

have appeared (1-5, 10, 11). However, the role of light in these effects was not considered. Very low concentrations of gilvocarcins (0.01 µg/ml) are detected as DNA damaging agents following illumination under normal laboratory conditions (Fig. 3, plate 4). Experiments performed by our colleagues on other microorganisms have indicated no instance in which gilvocarcin V exhibited toxicity in the absence of light. It seems likely therefore that similar effects reported by others were light-mediated.

Among antitumor agents, gilvocarcins appear to be unusual compounds. Unlike the hematoporphyrin derivative (HPD), an agent of current clinical interest that is retained by tumors and activated by laser light (16), the antitumor activity of gilvocarcins in mice was demonstrated in the absence of intentional irradiation. Other antitumor agents (such as daunorubicin and bleomycin) also demonstrate photodynamic effects (17, 18). However, unlike gilvocarcins, these agents have cytotoxic activity that is independent of light. An interesting case is that of camptothecin, the photoactivation of which appears to have been discovered 8 years after its clinical trials (19). The effect of light on the activity of this compound in humans can only be surmised, but the clinical experience with psoralens demonstrates that such effects can be dramatic.

Whether the observed in vivo antitumor activity of gilvocarcins was mediated by ambient light is not known. Gilvocarcin V showed activity against tumor cells administered intraperitoneally, a site not exposed to light, as well as against tumor cells transplanted under the skin (6); however, gilvocarcins are extremely light sensitive and some light penetration to internal organs in animals as small as mice might occur. Activation at the skin seems unlikely because of the absence of systemic toxicity (1, 4, 9, 10) and the requirement for activation at the target DNA (12) (Fig. 2a).

In vivo, activation could be enzymatic. The lack of systemic toxicity in animals may indicate that gilvocarcins undergo selective distribution into or activation at specific target tissues. Gilvocarcins might thus provide opportunities for several modes of cancer therapy, involving in vivo activation at the sites of tumors, or activation via external irradiation.

R. K. ELESAPURU
S. K. GONDA

National Cancer Institute—
Frederick Cancer Research Facility,
Fermentation Program,
Frederick, Maryland 21701

References and Notes

1. S. N. Sehgal and C. Vézina, 11th International Congress of Chemotherapy (1979), Abstract 851; S. N. Sehgal, H. Czerkawski, A. Kudelski, K. Pandev, R. Saucier, C. Vézina, *J. Antibiot.* **36**, 355 (1983).
2. K. Hatano, E. Higashide, M. Shibata, Y. Kamada, S. Horii, K. Mizuno, *Agr. Biol. Chem.* **44**, 1157 (1980).
3. H. Nakano, Y. Matsuda, K. Ito, S. Ohkubo, M. Morimoto, F. Tomita, *J. Antibiot.* **34**, 266 (1981).
4. D. M. Balitz *et al.*, *ibid.*, p. 1544.
5. T. T. Wei *et al.*, *ibid.* **35**, 529 (1982).
6. S. Horii, H. Fukase, E. Mizuta, K. Hatano, K. Mizuno, *Chem. Pharm. Bull.* **28**, 3601 (1980).
7. K. Takahashi, M. Yoshida, F. Tomita, K. Shirahata, *J. Antibiot.* **34**, 271 (1981).
8. J. A. Findlay, J.-S. Liu, L. Radics, S. Rakhit, *Can. J. Chem.* **59**, 3018 (1981).
9. U. Weiss, K. Yoshihira, R. J. Highet, R. J. White, T. T. Wei, *J. Antibiot.* **35**, 1194 (1982).
10. M. Morimoto, S. Okubo, F. Tomita, H. Marumo, *ibid.* **34**, 701 (1981).
11. F. Strelitz, H. Flon, I. N. Asheshov, *J. Bacteriol.* **69**, 280 (1955).
12. T. T. Wei, K. M. Byrne, D. Warnick-Pickle, M. Greenstein, *J. Antibiot.* **35**, 545 (1982); F. Tomita, K.-I. Takahashi, T. Tamaoki, *ibid.*, p. 1038.
13. R. K. Elespuru and M. B. Yarmolinsky, *Environ. Mutagenesis* **1**, 65 (1979).
14. B. R. Scott, M. A. Pathak, G. R. Mohn, *Mutat. Res.* **39**, 29 (1976).
15. D. Kanne, K. Straub, J. E. Hearst, H. Rapoport, *J. Am. Chem. Soc.* **104**, 6754 (1982).
16. *J. Am. Med. Assoc.* **248**, 2797 (1982).
17. P. J. Gray, D. E. Phillips, A. G. Wedd, *Photochem. Photobiol.* **36**, 49 (1982).
18. K. R. Douglas, N. Thakrar, S. J. Minter, R. W. Davies, C. Scazzocchio, *Cancer Lett.* **16**, 339 (1982).
19. J. W. Lown and H. H. Chen, *Biochem. Pharmacol.* **29**, 905 (1980).
20. R. K. Elespuru and R. J. White, *Cancer Res.* **43**, 2819 (1983).
21. We thank R. Pandey and K. Byrne for gilvocarcins, W. Morison for psoralens, W. Bradner for bleomycin, and A. Acierio for chartreusin, W. Morison and R. Pike for providing light sources, lamp housings, calibration of lamps, and measurement of emission spectra, W. Morison, K. Byrne, R. Wax, P. Hanawalt, C. Michejda, and colleagues at the FDA National Center for Devices and Radiological Health for useful discussions.

