain expression and, therefore, before an encounter between B cell and antigen. The antigen-independent pathway is triggered by the membrane form of the IgM heavy chain and establishes heavy chain exclusion.

REFERENCES AND NOTES

1. E. E. Max, in *Fundamental Immunology*, W. E. Paul, Ed. (Raven, New York, 1984), pp. 167–204; S. Tonegawa, *Nature (London)* **302**, 575 (1983).
2. J. Rogers *et al.*, *Cell* **20**, 303 (1980); F. Alt *et al.*, *ibid.*, p. 293.
3. D. Danner and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8658 (1985).
4. K. J. Nelson, J. Haimovitch, R. P. Perry, *Mol. Cell. Biol.* **3**, 1317 (1983); D. Yuan and P. W. Tucker, *J. Exp. Med.* **160**, 564 (1984).
5. A. Shimizu and T. Honjo, *Cell* **36**, 801 (1984).
6. B. G. Pernis, G. Chiappino, A. S. Kelus, P. G. H. Gell, *J. Exp. Med.* **122**, 853 (1965); J. S. Cebra, J. E. Colberg, S. Dray, *ibid.* **123**, 547 (1966).
7. S.-P. Kwan, E. Max, J. G. Seidman, P. Leder, M. D. Scharff, *Cell* **26**, 57 (1981); O. Bernard, N. Gough, J. Adams, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5812 (1981).
8. K. A. Ritchie, R. Brinster, U. Storb, *Nature (London)* **312**, 517 (1984).
9. D. Weaver, F. Constantini, T. Imanishi-Kari, D. Baltimore, *Cell* **42**, 117 (1985).
10. S. Rusconi and G. Kohler, *Nature (London)* **314**, 330 (1985).
11. U. Storb *et al.*, *J. Exp. Med.* **164**, 627 (1986).
12. K. I. Yamamura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2152 (1986).
13. R. D. Palmiter and R. L. Brinster, *Annu. Rev. Genet.* **20**, 465 (1986).
14. R. Grosschedl, D. Weaver, D. Baltimore, F. Constantini, *Cell* **38**, 647 (1984).

15. P. J. Maddon *et al.*, *ibid.* **47**, 333 (1986).
16. A. E. Reif and J. M. V. Allen, *J. Exp. Med.* **120**, 413 (1964).
17. A. F. Williams and J. Gagnon, *Science* **216**, 696 (1982).
18. S. Maruyana, H. Kubagawa, M. D. Cooper, *J. Immunol.* **135**, 192 (1985).
19. D. A. Melton, P. A. Krieg, M. R. Rebagliati, K. Zinn, M. R. Green, *Nucleic Acids Res.* **12**, 7035 (1984).
20. G. Lenoir, J. Preud'homme, A. Bernheim, R. Berger, *Nature (London)* **298**, 474 (1982).
21. J. R. Parnes and J. G. Seidman, *Cell* **29**, 611 (1982).
22. K. L. Holmes *et al.*, *J. Exp. Med.* **164**, 443 (1986).
23. J. Kung, S. O. Sharrow, D. G. Sieckman, R. Liberman, W. E. Paul, *J. Immunol.* **127**, 873 (1981).
24. R. L. Coffman, *Immunol. Rev.* **69**, 5 (1982).
25. J. A. Ledbetter and L. A. Herzenberg, *ibid.* **47**, 63 (1979).
26. T. Springer, G. Galfre, D. S. Secher, C. Milstein, *Eur. J. Immunol.* **9**, 301 (1979).
27. We thank R. Wallace for expert assistance in the transgenic experiments. Supported in part by grants from the American Business for Cancer Research Foundation and E. I. duPont de Nemours and Co., Inc.

8 January 1987; accepted 3 March 1987

Dual Infection of the Central Nervous System by AIDS Viruses with Distinct Cellular Tropisms

YOSHIO KOYANAGI, STEVEN MILES, RONALD T. MITSUYASU, JEAN E. MERRILL, HARRY V. VINTERS, IRVIN S. Y. CHEN

Human immunodeficiency virus (HIV) is the causative agent of the acquired immune deficiency syndrome (AIDS). A large number of AIDS patients show evidence of neurologic involvement, known as AIDS-related subacute encephalopathy, which has been correlated with the presence of HIV in the brain. In this study, two genetically distinct but related viruses were isolated from one patient from two different sources in the central nervous system: brain tissue and cerebrospinal fluid. Both viruses were found to replicate in peripheral blood lymphocytes, but only virus from brain tissue will efficiently infect macrophage/monocytes. The viruses also differ in their ability to infect a brain glioma explant culture. This infection of the brain-derived cells in vitro is generally nonproductive, and appears to be some form of persistent or latent infection. These results indicate that genetic variation of HIV in vivo may result in altered cell tropisms and possibly implicate strains of HIV with glial cell tropism in the pathogenesis of some neurologic disorders of AIDS.

ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is associated with a broad spectrum of clinical disorders (*1*). An increasing fraction of AIDS cases are recognized with disorders of the central nervous system (CNS) known as AIDS-related subacute encephalopathy (*2*). These disorders are correlated with the presence of HIV, the human immunodeficiency virus, in the CNS, which can be detected by virus isolation as well as by direct analysis by hybridization (*3, 4*). At least one of the cell types infected by HIV in brain tissue appears to be a macrophage/monocyte-like cell, presumably derived from infiltration of monocytes from the peripheral blood (*5*). However, some studies suggest that HIV can also infect cells of CNS origin (*6*).

We have studied virus from cerebrospinal fluid and brain tissue of patients with AIDS encephalopathy. In one patient, the virus in the cerebrospinal fluid was genotypically different from the virus isolated from brain tissue of the same individual. Furthermore, the cell-free virus isolated from the cerebrospinal fluid infected a primary brain glioma explant in culture. The virus from brain tissue efficiently infected macrophage/monocytes. These results suggest that genotypic variation in HIV within one individual may influence the pathogenesis of disease by conferring distinct tropisms for different systemic compartments.

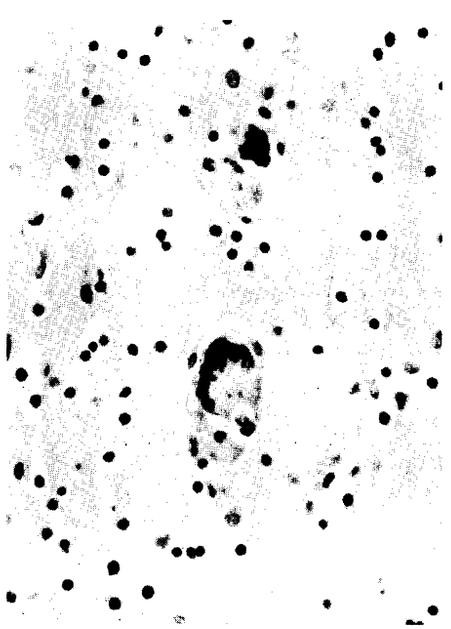

Fig. 1. Multinucleate giant cells in brain of patient J.R. Tissue samples from frontal lobe specimens were fixed with Formalin and stained with hematoxylin and eosin. The perivascular multinucleated giant cell syncytia are often observed in cases of AIDS encephalopathy (*5*).

Patient J.R. died with Kaposi's sarcoma and severe AIDS encephalopathy. The brain showed extensive leukoencephalopathy, and characteristic multinucleated giant cell syncytia were observed in pathologic specimens of frontal lobe brain tissue taken at autopsy (*7*). As shown in Fig. 1, these syncytia are morphologically similar to those previously described as being of macrophage/monocyte origin and shown to express HIV RNA detected by in situ hybridization (*5, 6*). Virus was isolated from various tissue sources by infection of lectin-activated normal human peripheral blood lymphocytes (PBL). The presence of infectious virus in the cultures was assayed by measuring reverse transcriptase (RT) activity in the supernatant fluid, and detecting viral antigens in the supernatant fluid by an enzyme-linked immunosorbent assay (ELISA; Cellular Products and Abbott) and viral DNA in the infected cells by Southern hybridization (*8*) (Fig. 2).

Viruses isolated from different sources were analyzed by restriction enzyme digestion. Viral DNA was isolated from infected PBL 11 to 14 days after cocultivation. This short period of growth in culture was chosen to minimize the possibility that selection for genetic changes would occur in vitro. Thus the viruses isolated would be most closely representative of virus resident in infected tissues.

Two genotypically distinct viruses were obtained from the CNS of patient J.R. The genome of virus isolated from the cell-free cerebrospinal fluid was distinct by restriction enzyme analysis from that of virus isolated from cells derived from the same sample (Fig. 2). Cerebrospinal fluid is generally free of cells in normal healthy individuals (*9*). The large number of cells in the cerebrospinal fluid in this case probably resulted either from cells of the CNS or from peripheral blood mononuclear cells infiltrating the cerebrospinal fluid, as is often observed in CNS disorders (*9*). We tested the possibility that the cell-associated

Y. Koyanagi, S. Miles, R. T. Mitsuyasu, I. S. Y. Chen, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, and Jonsson Comprehensive Cancer Center, Los Angeles, CA 90024.
J. E. Merrill, Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.
H. V. Vinters, Division of Neuropathology, Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024.