31 August 1983; accepted 6 October 1983

The Human Homologs of the *raf (mil)* Oncogene Are Located on Human Chromosomes 3 and 4

Abstract. Two human genes that are homologous to both the murine transforming gene (*oncogene*) *v-raf* and the chicken transforming gene *v-mil* have been mapped by means of human-rodent somatic cell hybrids to human chromosomes previously devoid of known oncogenes. One gene, *c-raf-2*, which appears to be a processed pseudogene, is located on chromosome 4. The other gene, *c-raf-1*, which appears to be the active gene, is located on chromosome 3 and has been regionally mapped by chromosomal *in situ* hybridization to 3p25. This assignment correlates with specific chromosomal abnormalities associated with certain human malignancies.

Transforming genes, or oncogenes, are a class of evolutionarily conserved genes that have been associated with the development of tumors in various vertebrate species (1). In some cases they are sufficiently conserved that invertebrate homologs have been identified (2). The fact that these genes are so well conserved suggests that they have an important role in the normal cell and that their tumorigenic properties arise from their abnormal expression. Most of the approximately 20 known oncogenes were originally identified as genes of nonprimate origin that were incorporated into acutely transforming retroviruses and have yet to be directly implicated in human disease. One possible approach to defining their involvement in human cancer is based on the observation (3) that certain types of human tumors are associated with specific chromosomal rearrangements. This observation led Klein (4) to suggest that such tumors arise from the transposition of transforming genes to a chromosomal location where their regulation is altered. This hypothesis has received considerable support from reports (5) that the characteristic translocation in Burkitt lymphoma of a portion of chromosome 8 to

chromosome 14, t(8;14) (q24;q23), places the *myc* gene in proximity to the immunoglobulin heavy chain genes. If tumor-specific rearrangements activate oncogenes, it might be possible to use the chromosomal location of an oncogene to focus on a few types of human tumors as the most probable candidates for involvement of the oncogene. In this report, we apply this concept to the recently characterized *raf* and *mil* oncogenes, which are of murine origin (6) and avian origin (7), respectively.

The *raf* oncogene was isolated as the transforming gene of 3611-MSV, an acutely transforming, replication-defective, murine retrovirus. This virus transforms fibroblasts and epithelial cells in culture and induces fibrosarcomas in newborn mice. The *v-raf* does not encode a tyrosine-specific protein kinase, which is common to several oncogenes (6, 8). The *mil* oncogene has been identified as a second oncogene in the avian retrovirus MH2, which contains the *myc* oncogene. In contrast to other *myc*-containing retroviruses, the MH2 virus is associated with a high incidence of liver and kidney carcinomas. This carcinoma-inducing potential could be a characteristic of the *mil* oncogene or a cooperative

effect of the *mil* and *myc* oncogenes. We recently found that the *raf* and *mil* oncogenes are the murine and avian forms of the same gene (9). Using the viral *raf* gene, *v-raf*, we identified two related genes in human DNA and characterized them by molecular cloning (10). One gene, *c-raf-1*, spans at least 16 kilobases (kb), contains at least eight introns, and is homologous to the full 1.2 kb of *v-raf*. The second gene, *c-raf-2*, lacks the introns present in *c-raf-1* and is a pseudo-gene because its *v-raf*-related sequence contains no open reading frame of more than 250 nucleotides. These two genes are the only human genes closely related to *v-raf* as judged by low-stringency DNA hybridization, and both genes are also homologous to the viral *mil* gene.

Thirty-five somatic cell hybrids made with normal human lymphocytes and either mouse or Chinese hamster cell lines (11) were used to determine the location of the human genes. Since the hybrids contain a full complement of rodent chromosomes with various partial complements of human chromosomes, they can be used to establish an unambiguous correlation between the presence of a human gene such as *c-raf-1* and the presence of a particular human chromosome. The human chromosomes present in each hybrid were identified by isozyme and karyotype analyses (11). The presence or absence of *c-raf-1* and *c-raf-2* in

Table 1. Correlation between the presence of the *c-raf-1* and *c-raf-2* genes and individual human chromosomes in rodent-human somatic cell hybrids.

Human chromosome	Percent discordance with	
	<i>c-raf-1</i>	<i>c-raf-2</i>
1	29	59
2	24	47
3	3	41
4	37	3
5	32	56
6	38	35
7	26	50
8	32	44
9	32	44
10	24	44
11	40	29
12	35	53
13	41	41
14	40	38
15	37	47
16	40	44
17	28	55
18	35	41
19	32	50
20	34	50
21	42	53
22	40	38
X	74	35

the hybrids was determined by Southern blot analysis (12) of their DNA's.

The human *c-raf-1* gene contains two characteristic Hpa I fragments of 5.9 and 3.6 kb that hybridize to the *v-raf*-specific probe. The 5.9-kb fragment is easily distinguished from the *v-raf*-related Hpa I

fragments of mouse and hamster DNA's (Fig. 1A) and can be used to score the hybrids for the presence of *c-raf-1*. The 3.6-kb band is obscured in the mouse hybrids by a mouse band of the same size but can also be used to score Chinese hamster hybrids for the presence of *c-raf-1*. As expected, the two bands are 100 percent concordant in the Chinese hamster hybrids. Although the rodent bands sometimes obscure the 3.6-kb human band, their presence provides an internal control on the amount of DNA in each lane. The results (Table 1) for the 22 Chinese hamster-human hybrids and 13 mouse-human hybrids indicate a clear correlation (97 percent) between the presence of *c-raf-1* and chromosome 3. The discordance of a single hybrid, 80H1A, was confirmed by analysis of a different cell passage, 80H1D, of the same hybrid. The fact that this hybrid is positive for *c-raf-1* but negative for chromosome 3 and its isozyme marker aminocyclase I may be explained by a rearranged human chromosome. Such a rearranged chromosome was detected by G-11 banding, which distinguishes human chromosomes from rodent chromosomes. However, its chromosomal origin was not readily identifiable by G banding. All other chromosomes show a substantial discordance (> 24 percent) with *c-raf-1*. Thus, we can assign *c-raf-1* to human chromosome 3.

The 19-kb Hpa I fragment of *c-raf-2* is too faint to be detected reliably with a *v-raf* probe. We have therefore used a 2.2-kb fragment of the *c-raf-2* gene as a probe for mapping its location. This fragment contains the *v-raf*-related sequence as well as an adjacent single-

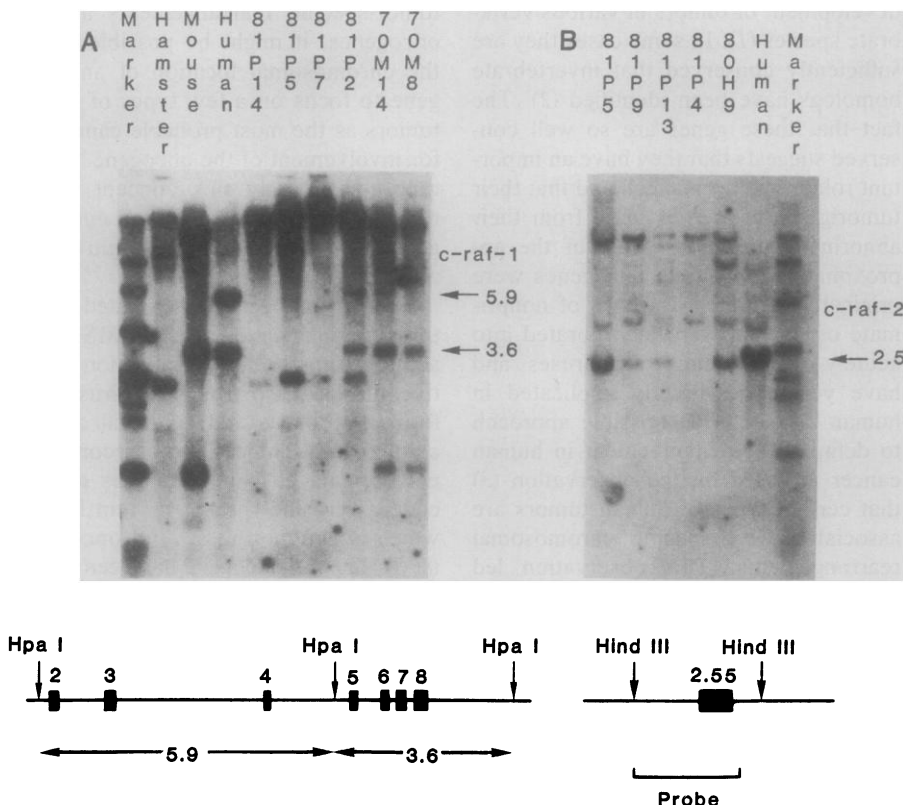


Fig. 1. Southern blot demonstrating *raf*-related sequences in human, mouse, and hamster DNA's and human-rodent hybrid DNA's. (A) All DNA's were digested with Hpa I, subjected to electrophoresis on 0.7 percent agarose gels, blotted onto nitrocellulose, and hybridized to a *v-raf*-specific probe as described (6). The bands due to human *c-raf-1* are 5.9 and 3.6 kb and are identified on the map of the gene shown below the blot. The exons showing homology to *v-raf* are shown as boxes and are numbered in the 5' to 3' direction. The 81 series of hybrids are all human-hamster hybrids with *c-raf-1* present in only 81P2. The 70 series of hybrids are human-mouse hybrids with *c-raf-1* present in 70M14 but absent in 70M8 as judged by the 5.9-kb band. (B) DNA's were digested with Hind III and blotted as in (A). Hybridization with the *c-raf-2* probe (for location see the partial map of the *c-raf-2* gene below the blot) produces a strong 2.55-kb *c-raf-2* band in human DNA as well as faint *c-raf-1* bands. All the hybrids shown are positive for *c-raf-2* except 81P4. The markers are end-labeled Hind III fragments of DNA plus Taq I fragments of ϕ X174 DNA.

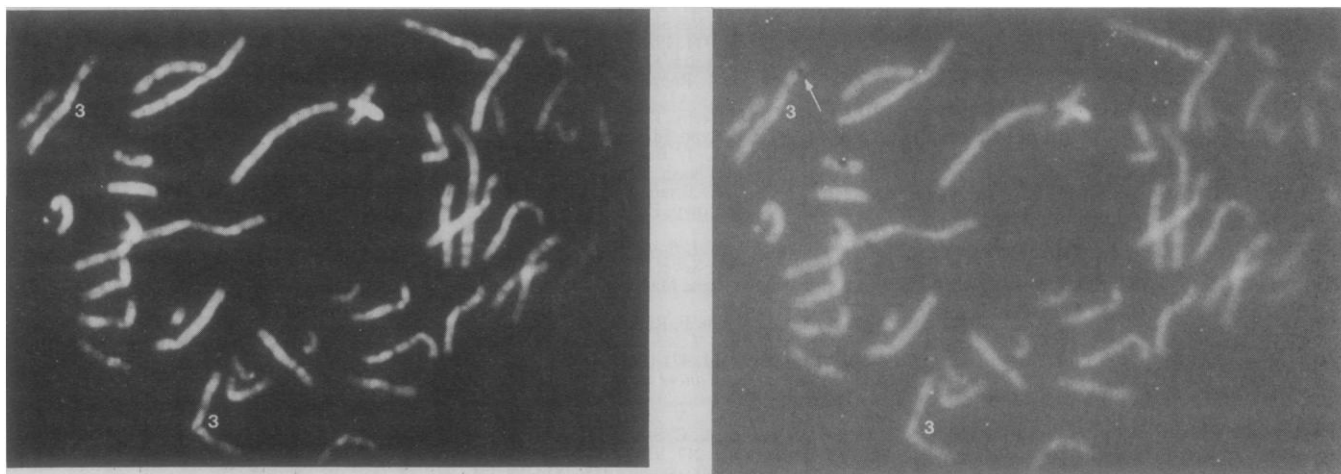


Fig. 2. In situ hybridization of the *c-raf-1* probe to normal human male metaphase chromosomes with use of a modification (21) of the method of Harper and Saunders (22). The plasmid containing single-copy sequences from the 3' flanking region of *c-raf-1* was labeled with all four tritiated nucleotides to a specific activity of 1.0×10^7 count/min per microgram. The chromosome preparations were hybridized overnight, exposed to photographic emulsion for 1 week, and then stained with quinacrine mustard dihydrochloride. A representative partial metaphase spread is shown as seen by incident fluorescent lighting (left) and by a combination of incident fluorescent and transmitted visible light (right) for visualization of silver grains. Note the grain near the end of the short arm of one of the two chromosomes labeled 3 at band p25.

copy sequence. A Hind III digest of human DNA hybridized to this probe produces a characteristic 2.6-kb band for the *c-raf-2* gene, which can be distinguished from the 6.8-, 3.5-, and 1.8-kb bands of *c-raf-1* as well as from the related bands in mouse and Chinese hamster DNA's (Fig. 1B). The results obtained with 34 hybrids (Table 1) indicate that *c-raf-2* is on chromosome 4. The discordance of a single hybrid, 80H9A, was confirmed in a different passage as positive for *c-raf-2* but negative for chromosome 4 and its two isozyme markers. This hybrid also contained a rearranged human chromosome that resembled chromosome 4 but was not identical to chromosome 4. Thus, it may have contained the *c-raf-2* portion of chromosome 4. All other chromosomes show a greater than 35 percent discordance with *c-raf-2*. We can therefore assign *c-raf-2* to chromosome 4. Thus, both human *c-raf* genes are located on chromosomes to which oncogenes have not previously been mapped (13). The presence of the pseudogene *c-raf-2* on a different chromosome from the one containing *c-raf-1*, together with a lack of introns, suggests that *c-raf-2* is a processed pseudogene (14).

There are numerous reports of chromosome 3 abnormalities in human tumors [see references in (15)] that lack any clear specificity. There are, however, three types of tumors that involve specific rearrangements of chromosome 3, all on the short arm of the chromosome: familial renal cell carcinoma with translocations at 3p21 and 3p13-14 (16), small-cell lung carcinomas with deletions

of various sizes spanning the region 3p14-3pter (17), and mixed parotid gland tumors with translocations at 3p21 and 3p25 (18). All three tumors involve epithelial cells that might be expected to be transformed by *raf* (*mil*). To determine the locus of the *c-raf-1* gene more precisely and to evaluate whether it is near any of these tumor-specific rearrangements, we have mapped its position by chromosomal in situ hybridization. A plasmid containing a 2.5-kb Eco RI fragment from the 3' flanking regions of *c-raf-1* was used as a probe to hybridize to normal male metaphase chromosomes (Fig. 2). Analysis of 70 chromosomal

spreads revealed that approximately 9 percent of all silver grains were located on or directly beside chromosome 3 at band p25 (Fig. 3) and that approximately 16 percent of all cells showed hybridization at 3p25. Statistical analysis of these data by the Poisson distribution with the number of grains per chromosome band adjusted for the relative size of the band in a 400-band idiogram (19) revealed a highly significant ($P < 10^{-33}$) distribution at 3p25. This location suggests that the *c-raf-1* locus may be involved in the mixed parotid gland tumors with the t(3;8) (p25;q21) translocation (17). It is likely from its position that the *c-raf-1*

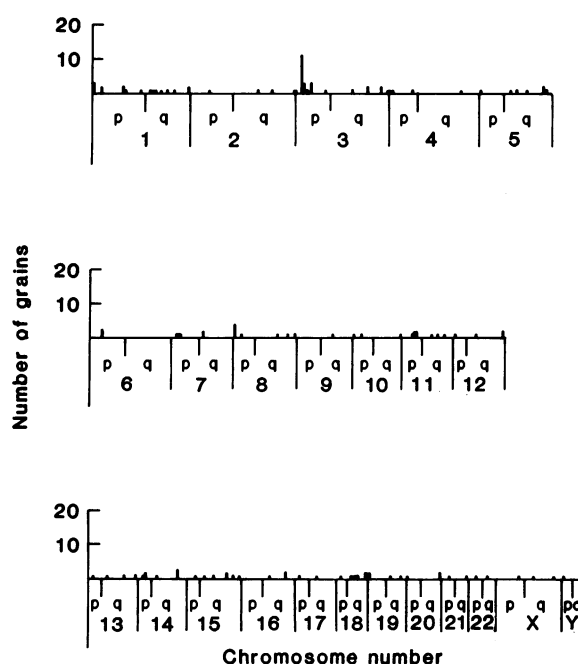


Fig. 3. Distribution of silver grains from 70 chromosome spreads. The total number of grains per chromosome band is plotted against band position in a 400-band idiogram.

gene is not rearranged in either small-cell lung carcinoma or renal cell carcinoma. However, most 3p deletions in small-cell lung carcinoma could result in a hemizygous state for the *c-ras*-1 gene. Whether this alteration reveals the presence of a deleterious recessive gene, as has been proposed for retinoblastoma (20), remains to be determined.

TOM BONNER*
STEPHEN J. O'BRIEN
WILLIAM G. NASH
ULF R. RAPP

Laboratory of Viral Carcinogenesis,
National Cancer Institute,
Frederick, Maryland 21701

CYNTHIA C. MORTON
PHILIP LEDER

Department of Genetics,
Harvard Medical School,
Boston, Massachusetts 02115

References and Notes

1. J. M. Bishop, *Annu. Rev. Genet.* **52**, 301 (1983).
2. B.-Z. Shilo and R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6789 (1981); H. Hoffman-Falk, P. Eirat, B.-Z. Shilo, F. M. Hoffman, *Cell* **32**, 589 (1983).
3. J. D. Rowley, *Nature (London)* **301**, 290 (1983); J. J. Yunis, *Science* **221**, 227 (1983).
4. G. Klein, *Nature (London)* **294**, 313 (1981).
5. R. Taub *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982); R. Dalla-Favera, S. Martinotti, R. C. Gallo, J. Erikson, C. M. Croce, *Science* **219**, 963 (1983).
6. U. R. Rapp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4218 (1983).
7. H. W. Jansen, B. Ruckert, R. Lurz, K. Bister, *EMBO J.* **2**, 1969 (1983); H. W. Jansen, T. Patchinsky, K. Bister, *J. Virol.* **48**, 61 (1983).
8. U. R. Rapp, F. H. Reynolds, Jr., J. R. Stephenson, *J. Virol.* **45**, 914 (1983).
9. H. W. Jansen, R. Lurz, K. Bister, T. I. Bonner, G. E. Mark, U. R. Rapp, *Nature (London)*, in press.
10. T. I. Bonner, S. Kerby, P. Suttrave, M. Gunnell, G. Mark, U. R. Rapp, in preparation.
11. S. J. O'Brien, T. I. Bonner, M. Cohen, C. O'Connell, W. G. Nash, *Nature (London)* **303**, 74 (1983); S. J. O'Brien, W. G. Nash, J. L. Goodwin, D. R. Lowy, E. H. Chang, *ibid.* **302**, 839 (1983).
12. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
13. Proceedings of the Seventh International Workshop on Human Gene Mapping, *Cytogenet. Cell Genet.*, in press.
14. A. Leder, D. Swan, F. Ruddle, P. D'Eustachio, P. Leder, *Nature (London)* **293**, 196 (1981); P. A. Sharp, *ibid.* **301**, 471 (1983).
15. A. A. Sandberg, *Cancer Genet. Cytogenet.* **7**, 1 (1982).
16. A. J. Cohen *et al.*, *N. Engl. J. Med.* **301**, 592 (1979); S. Pathak, L. C. Strong, R. E. Ferrell, A. Trindade, *Science* **217**, 939 (1982).
17. J. Whang-Peng *et al.*, *Science* **215**, 181 (1982); J. Whang-Peng *et al.*, *Cancer Genet. Cytogenet.* **6**, 119 (1982).
18. J. Mark, R. Dahlenfors, C. Ekedahl, G. Stenman, *Cancer Genet. Cytogenet.* **2**, 231 (1980); J. Mark, R. Dahlenfors, C. Ekedahl, *Hereditas* **96**, 141 (1982).
19. D. G. Harden, J. E. Lindsten, K. Buckton, H. P. Klinger, Eds., *Cytogenet. Cell Genet.* **31**, 5 (1981).
20. W. F. Benedict, A. L. Murphree, A. Banerjee, C. A. Spina, M. C. Sparkes, R. S. Sparkes, *Science* **219**, 973 (1983).
21. I. R. Kirsch, C. C. Morton, K. Nakahara, P. Leder, *ibid.* **216**, 301 (1982).
22. M. E. Harper and G. S. Saunders, *Chromosoma* **83**, 431 (1981).
23. We thank Steve Kerby, Janie Simonson, Mary Eichelberger, and Richard Bauer for technical assistance.

* Present address: Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Md. 20205.

4 August 1983; accepted 31 October 1983

Transmission of Simian Acquired Immunodeficiency Syndrome (SAIDS) with Blood or Filtered Plasma

Abstract. *Simian acquired immunodeficiency syndrome (SAIDS), a disease clinically and pathologically similar to acquired immunodeficiency syndrome in humans, was transmitted from diseased rhesus monkeys (Macaca mulatta) to normal monkeys by inoculation with heparinized whole blood or plasma that had been passed through filters of 0.45 micrometer pore size. This suggests that the causative agent is small and most probably a virus. No viruses, however, were isolated by standard cell culture techniques from the blood or filtered plasma which caused SAIDS. Both cellular and humoral immunity were markedly depressed in animals with advanced SAIDS.*

Many different infectious agents have been isolated from patients with acquired immunodeficiency syndrome (AIDS), but none of these has been clearly implicated as the cause of this disease. The difficulty of identifying the cause of AIDS has been compounded by the lack of a susceptible experimental animal. A spontaneous outbreak of a disease clinically and pathologically similar to AIDS in humans was recently described in rhesus monkeys (*Macaca mulatta*) housed in an outdoor corral at the California Primate Research Center of the University of California, Davis (CPRC) (1). Affected animals had symp-

toms similar to those of AIDS victims including profound immunosuppression, lymphadenopathy, splenomegaly, multiple opportunistic infections, persistent diarrhea, chronic wasting, and high mortality. Some animals also had cutaneous fibrosarcomas. A similar immunosuppressive disease was reported to occur in macaque monkeys housed at the New England Primate Research Center in Southborough, Massachusetts (2). An understanding of SAIDS is important so that methods can be developed to protect nonhuman primates from this devastating disease. Such studies may also provide clues to the etiology and patho-

genesis of AIDS in humans and serve as a useful model for investigation of prophylaxis and therapy.

We recently reported on the experimental transmission of SAIDS from two animals at the CPRC to four rhesus monkeys at the National Institutes of Health (NIH) that were negative for cytomegalovirus (CMV) antibody (3). Inocula for these studies were mixtures of unfiltered supernatant fluids from 10 percent homogenates of various organs with or without buffy coat cells from blood. In this report we narrow our focus on the cause of SAIDS by describing transmission of the syndrome to rhesus monkeys using whole blood or filtered plasma from diseased animals.

These studies were carried out at two geographically separated sites, NIH and CPRC, with inocula from different donor animals. The experiments were performed independently but the data were shared.

Four juvenile rhesus monkeys were each inoculated intravenously with 0.9 ml of heparinized whole blood from either of two moribund donor animals with experimentally transmitted SAIDS (Table 1). The clinical history and pathology of the donor animals were described previously (3). Monkeys 1 and 2, inoculated at NIH, were 8.5 and 8 months of age, respectively, and monkeys 3 and 4, inoculated at CPRC, were both 11 months of age. All four inoculated animals developed SAIDS; three of them became moribund and died 2 and 3 months after inoculation. Animal 1 remains alive with persistent generalized lymphadenopathy and splenomegaly 5 months after inoculation (Table 1).

In an attempt to characterize the SAIDS agent, plasma from two donor animals with advanced disease was filtered, sequentially, through two 0.45- μ m Millipore filters to minimize the chance of filter failure. At CPRC, the integrity of the filters was further verified by retention of a mixture of *Staphylococcus aureus* and *Escherichia coli* by the same filter used to filter the SAIDS plasma. Confluent growth of the bacteria occurred on agar medium prior to filtration of the mixture, but no growth by either organism was seen after filtration.

Four juvenile rhesus monkeys were each inoculated with 3 ml of the filtered plasma from two animals with SAIDS (Table 1). Monkeys 5 and 6, inoculated at NIH, were negative for antibody to rhesus monkey CMV and were 11 and 8 months of age, respectively. At CPRC, animals 7 and 8, both 14 months of age, had antibody to rhesus monkey CMV. Two to four weeks after inoculation with